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Review

Müller Cell Metabolic Signatures: Evolutionary Conservation and Disruption in Disease

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Müller cells are glia that play important regulatory roles in retinal metabolism. These roles have been evolutionarily conserved across at least 300 million years. Müller cells have a tightly locked metabolic signature in the healthy retina, which rapidly degrades in response to insult and disease. This variation in metabolic signature occurs in a chaotic fashion, involving some central metabolic pathways. The cause of this divergence of Müller cells, from a single class with a unique metabolic signature to numerous separable metabolic classes, is currently unknown and illuminates potential alternative metabolic pathways that may be revealed in disease. Understanding the impacts of this heterogeneity on degenerate retinas and the implications for the metabolic support of surrounding neurons will be critical to long-term integration of retinal therapeutics for the restoration of visual perception following photoreceptor degeneration.

Highlights

Müller cells are the primary macroglia of the retina, that have a highly conserved metabolic signature across species.

During degeneration, Müller cells' metabolic signatures diverge and become chaotic.

The glutamate cycle is disrupted during retinal degeneration, leading to levels of glutamate and glutamine varying irrespective of glutamine synthetase levels.

Factors contributing to Müller cell metabolic homogeneity in health and heterogeneity in disease are currently matters of speculation requiring further investigation.

Introduction

The retina is a thin, multilaminar extension of the central nervous system (CNS) located at the back of the eye. This neural tissue is responsible for the detection and initial processing of visual primitives including luminance, contrast, direction, and velocity before sending them through the optic nerve to other areas of CNS for further processing. The retina is highly organized and compact and, like the rest of CNS, comprises neurons and glia. The largest population of glia in the retina, and the only retina-specific glia, is the Müller cell (Box 1). This review focuses on the metabolism of Müller cells, which are found in every vertebrate retina described to date, and features retinas from multiple vertebrate species (both healthy and diseased) and characterizes them based on their metabolic phenotype. Metabolic phenotyping, put simply, describes an analysis of combinations of small molecules and proteins allowing the characterization of unique cell classes (Box 2). Müller cells have a remarkably stable and homogeneous metabolic phenotype in healthy retinas across vertebrate species, indicating high levels of conservation through evolution. However, this stability becomes chaotic in retinal disease. While the regulatory mechanisms responsible for this homogeneity in Müller cells across the retina and what specific alterations lead to the loss of this homogeneity in disease are not understood, the metabolic precision can be measured and is an area of active exploration. Identifying precipitating factors leading to metabolic changes in disease, and what impact these changes have on the ability of Müller cells to support neuronal function, not only has significance in understanding the progression of retinal disease but will also be fundamental in designing therapeutics compatible with the diseased retina.

Retinal Function and Metabolic Support

Retinal neurons can be broadly split into outer and inner retinal neurons based on retinal stratification. Photoreceptors, found in the outer retinal layers, are highly specialized neurons responsible for the initial detection of light and the transduction of that signal to the first synapse in visual processing. Signals are then propagated from photoreceptors to the inner retinal neurons, which are responsible for the shaping and refinement of the visual primitives before they are transmitted into the brain for further processing. Inner retinal processing entails refinement of signals through

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Box 1. Retinal Glia

Glia are a prominent feature of the nervous system, initially described in the early to mid-1900s as holding neurons together as eponymous glue. Since this time, numerous types of and functional differences between glia have been described [70], although many functions and mechanisms remain a mystery. Broadly, oligodendrocytes (CNS) and Schwann cells (peripheral nervous system) provide myelin to insulate long axons. Microglia exist in numerous states and function as the resident immune cells of the nervous system involved in processes from development to synapse maintenance to the clearance of debris in injury and disease [71]. Astrocytes are probably the most heavily studied glia, consisting of numerous types and performing diverse functions including maintenance of the blood–brain barrier, regulation of ion homeostasis, clearance of extrasynaptic neurotransmitters, and synaptic function modulation [72,73].

