Pyruvate kinase and aspartate-glutamate carrier distributions reveal key metabolic links between neurons and glia in retina

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Symbiotic relationships between neurons and glia must adapt to structures, functions, and metabolic roles of the tissues they are in. We show here that Müller glia in retinas have specific enzyme deficiencies that can enhance their ability to synthesize Gln. The metabolic cost of these deficiencies is that they impair the Müller cell's ability to metabolize Glc. We show here that the cells can compensate for this deficiency by using metabolites produced by neurons. Müller glia are deficient for pyruvate kinase (PK) and for aspartate/glutamate carrier 1 (AGC1), a key component of the malate-aspartate shuttle. In contrast, photoreceptor neurons express AGC1 and the M2 isoform of pyruvate kinase, which is commonly associated with aerobic glycolysis in tumors, proliferating cells, and some other cell types. Our findings reveal a previously unidentified type of metabolic relationship between neurons and glia. Müller glia compensate for their unique metabolic adaptations by using lactate and aspartate from neurons as surrogates for their missing PK and AGC1.

glutamine metabolism | aerobic glycolysis | retina | Müller glia | photoreceptors

Aerobic glycolysis is a metabolic adaptation that proliferating cells use to meet anabolic demands (1, 2). In tumors, it is called the "Warburg effect." Tumors convert ∼90% of Glc they consume to lactate (Lac). The brain converts only 2–25% of the Glc it uses to Lac (3).

In retinas of vertebrate animals, energy is produced in a way that resembles tumor metabolism more than brain metabolism. Aerobic glycolysis accounts for 80–96% of Glc used by retinas (4–7). Retinas are made up of neurons and glia (8). The outermost layer is occupied by photoreceptors (PRs). The inner layers are a diverse collection of signal processing neurons. Müller glia spans the thickness of the retina. The site of aerobic glycolysis in retina has not been established.

Exchange of fuels is an important part of the relationship between neurons and glia (9–12). Transfer of metabolites between intracellular compartments also is important. Glycolysis is supported by reoxidation of cytosolic NADH, which can be catalyzed by lactate dehydrogenase (LDH) or by the malate-aspartate shuttle (MAS). PRs and other neurons in retinas express aspartate/glutamate carrier 1 (AGC1; also known as "Aralar") (13), a mitochondrial aspartate/glutamate carrier that has a key role in the MAS. However, Müller cells (MCs) are AGC1-deficient (13). The significance of the distribution of AGC1 has been enigmatic.

Aerobic metabolism in tumors is linked to expression of the M2 isoform of pyruvate kinase, PKM2 (14, 15). Pyruvate kinase (PK) catalyzes the final step in glycolysis, synthesis of Pyr (16). Liver (PKL) and erythrocyte (PKR) isoforms are splice variants of the PKLR gene, and PKM1 and PKM2 are splice variants of the PKM gene. A unique feature of PKM2 is that it is responsive to allosteric and posttranslational regulators (16). PKM2 expression in cancer cells correlates with reduced yield of ATP from Glc and accumulation of glycolytic intermediates. PKM2 also favors synthesis of Ser and pentose phosphate pathway intermediates that support anabolic activity (17).

Association of PKM2 with aerobic glycolysis in tumors motivated us to explore the relationship between PKM2 and aerobic glycolysis in retina. We found that PKM2 is abundant and only in the outer retina, implicating PRs as a primary site of aerobic glycolysis. Unexpectedly, we also found MCs are deficient for all isoforms of PK.

The deficiency of MCs for AGC1 and PK led us to investigate metabolic relationships between PRs and MCs. We found cultured MCs have an impaired ability to metabolize Glc, but they metabolize Asp effectively. Our findings reveal that metabolic benefits of down-regulating PK and AGC1 in MCs have led to specific metabolic adaptations in the retina. Rather than use Glc to fuel their mitochondria, MCs use Lac and Asp from neurons as surrogates for their missing PK and AGC1 activities.

Results

Glycolytic Enzyme Activities in Mouse Retinas. We compared maximum specific activities of glycolytic enzymes in homogenates of retina, muscle, brain, liver, a transformed MC line (RMC-1) (18), and HeLa cells. These values represent maximum possible activity for each enzyme, and not the activity it would have in the cell. The activities generally increase from the first step to the

Significance

Aerobic glycolysis is a metabolic adaptation that helps cells in a tumor meet high anabolic demands. The M2 isoform of pyruvate kinase (PKM2) is associated with aerobic glycolysis in cancer cells. Aerobic glycolysis also accounts for most of the Glc metabolized in retinas. We find that photoreceptors (PRs) in retinas, like cancer cells in tumors, express PKM2. We also found very little expression of pyruvate kinase (PK) in Müller glia. We present metabolic flux analyses that show a metabolic relationship between PRs and Müller cells (MCs) that is different from the relationship between some neurons and astrocytes in brain. To compensate for PK deficiency and aspartate/glutamate carrier 1 deficiencies, MCs can fuel their mitochondria with lactate and aspartate produced by PRs.