In the retina, there are three primary types of glial cells: microglia, astrocytes, and Müller cells [74], where Müller cells are found solely in the retina. Oligodendrocytes are not typically included as retinal glia because axons in the retina are typically not myelinated. Microglia in the retina are found distributed throughout the retinal layers especially at the edges of the plexiform layers. Retinal microglia perform functions similar to those observed in the brain, where they exist in multiple states and can rapidly react and move towards sites of injury and disease, and act in a surveillance capacity in the healthy retina. Astrocytes are largely absent in avascular retinas (e.g., rabbits) but are found along the optic fiber layer. In vascular retinas, astrocytes are found largely in connection with the distribution of blood vessels, where astrocytes contribute to the blood–retina barrier. Retinal astrocytes are unique from those of the brain in that they are found in lower numbers and express lower levels of [75] or no [76] GS, indicating that their contributions to metabolic support differ from those in the brain. Instead, retinal astrocytes are found to express high levels of vascular endothelial growth factor (VEGF) and may be associated with the promotion of vascularization in healthy and pathological retinas [77]. Müller cells are the most prominent retinal glia found in all species. Müller cells perform many of the same functions in the retina as astrocytes in the brain, including ion homeostasis, regulation of extracellular space, and removal of extrasynaptic neurotransmitters [4]. In addition, Müller cells form barriers in the retina, including the outer limiting membrane and the inner limiting membrane, and contribute to the blood–retina barrier.

both feedforward and feedback loops among multiple classes of neurons: horizontal cells, bipolar cells, amacrine cells, and ganglion cells. For more information of retinal visual processing, see [1]. In terms of metabolism, the retina, and in particular the photoreceptor/retinal pigment epithelium (RPE) interface is the most metabolically demanding interface in vertebrates [2,3]. The metabolic demands of the retina are primarily split between the RPE and Müller cells, as astrocytes are relatively sparse in the retina. The RPE primarily serves as a source of energy, in the recycling of visual cycle components, and in waste management for the photoreceptors. Müller cells, by contrast, serve as structural, homeostatic, and metabolic support in part for photoreceptors but primarily for the neurons of the inner retina [4].

Müller Cells and the Glutamate Cycle

Glutamate is a nonessential amino acid central to numerous metabolic and neurotransmitter processes in cells [5–7]. In the nervous system, glutamate is the most common excitatory neurotransmitter [8,9]. Glutamate is also a precursor for the synthesis of GABA, the most prevalent inhibitory neurotransmitter [10,11]. Although the signaling by glutamate as a neurotransmitter is often the most referenced function in the nervous system, it is important to recognize that glutamate plays a central role in numerous other biochemical functions. Some of these functions of glutamate include: assimilation and distribution of nitrogen [12,13]; being a source of amines for numerous amino acids [14,15]; as a carbon source for the TCA cycle via breakdown into α-ketoglutarate [16,17]; and, of course, as a common amino acid used in protein synthesis [5,18].

In the glutamate cycle of the retina (Figure 1), glutamate is released from excitatory neurons then Müller cell processes surrounding the synaptic cleft transport the extracellular glutamate into Müller cells, primarily via excitatory amino acid transporter 1 (EAAT1), also referred to as glutamate/aspartate transporter (GLAST) [19]. Upon entering the Müller cell, glutamine synthetase (GS) rapidly converts glutamate to glutamine by amidation via an ATP-dependent process [20]. Of note, only macroglia of the retina contain GS, with Müller cells containing the highest levels [21,22]. Following conversion to glutamine within the Müller cell, glutamine is exported into the extracellular space by glial SN1 [23,24]. In all neurons, but especially in GABAergic and

glutamatergic neurons, glutamine can be converted back to glutamate by glutaminase (EC 3.5.1.2) [25]. Glutamate can now be loaded into vesicles via vesicular glutamate transporters (VGLUTs) and released on depolarization or converted to GABA by glutamate decarboxylase (EC 4.1.1.15) [26] and loaded into vesicles via VGAT (which transports both GABA and glycine) [27]. The role of Müller glia is therefore integral to both extracellular glutamate clearance and the synthesis of glutamate and GABA within the retina [28]. It is this transport of glutamate and its rapid amidation to glutamine that is believed to be responsible for the high glutamine and medium to low glutamate components of the Müller cell metabolic signature.

The above mechanism is the most cited pathway for glutamate recycling following synaptic release, but it is important to note that there are differences in the dependence of outer retinal versus inner retinal neurons with respect to this cycle [29]. Glutamate can be transported into neurons by glutamate transporters expressed within the synaptic cleft [19]. This is especially true in the outer retina where high levels of photoreceptor EAAT5 expression lead to the transport of large amounts of glutamate back into the photoreceptor from the synaptic cleft. Therefore, much of the glutamate recycling of the outer retina occurs independent of Müller cells [30]. In the inner retina, however, Müller cell transport of glutamate that diffuses beyond the synaptic cleft is important to prevent off-target signaling [31] and recycle the glutamate for inner retinal neurons [32].

Müller Cells and Taurine

Müller cells are responsible for regulating their surrounding microenvironment, including the osmolarity of the extracellular space. In large part this is done through the rapid removal of released neurotransmitters and K^+ from the extracellular space [4]. In the healthy retina, Müller cells also regulate their own cell volume closely and do not swell in response to neurotransmitter release the way neurons do. This is, in part, due to the transmembrane transport of taurine and release by Müller cells under hypoosmotic conditions[33,34].

Taurine is a sulfonic acid commonly referred to as a conditional amino acid. In contrast to conventional amino acids, taurine has its amine group on the β-carbon (rather than the α-carbon) and is not used in the assembly of proteins. Although taurine can be synthesized from cysteine, it is not synthesized at high enough levels to account for the large quantities found in mammals. It is therefore necessary to obtain it from the diet [35]. Also, despite its high levels in CNS tissues, taurine is not used as a conventional neurotransmitter as, to date, no taurine receptor has been identified

Box 2. Computational Molecular Phenotyping (CMP)

For decades, the role of metabolism in cellular systems has been predominantly assessed using tissue homogenates, yielding information about total concentrations of small molecules and enzymes across an entire tissue. Alternatively, tissues may be dissociated to determine the concentrations of metabolites in a single cell type, although dissociation often leads to metabolite concentration changes compared with intact tissue. To better understand the combinations of small molecules contained in cells and how they respond to insult and disease, a method for individual-cell metabolic characterization in intact tissue is necessary.

Techniques originating in the 1970s and 1980s demonstrated differing levels of neurotransmitters and neuropeptides in individual cells by using rapid fixation with mixed aldehydes, trapping small molecules (including amino acids) in their cellular compartments, and probing with various antibodies [78–80]. This was further expanded by serial sectioning of fixed tissues, exposing epitopes of small molecules in multiple sections through a single cell without having to dissociate the tissue. Registering the serial sections, and probing for various small molecules, allows one to identify the metabolic profile of individual cells in a complex tissue. This can be further expanded on using multidimensional pattern recognition via computer algorithms for more robust classification, in which the quantitative levels of all small molecules comprise the signature of a class (Figure I) [43]. This method of cell classification and metabolic evaluation is referred to as CMP [81].

The nervous system is an extensively heterocellular system with complex topologies, in which small molecules act within cells and on neighboring cells through tightly regulated cell- and tissue specific mechanisms. Using CMP approaches, we identified a robust *n*-dimensional small-molecule and protein signature of each primary cell class of the retina in multiple species [42,43,48,54,81–87], although this technology can be utilized in any small-molecule-containing tissue. Cell-class signatures are driven by the metabolic demands, relationships, and neurotransmitter functions of each unique cell class. These signatures may change as metabolic demands are altered following trauma or disease, providing insight into the metabolic state of the total tissue as well as individual cell classes [88].