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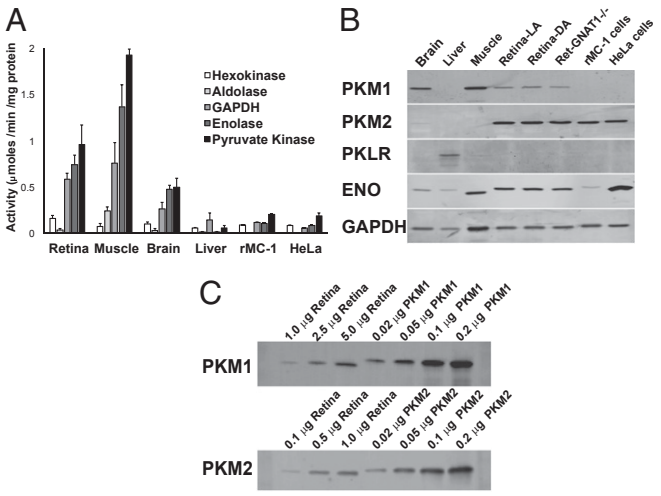

Fig. 1. Glycolytic enzymes in retina. (*A*) Activities of glycolytic enzymes in homogenates of retina, muscle, liver, and brain. Activities were measured at substrate concentrations above the reported K_m for each enzyme. (*B*) Immunoblot analysis of PK isoforms in homogenates of whole brain, liver, muscle, and retina. DA, dark-adapted; LA, light-adapted. GNAT1$^{-/-}$ retinas were included because they are unresponsive to light. rMC-1 is an SV-40 transformed MC line (18), and HeLa is a cancer cell line. The blot is representative of two experiments. Each lane is loaded with 5 μg of protein. (*C*) Quantitative immunoblot analysis comparing retina homogenate with known amounts of purified PKM1 and PKM2.

final step of glycolysis. PK has the highest activity in all tissues except liver (Fig. 1*A*).

PKM2 Is Abundant in Retina. We evaluated PK expression in brain, liver, muscle, and retina with antibodies specific for PKLR, PKM1, and PKM2. PKLR is in homogenates of liver but not retina (Fig. 1*B*). Consistent with this finding, no PKLR was found in a proteomic analysis of rat retina (19). PKM1 is in brain, muscle, and retina, whereas PKM2 is most abundant in retina (Fig. 1*B*). We quantified PKM1 and PKM2 in mouse retina by calibration with pure recombinant PKM1 and PKM2 (concentration determined by amino acid analysis) (Fig. 1*C*). Our analysis showed there are ∼150 pmol of PKM2 and ∼26 pmol of PKM1 per mouse retina. PKM2 is present at 25% of the amount of rhodopsin, the most abundant protein in PRs (∼600 pmol per retina) (20, 21).

PKM2 Is Only in PRs. We evaluated the distribution of PK in light-adapted retinas by assaying PK activity in serial sections of frozen unfixed rat retinas (22, 23) (Fig. 2*A*). We noted two distinct peaks of activity. Rhodopsin and synaptotagmin marked outer segments and synaptic terminals of PRs. Immunoblot analysis (Fig. 2*A*, *Top*) detected PKM2 only in the outer retina. Immunohistochemical analysis showed that PKM2 is confined to cell bodies and synaptic terminals of PRs (Fig. 2*B*, *Left*), whereas PKM1 is in PRs and inner retinal neurons (Fig. 2*C*, *Left*).

PKM2 Is Regulated Allosterically but Not Influenced, by Light. PKM2 is sensitive to multiple types of regulation (16). We found that Phe (5 mM) inhibits PK activity in retinal homogenates. Fructose 1,6-bisphosphate (1 mM) stimulates it by 15% and is effective at reversing inhibition of PK activity caused by Phe (Fig. S1). We were unable to find differences between PK activity in homogenates prepared from light vs. dark-adapted retinas. Immunoblotting did not reveal any light vs. dark effects on Y105 phosphorylation (24) or on PKM1 or PKM2 expression.

PKM2 in cancer cells diverts glycolytic intermediates to Ser and Gly synthesis (25). We incubated retinas with U-^{13}C Glc and compared incorporation of ^{13}C into Lac vs. into Ser. In 5 min, 44 ± 2% of ^{12}C Lac is replaced by ^{13}C Lac in light vs. 52 ± 5% in darkness. In some cancer cells, labeling of Ser from ^{13}C Glc is detected within 10 min (17). We found no labeling of Ser from ^{13}C Glc in retinas even after 6 h. PKM2 in retina does not divert carbons from glycolysis into Ser to the extent it does in cancer cells. PKM2 is excluded from nuclei in retinas, so it also is unlikely to influence transcription (16).

MCs Are Deficient for All PK Isoforms. We evaluated PKM1 and PKM2 in MCs in retinas by colabeling retinas with an MC-specific marker, cellular retinaldehyde binding protein (CRALBP) (Fig. 2 *B* and *C*, *Middle*). Neither colocalized with CRALBP (Fig. 2 *B* and *C*, *Right*). To confirm our method detects glycolytic proteins in MCs, we also probed CRALBP-labeled sections with antibodies to enolase and GAPDH. Both colocalize with CRALBP (Fig. S2).