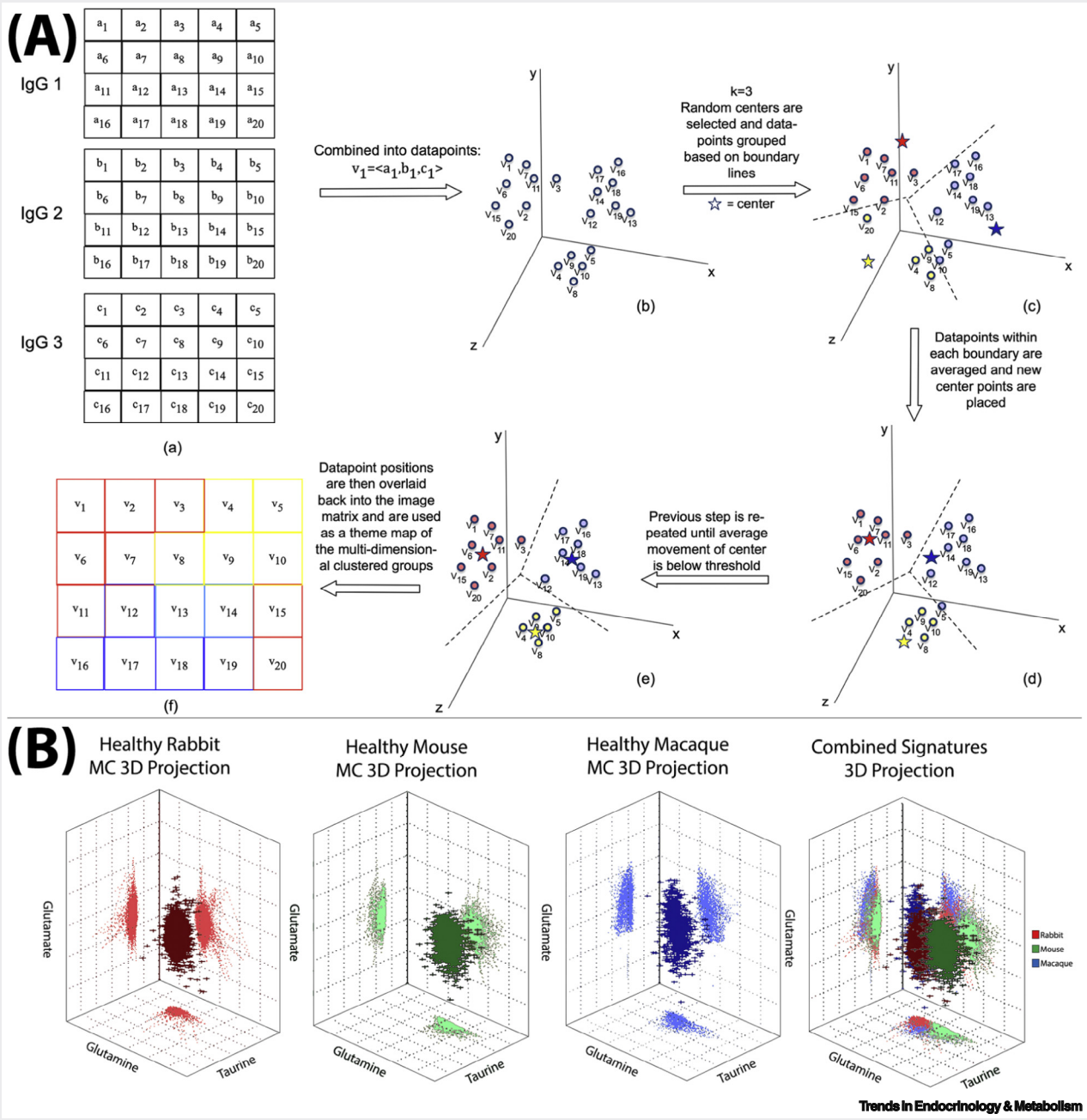

Figure I. Pattern Recognition of *n*-Dimensional Images and Identification of Metabolic Signatures. (A) Methodology for deriving metabolic signatures through image classification and separation of metabolic classes through *k*-mean cluster analysis. (B) 3D (TQE) metabolic profiles of Müller cells from three different mammalian species demonstrating similar relationships of amino acids across species.

though potential interactions with other receptors have been suggested. Instead, it appears to have important roles in cell volume regulation as an osmolyte and has been suggested as a neuroprotective agent [36,37]. In addition, lack of dietary taurine leads to retinal (especially photoreceptor) degeneration [38]. Taurine transport in Müller cells is regulated by the Na^+-coupled

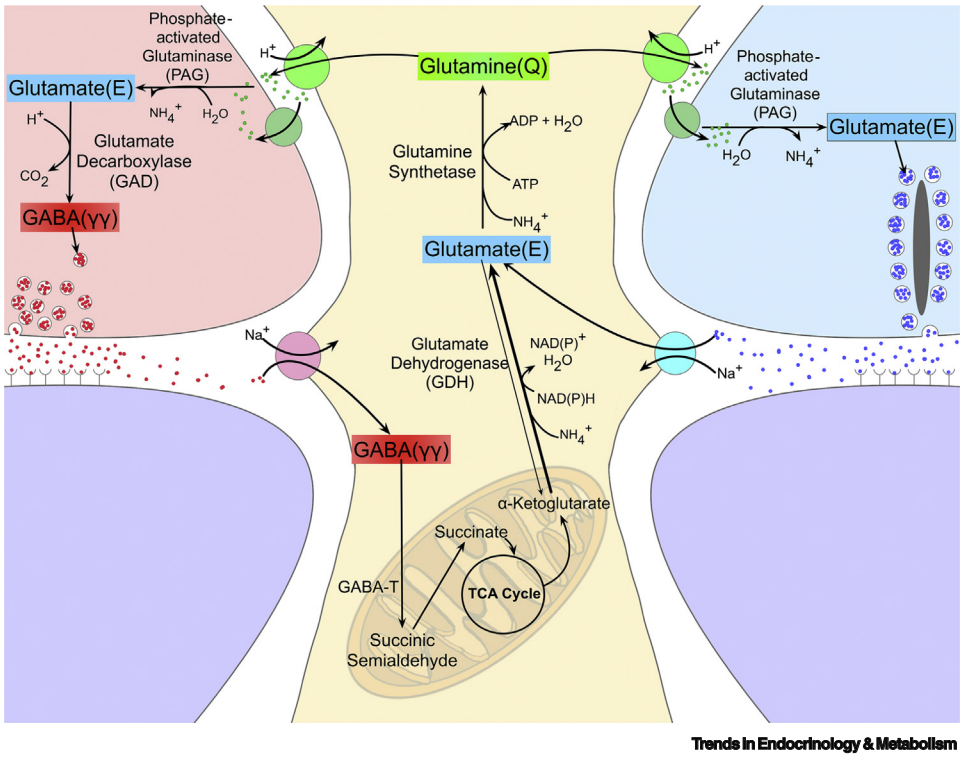

Figure 1. Retinal Glutamate Metabolic Pathway. Red cell indicates GABAergic cell (amacrine or subset of horizontal cells). Blue cell indicates glutamatergic cells (bipolar cells or photoreceptors). Yellow central cell indicates Müller cell. Circles indicate transporters.

taurine transporter (TauT). It transports using the Na^+–K^+ gradient to generate the high intracellular taurine concentration found in Müller cells, while extracellular levels are typically low [39].

Müller Cell Metabolic Signatures in Disease

Müller cells have a remarkably consistent small-molecule metabolic profile that is constant across all Müller cells in the retina across species [40–42]. Healthy Müller cells have a profile composed of high taurine (>10 mM), medium to high glutamine (0.3–1.0 mM), medium to low glutamate (~0.1–0.5 mM), and medium levels of GS [43]. However, this is not to say that there are no species differences in Müller cell metabolism. GABA transport by Müller cells has been observed only in mammals[44], but GABA is rapidly metabolized so it does not accumulate in Müller cells except under conditions of hypoxia [45]. Also, despite some genetic heterogeneity observed in culture experiments and a subset of Müller glia potentially retaining pluripotent capabilities [46], no metabolic subclasses have ever been observed in healthy retinas. In disease, Müller cells diverge into numerous separable subclasses of metabolic phenotypes [47,48] (Figure 2). This phenomenon occurs coincident with early hypertrophy of the Müller cells, in addition to global upregulation of glial fibrillary acidic protein (GFAP), and typically precedes any widespread neuronal loss. It is unknown whether the variation in metabolic state is stable or if we are observing metastable or metabolically oscillating signals. However, this metabolic heterogeneity persists throughout retinal degeneration and appears to progress in a chaotic fashion. In addition to the chaotic metabolic heterogeneity of Müller cell signatures, GS expression is also altered [49,50]. As noted in previous descriptions of the metabolic alterations occurring during retinal degeneration [47,50,51], GS