We also mined a published gene expression database derived from MCs isolated from mouse retinas (26). To evaluate how well this database reflects native MC expression, we examined genes expressed only in PRs (GNAT1 and IRBP) or only in MCs [glutamine synthetase (GLUL) and CRALBP]. Our analysis confirmed the accuracy of the database. GNAT1 and IRBP occur more frequently in PRs than in MCs, and GLUL and CRALBP occur more frequently in MCs than in PRs (Fig. S3). We also analyzed PKM and PKLR. PKM sequences are enriched in PRs, whereas they are 12-fold less abundant in MCs. PKLR is 50- to 100-fold less abundant than PKM in both PRs and MCs (Fig. S3).

Lac Production in Retina. Our findings that MCs do not express PK and that PRs express abundant PKM2 suggested that PRs, and not MCs, are the primary source of Lac. Retinas convert Glc to Lac at a very fast rate (4–7, 13). To determine if PRs are the major source of Lac, we compared retinas with PRs and retinas without PRs. Adult aryl hydrocarbon interacting protein-like 1 (AIPL1$^{-/-}$) mouse retinas have no PRs because AIPL1

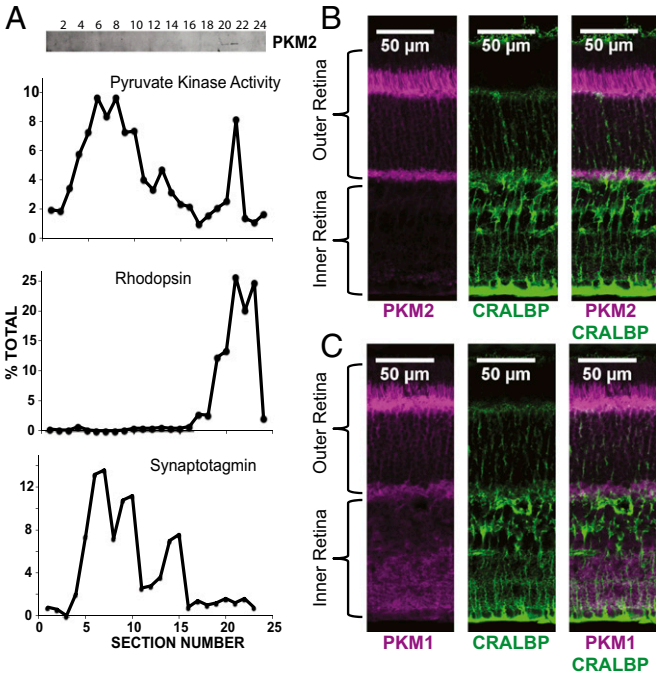

Fig. 2. Distribution of PK in retina. (*A*, *Top*) Distribution of PK activity in serial sections of rat retina. (*A*, *Middle*) Rhodopsin shows the location of PR outer segments. (*A*, *Bottom*) Synaptotagmin shows locations of synapses. Immunoblot shows PKM2 immunoreactivity. (*B*) Immunohistological localization of PKM2 (*Left*), CRALBP (*Middle*), and their overlap (*Right*) in mouse retina. (*C*) Immunohistological localization of PKM1 (*Left*), CRALBP (*Middle*), and their overlap (*Right*) in mouse retina.

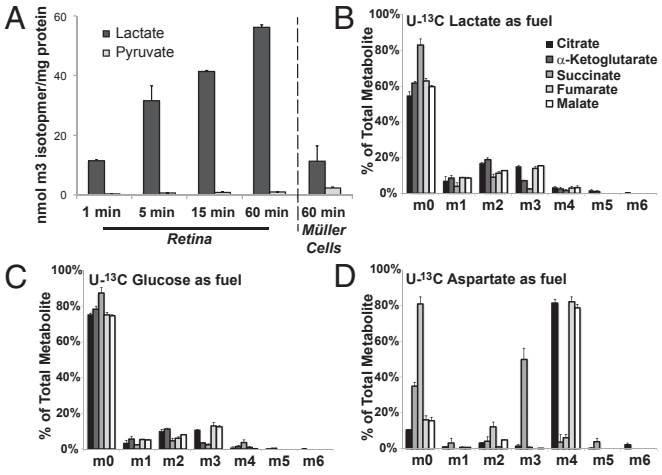

Fig. 3. Cultured MCs use Asp more effectively than Glc, Lac, and Gln. (*A*) Accumulation of M3 lactate and pyruvate from ^{13}C Glc in retinas and in cultured MCs. Cultured MCs do not make Lac fast enough for Müller glia to be the primary source of Lac from retinas. Cultured MCs were incubated for 1 h with 5 mM U-^{13}C Lac (*B*), 5 mM U-^{13}C Glc (*C*), and 5 mM U-^{13}C Asp (*D*). The ^{13}C enrichment is expressed as the percentage of total ion intensity of all isotopomers for each metabolite ($n = 3$). ^{13}C Asp is incorporated more effectively than any other fuel.

deficiency causes PR degeneration (27), so we extracted metabolites from mutant and control retinas and quantified them by GC/MS (Fig. S4). Taurine and docosahexaenoic acid, which are PR-specific metabolites, are reduced 90% and 83% in AIPL1$^{-/-}$ retinas. Lac is also decreased by 74%, consistent with PRs being the major site of Lac production. Gln accumulates, indicating PRs are a major site for Gln degradation.