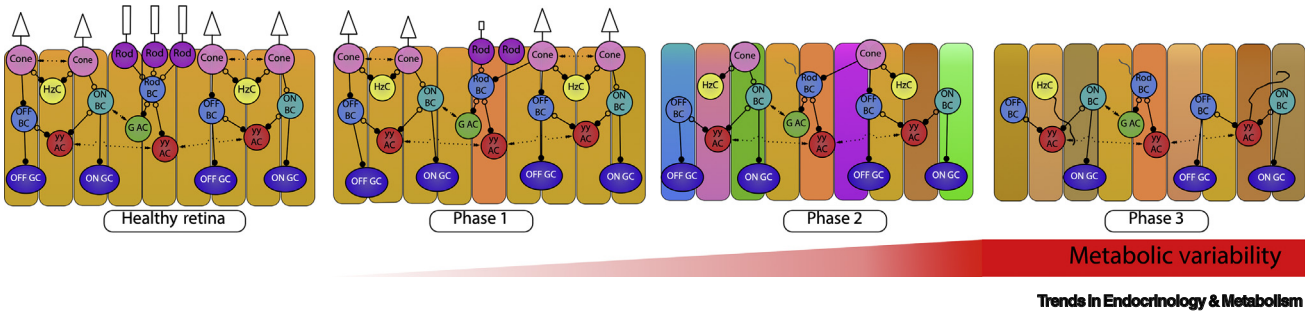

Figure 2. Müller Cell Metabolic Variability. Müller cells represented by rectangles; color signifies metabolic class and changing colors indicate metabolic changes. Circles and ovals indicate retinal neurons (pink, photoreceptors; yellow, horizontal cells; blue, OFF-cone and rod bipolar cells; teal, ON-cone bipolar cells; red, GABAergic amacrine cells; purple, ganglion cells). Unfilled circles between cells indicate sign-inverting synapses. Filled circles indicate sign-conserving synapses. Bidirectional arrows indicate gap junctions. Irregular lines indicate neurite sprouting. Abbreviations: yy AC, GABAergic amacrine cell; G AC, glycinergic amacrine cell; BC, bipolar cell; HzC, horizontal cell; GC, ganglion cell.

concentration/expression collapses throughout the degenerate retina in a mosaicked fashion. This alteration in GS occurs simultaneously with a global increase in glutamine and a stable global concentration of Müller cell glutamate. In healthy retinas, the levels of glutamate, glutamine, and GS (and thus their ratios) remain constant across Müller glia. In the degenerate retina, all three metabolite concentrations increase in their variability of expression, varying widely and independently of one another, in conflict with the standard model of glutamate–glutamine metabolism describing glutamate and glutamine levels in the retina as stoichiometrically related to the available GS [47,50,51].

The driving forces and mechanisms behind the metabolic heterogeneity of stressed Müller cells are topics of debate and continued research. In aged human and diseased retinas it is common to find slightly variable levels of glutathione across Müller cells [52]. This might be explained given the Müller cell's role in buffering against cellular damage caused by reactive oxygen species, with which we come in regular contact. Given the varied experiences of normal humans (smoking, inflammation, obesity, alcoholism, stress, circadian variations, etc.), we might accumulate enough damage to influence the Müller cell's ability to maintain a narrowly programmed/restricted range of small-molecule expression, or external forces could be influencing the Müller cells' normal performance envelope beyond what they are able to constrain. However, the increases in variability observed in glutamate, glutamine, taurine, and GS in the Müller cells of retinas undergoing disease is more dramatic. This variability has been consistent across all species and strains evaluated (mouse, rabbit, rat, pig, and human) [47,48,50,52–54]. If such changes were driven by the microenvironment – that is, if individual Müller cells changed their metabolic, gene, and protein expression in response to environmental cues generated by regional differences in photoreceptor degeneration – we would expect to find Müller cell metabolic variation along a gradient centered on regions of photoreceptor degeneration. This is not what we observe. Adjacent Müller cells appear to have individual metabolic profiles that vary irrespective of their surrounding environment in retinal degeneration. We do not know what drives the Müller cells to adopt such variability in their metabolic signatures; however, to this end, we do not know what causes the metabolism of Müller cells to be so homogeneous in the first place, and this begs further investigation.