Cultured MCs Are Less Effective Than Intact Retinas at Converting Glc to Lac. To evaluate contributions of MCs to Lac production, we used MCs isolated from young mouse retinas and cultured for 10–14 d. During that time, MCs grow to confluence, whereas neurons degenerate (28). We incubated cultured MCs with ^{13}C Glc and used GC/MS to measure the appearance of M3 isotopomers of Lac and Pyr (Fig. 3*A*). The time course of incorporation of ^{13}C from Glc into whole-mouse retina metabolites is shown for comparison. The ^{13}C from Glc is incorporated into Lac ∼60-fold faster in whole retinas than in cultured MCs. This finding indicates neurons, and not MCs, are the primary site of Lac synthesis in retinas.

Asp Is the Preferred Fuel for Cultured MCs. To quantify fuel preferences of cultured MCs, we incubated them for 1 h with either U-^{13}C Lac or U-^{13}C Glc and measured incorporation of ^{13}C into tricarboxylic acid cycle (TCA) intermediates (Fig. 3 *B* and *C*). Neither Lac nor Glc contributed much ^{13}C (Fig. 3 *B* and *C*). We also tested U-^{13}C Asp because Pardo et al. (29) found that other types of glial cells use Asp. We found Asp is the fuel used most effectively by cultured MCs to contribute carbons to TCA intermediates (Fig. 3*D*). An isotopomer analysis (Fig. S5) shows how cultured MCs convert U-^{13}C Asp into citrate, α-ketoglutarate, Glu, and Gln. MCs also use carbons from Asp to make malate and fumarate by reverse malate dehydrogenase and fumarase reactions. The cells label very little succinate. Rapid exchange of cytosolic malate for mitochondrial α-ketoglutarate limits the extent to which intermediates are recycled through the TCA cycle.

Asp Enhances Glycolysis in Cultured MCs. We reasoned that Asp might stimulate oxidation of Glc by enhancing regeneration of cytosolic NAD$^+$. Asp entering MCs can be transaminated to oxaloacetate, which then oxidizes NADH. We tested this reasoning by incubating cultured MCs with U-^{13}C Glc with vs. without Asp, and found Asp enhances incorporation of ^{13}C into Pyr and Lac (Fig. 4*A*).

Asp Enhances Incorporation of Carbons from Glc and Lac into Mitochondrial Intermediates. Asp also enhances incorporation into citrate of two ^{13}Cs from either U-^{13}C Glc or U-^{13}C Lac (Fig. 4*B*). We also noted evidence of Pyr carboxylase activity. In the presence of U-^{13}C Glc or U-^{13}C Lac, ∼10% of malate and citrate in retina incorporates three ^{13}Cs (Fig. 4*C*). The M3 isotopomers are formed when M3 Pyr is incorporated into mitochondrial intermediates by Pyr carboxylase. The contribution of Pyr carboxylase becomes minor when unlabeled Asp is present (Fig. 4*C*).

Asp and Lac Enhance Gln Synthesis in Cultured MCs and in Retina. MCs can synthesize Gln. However, when we use Glc as the only fuel, neither cultured MCs (Fig. 4*D*) nor intact retinas (7) released much Gln into the culture medium. We found sixfold more Gln in the medium when Glc is supplemented with Asp. When Asp is provided with Lac instead of Glc, Gln rose 42-fold compared with Glc alone (Fig. 4*D*).

To confirm that MCs use Lac and Asp in a more physiological context, we incubated whole intact retinas with ^{13}C Lac for 2 h with vs. without Asp. We measured Gln in the retinas instead of in the medium because Gln release is undetectable from light-adapted retinas (7). Asp caused a 144-fold increase in labeling of Gln, which is made only in MCs (Fig. 4*E*). Labeling of TCA intermediates, which occurs in all retinal cells, was stimulated only 1.5-fold to sevenfold by Asp (right side of Fig. 4*E*). We also incubated retinas with 5 mM Lac, together with 5 mM Asp, dimethyl α-ketoglutarate, dimethyl succinate, dimethyl fumarate, Asn, or Pro. Asp and Asn were the most effective at raising levels of Gln (Fig. 4*F*).

MCs Lack Biochemical Pathways Needed to Sustain Glycolysis. Neither ^{13}C Glc alone nor ^{13}C Lac alone is an effective source of carbon for mitochondrial intermediates (Fig. 3 *B* and *C*). The limited ability of cultured MCs to incorporate ^{13}C from Glc or Lac could mean they cannot make cytosolic NAD$^+$ fast enough to sustain oxidation of these fuels.

To sustain glycolysis, a cell must reoxidize NADH. LDH can regenerate NAD$^+$ by converting Pyr to Lac. However, MCs are PK-deficient, so they cannot make enough Pyr to support this conversion. MAS activity would be an alternative way to regenerate NAD$^+$ (30), but AGC1, a key component of the MAS, is absent from MCs in retinas (13) (Fig. S2). Consistent with this localization data, microarray data revealed low AGC1 expression in MCs (0.031% of total transcripts in PRs vs. 0.0003% in MCs) (26). The glycerol-3-phosphate shuttle (30) is unlikely to play a major role because transcripts encoding the two glycerol-3-phosphate dehydrogenases are of low abundance in both PRs and MCs (each is ∼0.0006% of total transcripts) (26).

Caveats Associated with Cultured MCs. The experiments we have described so far were done mostly with MCs dissociated from p12 retinas and cultured for 10–14 d. MCs under these conditions partially dedifferentiate (28). They retain some native characteristics but lose others.

We evaluated how much dedifferentiation occurs in our cultured MCs. The MC-specific proteins CRALBP and glutamine synthetase (GS) are present (Fig. S6), but only at 65 ± 5% and 8 ± 4% of levels in whole retina. AGC1 is present at 37 ± 5% and PKM2 is present at 39 ± 8% of whole-retina levels (±SEM, n = 4). This finding confirms cultured MCs partially dedifferentiate. We also noted conversion of Glc into Lac and TCA intermediates by cultured MCs (Fig. 4 *A*–*C*), which we would not expect based on immunocytochemical analysis of intact retinas. Fig. 2 in this report shows PKM2 is undetectable in MCs within retinas, and a previous analysis (13) and ours (Fig. S2) detected no AGC1 in MCs in intact retinas.

Our findings with intact retinas and cultured MCs suggest a previously unidentified metabolic relationship in which MCs use Lac and Asp made by PRs. However, cultured MCs only partially represent authentic MCs, so we developed an independent strategy to analyze metabolic relationships between PRs and MCs in intact retinas.

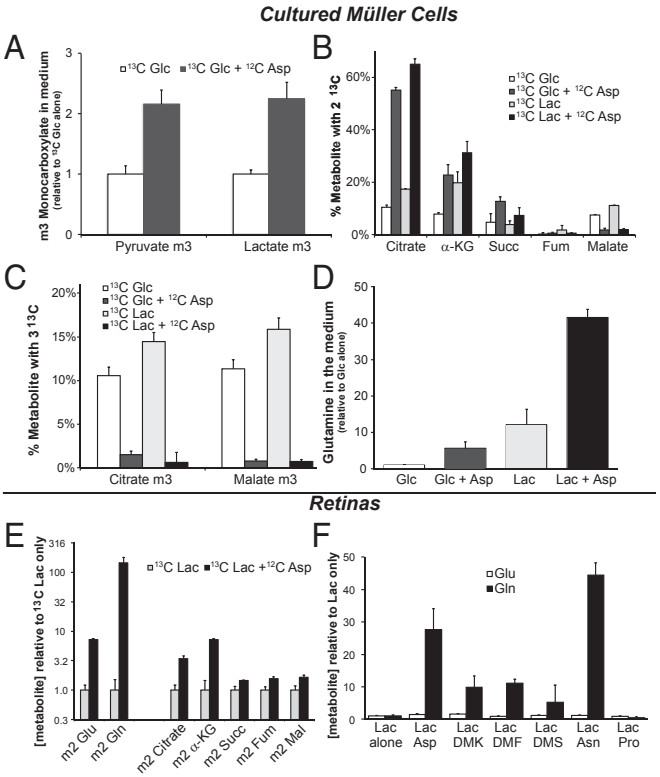

Fig. 4. Asp stimulates Glc oxidation. (*A*) Isolated MCs were incubated with 5 mM U-^{13}C Glc with or without 5 mM unlabeled Asp for 1 h. The medium was collected and analyzed by GC/MS. (*B*) Isolated MCs were incubated with 5 mM U-^{13}C Glc or with U-^{13}C Lac (5 mM) with vs. without 5 mM unlabeled Asp for 1 h. The percentage of each metabolite in the M2 isotopomer is shown ($n = 3$). (*C*) Pyr carboxylase activity is a significant source of citrate and malate, but only in the absence of Asp ($n = 3$). The M3 isotopomers of citrate and malate are made by carboxylation of Pyr. Total citrate and malate increased only ~70% and ~7%, respectively, when Asp was included. (*D*) Asp (5 mM) increases synthesis and release of Gln into the MC culture medium ($n = 3$). (*E*) Asp stimulates synthesis of Gln from U-^{13}C Lac by MCs in intact retinas (note log scale on *y* axis). (*F*) Asp and Asn are the most effective anapleurotic substrates for raising the concentration of Gln in retinas. Retinas were incubated with 5 mM lactate and 5 mM additional substrate for 1.5 h ($n = 3$). The effect on Glu is much smaller. In mouse retinas incubated with Lac alone, the Glu pool size is ~130-fold larger than the Gln pool size.

Metabolic Fate of ^{13}C-Labeled Gln in Intact Retinas Confirms a Role for Asp in Neuron/MC Symbiosis. We designed a pulse–chase strategy to test our hypothesis that Asp transports oxidizing power and carbons from neurons to MCs in intact retinas.