Müller Cell Glutamate Pathways in Degenerated Tissue

The global increases in glutamine occurring coincidentally with the loss of GS is a perplexing phenomenon warranting further examination of glutamate metabolism in Müller cells. Previous

suggestions that Müller cell glutamate transport fails early in retinal degeneration [28,55]. It has been previously hypothesized that the decrease in GS is related to a loss of EAAT1, the main glutamate transporter found on Müller cells [56,57]. In this model of metabolic changes in Müller cells in response to disease, a decrease in glutamate transport leads to a subsequent decrease in GS production, thereby leading to a decrease in glutamine synthesis. To explain the increases in glutamine, we hypothesize that in this model the glutamine transporter of Müller cells is also reduced in some capacity leading to the increase in intracellular glutamine in Müller cells (Figure 3A). It is this model of reduced glutamate sequestration by Müller cells, which is foundational to the potential of glutamate excitotoxicity leading to subsequent neurodegeneration observed post-photoreceptor degeneration [28]. Conflicting studies have shown that an increase of GLAST/EAAT1 expression is a potential component of retinal disease [58], but in this case glutamate transport alone does not adequately explain the metabolic signatures observed, nor do they explain the phenotypic heterogeneity. We therefore propose the possibility of two alternative pathways accounting for the differing metabolic signatures of Müller cells in the degenerated retina. One possibility is that Müller cells with depressed GS activity unmask less energetically favored metabolic pathways, including a strengthening of the oxidative deamination direction of the GDH reaction forming α-ketoglutarate from glutamate [59,60], which could then enter the TCA cycle (Figure 3B). This pathway could help to support the changes in energy demand from the neural retina, as numerous plasticity events occur during remodeling [51,61]. Another potentially unmasked mechanism is an alternative synthesis pathway of glutamate into glutamine through a currently unknown enzyme or pathway other than GS (Figure 3C) [14]. We argue that the glutamate–glutamine cycle should be considered less of a linear stoichiometric biochemical pathway and more of a nonlinear intersection with many other metabolic pathways. The amount of glutamate production contributed by one pathway over another is likely to change depending on the metabolic demands of a cell and,