PRs are the primary site of Gln catabolism, and MCs are the primary site of Gln synthesis. Microarray data (26) confirm this enzyme distribution. Glutaminase (GLS) transcripts occur at an approximately eightfold higher frequency in PRs than in MCs. In contrast, transcripts encoding GLUL occur 50-fold more frequently in MCs than in PRs. Based on these distributions and on the ability of Asp and Lac to enhance Gln synthesis, we hypothesized the pathway in Fig. 5*A*. Gln is imported into PRs. GLS converts it to Glu, which is transaminated to α-ketoglutarate. A small fraction (7) of this α-ketoglutarate is oxidized to succinate, to malate, and to oxaloacetate, and is then transaminated to Asp. Our model predicts some of this Asp exits the neuron to be imported into MCs. Once in the MC cytosol, Asp is transaminated to oxaloacetate, which then oxidizes cytosolic NADH. This sequence of reactions is how Asp helps maintain cytosolic NAD^+ to support oxidation of Glc and Lac.

Malate from reduction of oxaloacetate in the MC then enters mitochondria, where NAD^+ oxidizes it to oxaloacetate. Oxaloacetate is trapped in the MC mitochondria because MCs lack AGC1, so it is used there to make citrate, which then is oxidized to α-ketoglutarate and Glu. Ultimately, in an MC-specific reaction, the Glu is made into Gln. The net result is that carbons from Gln initially taken up by PRs are used to resynthesize Gln in MCs. Fig. 5*A* tracks the flow of carbons in this model.

To test the strategy, we treated retinas for 5 min with U-^{13}C Gln (M5), followed by incubation with 5 mM unlabeled Lac. At various times, retinas were harvested and metabolites were extracted and analyzed by GC/MS. Fig. 5*B* (*Upper*) shows that M5 Gln converts rapidly to M5 Glu. The lingering M5 Glu and M5 α-ketoglutarate are consistent with our previously reported finding that α-ketoglutarate in retinas is protected from oxidation in neurons (7). Fig. 5*B* (*Lower*) shows how M4 Asp accumulates and then decays as M3 Glu and then M3 Gln accumulate. Conversion of M4 Asp into M3 Gln confirms that the subsequent reactions (Fig. 5*A*) occur in MCs, because only MCs express GS. Overall, these results support the model in Fig. 5*A* in which Asp from neurons supplies carbons and oxidizing power for synthesis of Gln by MCs.

Confirmation That Asp Can Be a Surrogate for the Missing MAS in MCs. An alternative interpretation of our results could be that U-^{13}C Gln is used directly by MCs, where it is made directly into M3 Gln. This sequence of reactions would not require import of extracellular Asp into Müller glia. This alternative explanation is unlikely, because the role of MCs is to synthesize Gln rather than break it down. Nevertheless, to address this alternative explanation, we performed the following control experiments that test whether or not Gln synthesis in MCs depends on import of extracellular Asp:

i) We added (2S, 3S)-3-{3-[4-(trifluoromethyl)benzoylamino] benzyloxy]aspartate to retinas to inhibit EAAT1, a transporter for Asp into MCs (31). Fig. 6*A* shows it inhibits formation of M3 Gln from M5 Gln without affecting other metabolites.

ii) We treated retinas with carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase, a multienzyme complex that converts Asp to *N*-carbamoyl aspartate (32), to deplete extracellular Asp. Fig. 6*B* shows that it inhibits M3 Gln formation without affecting other metabolites.

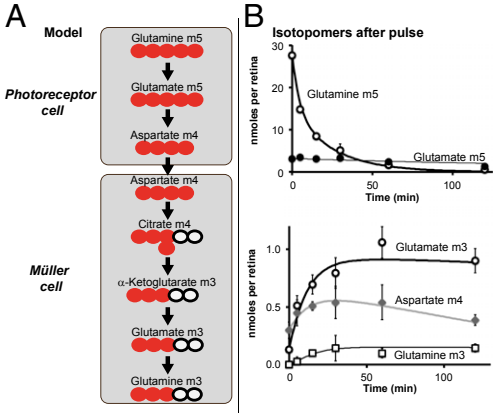

Fig. 5. Pulse–chase analysis of U-^{13}C Gln in retina. (*A*) Schematic model for the role of Asp as a carrier of oxidizing power between retinal neurons and glia. Red circles represent the ^{13}C carbons, and black circles represent the ^{12}C carbons. (*B*) ^{13}C labeling of Asp, Glu, and Gln from the pulse of U-^{13}C Gln. (*Upper*) The M5 Gln and Glu are derived directly from the pulse of 5 mM U-^{13}C Gln. After 5 min, the medium was changed to 5 mM unlabeled Lac with no added Gln. Unlabeled Gln was not included in the chase because the intense signal from the added Gln would have obscured the isotopomer signals we intended to quantify. The retinas were subsequently harvested at the indicated times after the pulse. (*Lower*) The M4 Asp derived from oxidation of Glu via the TCA. The M3 Glu is made by further oxidation via citrate, and M3 Gln is made only in MCs by Gln synthetase. ($n = 6$).

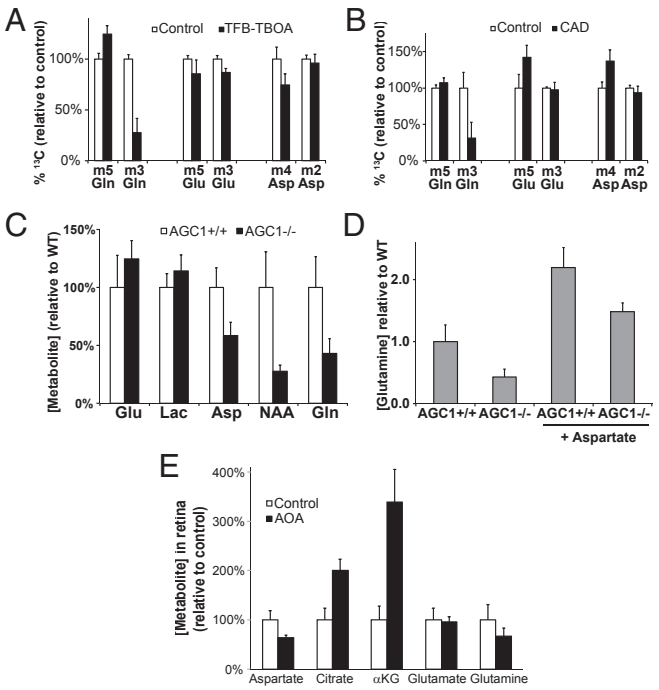

Fig. 6. Inhibition of Asp transfer to MCs inhibits Gln synthesis in retina. (*A*) Inhibition of EAAT1 by (2S, 3S)-3-{3-[4-(trifluoromethyl)benzoylamino]benzyloxy}aspartate (TFB-TBOA; 10 μM). (*B*) Depletion of extracellular Asp by carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase (CAD). The medium contained CAD protein (6 μg/mL) and ATP (0.1 mM). (*C*) Asp, *N*-acetyl aspartate (NAA) and Gln are diminished in AGC1$^{-/-}$ mouse retinas. (*D*) Asp boosts Gln levels in AGC1-deficient retinas ($n = 4$). (*E*) AOA, an aminotransferase inhibitor, inhibits formation of Asp and promotes formation of citrate, consistent with sequestering oxaloacetate in mitochondria so that it is more likely to be made into citrate. By preventing formation of Asp and by blocking aminotransferase activity in MCs, AOA (1 mM) also causes accumulation of α-ketoglutarate (αKG) and lowers Gln ($n = 3$).

iii) We compared Gln in normal retinas and AGC1$^{-/-}$ retinas. AGC1 is required for Asp synthesized in neurons to escape from the matrix. Our model predicts AGC1 deficiency should limit synthesis of Gln by MCs by limiting release of Asp from neurons. Fig. 6*C* shows that Asp and Gln in AGC1$^{-/-}$ retinas are diminished compared with controls, consistent with our model. *N*-acetyl aspartate also is diminished in the absence of AGC1, consistent with a previous analysis of AGC1 deficiency in brain (33).

iv) Our findings suggest AGC1 deficiency limits Gln synthesis by preventing PRs from exporting Asp for import by MCs. We tested this explanation by adding exogenous Asp to AGC1$^{-/-}$ retinas. Fig. 6*D* shows Asp restores Gln synthesis in AGC1$^{-/-}$ retinas, consistent with our model.

v) Aminotransferases play key roles in both PRs and MCs. We incubated retinas under conditions similar to those conditions in Fig. 5, but we also included 1 mM aminooxyacetate (AOA) to inhibit aminotransferase activities. Fig. 6*E* shows AOA lowers Asp and Gln, consistent with our model.

These controls confirmed the importance of Asp as a source of carbon for Gln synthesis by MCs in intact retinas.

Discussion

Metabolic Requirements in Retina Are Different from in Brain. Metabolic relationships between neurons and astrocytes in brain have been studied extensively (10, 34). However, retina and brain have different functions and structures that influence the nature of the interactions between their neurons and glia:

i) Aerobic glycolysis accounts for 2–25% of Glc metabolism in brain (3), but it accounts for 80–96% of it in retina (4–6). PKM2 is barely detectable in brain but abundant in retina.

ii) Brain astrocytes have direct access to nutrients and O_2 from vasculature, but some neurons do not. Those neurons rely on astrocytes for fuel (10, 34). In retina, PRs and MCs have direct and equal access to nutrients and O_2 from the interphotoreceptor matrix (IPM) that bathes the retina surface (35).

iii) Neurons in brain fire intermittently, releasing Glu primarily when they are stimulated. In darkness, PRs are constantly depolarized such that they release a continuous flow of Glu at their terminals.

iv) Glu released from neuronal synapses in brain can diffuse from synapses to be recovered by astrocytes. PR synapses are encapsulated structures with efficient EAAT5 transporters that recover Glu before it can reach MCs (36). Brain slices, but not intact retinas, release Gln into the culture medium (7).

Why Do PRs Express So Much PKM2? PKM2 in a mouse retina is 25% as abundant as rhodopsin, the most prominent protein in PRs. It is the isoform of PK most suited for aerobic glycolysis in cancer cells (2, 16) because its activity is poised for either positive or negative regulation. We have not found evidence that PKM2 is regulated by light, but there could be conditions we have not yet explored where its ability to be regulated imparts a selective advantage. PKM2 basal activity is lower than that of other isoforms (25), but it is so abundant in PRs that retinas convert ~90% of the Glc they use to Lac.

Why Do MCs Have a PK Deficiency? The advantage to a MC of low PK expression is that intermediates of glycolysis become available for anabolic activities. The disadvantage is that MCs become dependent on other cells to fuel their mitochondria.