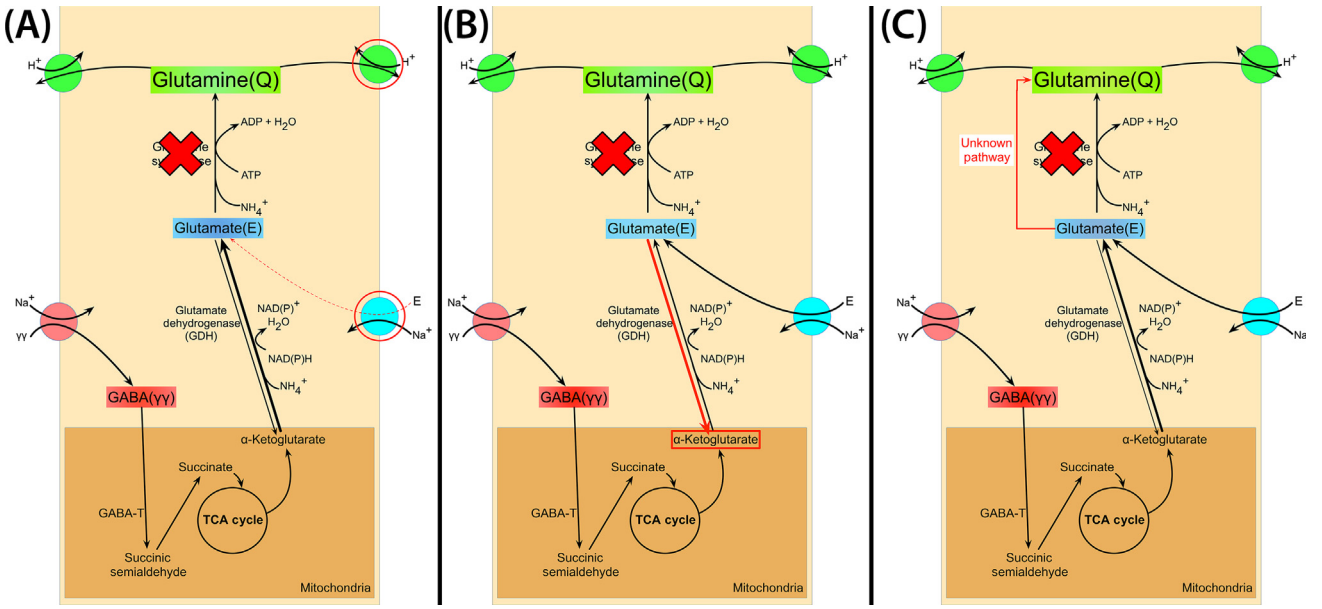

Figure 3. Potential Glutamate Metabolic Pathways in Retinal Degeneration. Tan rectangle indicates the Müller cell boundaries, with brown box indicating mitochondria. Red markings indicate changes to the normal pathway uncovered by retinal degeneration and remodeling. (A) Reduction in glutamate transport by excitatory amino acid transporter 1 (EAAT1). (B) Increase in GDH pathway forming α-ketoglutarate. (C) Uncovering of another transaminase pathway in the absence of glutamine synthetase expression.

in the case of glia, the local microenvironment. Each of these pathways will require further experimentation to understand or unmask, and find the limits of physiological glutamate that can be endogenously handled before excitotoxicity occurs.

Concluding Remarks

The loss of a unified metabolic phenotype in Müller cells in response to widespread photoreceptor death is not in and of itself particularly remarkable. Müller cells are well preserved in the evolutionary record, with the same general signature and function being observed across the animal kingdom, from avians to reptiles and mammals. One would expect cells that are substantially similar in terms of metabolic signatures, morphological and physiological function across almost 300 million years of evolution to have accumulated redundant metabolic pathways and to have substantial metabolic support functions that could compensate for changes in metabolic demand brought on by environmental conditions, aging, or perhaps trauma. What is surprising is that a cell class with such a robust metabolic phenotype, conserved across species, diverges into numerous statistically separable metabolic classes with seemingly no relation to one another in disease. Is there a signal that coordinates Müller cells in terms of metabolism that is lost in disease or are the mechanisms maintaining tight control of the metabolic envelope compromised in disease? We also do not know whether the signals that we observe are constant or whether we are viewing a periodic variability in metabolism within individual Müller glia, because CMP provides only individual temporal snapshots of the metabolic state.

We hypothesize that a Müller cell synchrony signal exists that regulates metabolism and synchronizes cells across the normal retina. We would expect that it functions based on a number of factors present in the intact retina, perhaps additional photoreceptor signals such as melatonin [62] or endothelin as first described by Rattner and Nathans [63]. Many studies attempting to identify potential factors leading to Müller cell regulation use dissociated Müller cells [46,64–68]. If Müller cells are somehow synchronously regulated, dissociated cells are likely to express phenotypes differing from those found *in vivo*. Gene expression studies have indicated heterogeneity in dissociated mouse Müller cells [46]. Although the level of diversity in Müller cells and the potential for multiple types is still under debate for adult Müller cells, it is likely that they also respond to circadian rhythm signals that may be corrupted in degenerate retinas. Müller cells have vasopressin receptors, which facilitate their response to the circadian variations in vasopressin secretion by some amacrine cells [69] and in the absence of these signals metabolism may be compromised through incomplete hormonal feedback.

Outstanding Questions

What factors lead to Müller cell metabolic coordination in healthy retinas across species?

What factors in Müller cell metabolic coordination are altered in disease?

Are there pathways of glutamate metabolism that become more energetically favorable in disease and are they sufficient to support increased signaling activity associated with therapeutic interventions?

How do the microenvironments surrounding the various metabolic classes of Müller cells differ between one another?

Acknowledgments

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