MCs may not be completely devoid of PK. Previous studies of MCs treated with protease and mechanically dissociated from guinea pig retinas showed that they make Lac (37, 38). Those studies found less Lac release from a complex of MCs and PRs than from isolated MCs. They inferred a net flow of lactate from MCs to PRs, but the analysis did not show it directly. The definitive conclusion from our studies is that MCs express much less PK than PRs.

Why Is AGC1 in Neurons but Not in Glia? AGC1 in neurons shuttles Asp out of the matrix to oxidize NADH in the cytoplasm. A key

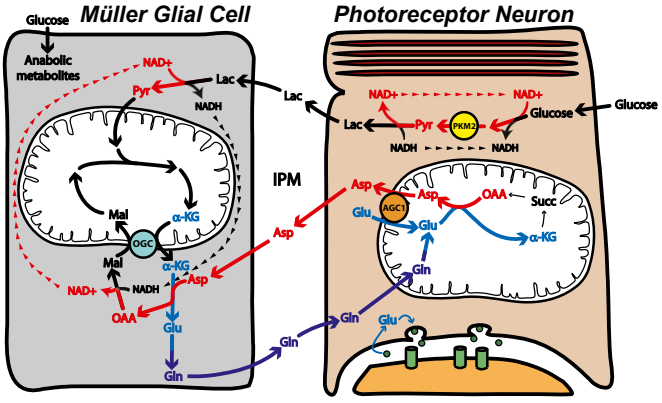

Fig. 7. Model for relationship between PRs and MCs. MCs are PK-deficient, so glycolytic intermediates can be used for anabolic activities. MCs are AGC1-deficient, so mitochondrial oxaloacetate in MCs is not diverted away from the pathway that leads to Gln synthesis. To compensate for these metabolic deficiencies, PRs produce Lac that MCs can oxidize to Pyr to fuel their mitochondria. PRs also export Asp that MCs can use to oxidize Lac. Nitrogen for the amino group of Gln comes from Asp, and nitrogen for the amide group of Gln comes from NH_4^+. Mal, malate; OAA, oxaloacetate; OGC, oxoglutarate carrier; Succ, succinate. Double-membrane compartments within the cells represent mitochondria. Green circles represent Glu, and green cylinders represent Glu receptors.

function of MCs is to synthesize Gln. AGC1-mediated efflux of Asp from the matrix would divert oxaloacetate away from synthesis of α-ketoglutarate, the precursor for Gln. The advantage to an MC of low AGC1 is that Asp is not diverted from the path to Gln.

Role of Lac in the Symbiotic Relationship Between PRs and MCs. Our findings are most consistent with the model in Fig. 7. PRs and MCs have direct access to the IPM that bathes the surface of the retina (35). MCs are PK-deficient, so they cannot make much Pyr directly from Glc. However, like cells in a tumor (39), MCs and PRs express different amounts of PK. PRs express abundant PKM2, making them well-equipped to make Lac from Glc. Lac can be released from PRs via MCT1 (40) and imported into MCs via MCT4 (41).

Role of Asp in the Symbiotic Relationship Between PRs and MCs. For MCs to oxidize the Lac they import from PRs, they require an oxidant. MCs are PK-deficient, so they cannot generate the Pyr that LDH needs to oxidize NADH. MCs also are AGC1-deficient, so they cannot use MAS activity to oxidize NADH. Exogenous Asp can provide the needed oxidizing power. As outlined in Fig. 7, oxaloacetate derived from Asp is a substrate that cytosolic malate dehydrogenase can use to oxidize NADH to NAD^+. Malate from this reaction can enter MC mitochondria via oxoglutarate carrier (13) and be oxidized to oxaloacetate. The absence of AGC1 in MC mitochondria prevents oxaloacetate from escaping. Instead, it is directed toward synthesis of citrate; isocitrate; α-ketoglutarate; and, ultimately, Gln. Our findings reveal how Lac and Asp can provide a surrogate for the missing PK and AGC1 activities in MCs. Asp promotes oxidation of NADH to support Lac consumption. Asp also provides NH_2 to convert α-ketoglutarate to Glu (29), and both Asp and Lac provide carbons to synthesize Gln.

Experimental Procedures

Retina Tissue Culture. Mice were euthanized by cervical dislocation. Retinas were isolated and cultured as previously described (42). Retina explants were incubated with 5 mM Glc or 5 mM other ^{13}C tracers in a 5% (vol/vol) CO_2 incubator at 37 °C.

Antibodies. PKM1/rabbit (1:1,000, 7067; Cell Signaling), PKM2/rabbit (1:1,000, 4053; Novus Biologicals and Cell Signaling), phospho-PKM2 (Tyr105) (1:1,000, 3827; Cell Signaling), and GAPDH/goat–anti-GAPDH (1:1,000, sc-20357; Santa Cruz Biotechnology) antibodies were used. CRALBP antibody was obtained from Jack Saari (University of Washington, Seattle). Anti-AGC1 was obtained from the laboratory of one of the authors (J.S.) (43). LICOR goat anti-rabbit 800 and LICOR donkey anti-goat 680 were used as secondary antibodies.

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