Trends in Cell Biology

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Review

Metabolic Regulation of Cell Fate and Function

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Increasing evidence implicates metabolic pathways as key regulators of cell fate and function. Although the metabolism of glucose, amino acids, and fatty acids is essential to maintain overall energy homeostasis, the choice of a given metabolic pathway and the levels of particular substrates and intermediates increasingly appear to modulate specific cellular activities. This connection is likely related to the growing appreciation that molecules such as acetyl-CoA act as a shared currency between metabolic flux and chromatin modification. We review recent evidence for a role of metabolism in modulating cellular function in four distinct contexts. These areas include the immune system, the tumor microenvironment, the fibrotic response, and stem cell function. Together, these examples suggest that metabolic pathways do not simply provide the fuel that powers cellular activities but instead help to shape and determine cellular identity.

Highlights

Metabolites such as acetyl-CoA directly connect metabolism to the regulation of protein function and chromatin modification.

The metabolic substrates that cells use provide more than bioenergetics and help to shape cellular identity.

Understanding the connection between metabolism and cell fate could usher in a wave of new therapies.

Introduction

'Tell me what you eat and I will tell you what you are'

[Jean Anthelme Brillat-Savarin, 1826]

The internet and magazines are filled with a dazzling array of metabolic intermediates and byproducts whose consumption would appear to cure all that ails us, and in some cases solve problems we did not even know to exist. Indeed, with regards to our own health, perhaps nothing holds the public imagination more than the notion that what we consume determines our well-being. Remarkably, the translation of the notion 'you are what you eat' has been slow to permeate our understanding of intracellular metabolism. For many years the substrates a cell could consume (e.g., glucose, fatty acids, etc.) were all viewed as being essentially indistinguishable. In this framework, the only function of the complex and interconnected web of metabolism was to generate sufficient cellular ATP. That view, however, has substantially changed over the past decade and this bioenergetics-centered model has been replaced by a much more nuanced understanding of cellular metabolism. The growing realization that histone modification relies on the byproducts of one-carbon metabolism and acetyl-CoA production, and that metabolic intermediaries such as α-ketoglutarate act as regulators of chromatin-modifying enzymes, suggest tight coupling between metabolic flux and cellular fate. We review here the recent exploration of this concept in four related but discrete areas. First, we briefly discuss the perhaps best-studied example, namely the role that metabolism plays in shaping the immune response. Next, we examine the harsh world of the tumor microenvironment where competition for substrates limits body defenses and where understanding the metabolic interplay between tumor and stroma might lead to therapeutic advances. We then turn to how metabolism might regulate the processes of fibrosis, a condition linked to the pathology of many chronic diseases. Finally, we discuss recent insights into how metabolism can regulate the fate of stem and progenitor cells as well as their lineage specification. Together, these recent advances highlight the recurring theme that metabolism provides not only the energy to run the cell but also crucial determinants of cell fate.

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Immunometabolism

There is growing appreciation that the highly specialized function of various immune cells is intimately linked to their underlying metabolism. The burgeoning field of immunometabolism has been the subject of several excellent reviews [1,2]. In brief, perturbations of anaerobic glycolysis, fatty acid synthesis, fatty acid oxidation, glutaminolysis, or mitochondrial oxidative phosphorylation have been increasingly used to understand how metabolism is coupled to the function of T cells, macrophages, and natural killer (NK) cells. Although these approaches initially relied on pharmacological perturbations of metabolism, genetic models are increasingly being employed. This genetic approach has sometimes led to a re-evaluation of the precise role that specific metabolic pathways play in immune function [3]. The majority of studies have focused on major pathways such as glycolysis and fatty acid oxidation, but the influence of metabolism likely extends beyond these core pathways. In particular, the regulation of *S*-adenosyl methionine (SAM), which serves as the primary methyl donor for histone modification, is likely to be a central node connecting metabolic flux with immune function [4]. The generation of SAM involves the complex web of interconnected pathways known as one-carbon metabolism. This involves both the methionine and folate cycles, the former of which is linked to glutathione production and the maintenance of redox homeostasis. One of the major carbon donors for this pathway is the non-essential amino acid serine. In this regard, genes involved in serine metabolism are markedly upregulated when $CD8^+$ T effector cells are stimulated [5]. This upregulation appears to be required for T cell expansion not solely for bioenergetic needs but instead to meet a surge in nucleotide synthesis [5]. Interestingly, mice fed a serine/glycine free diet had impaired *in vivo* T cell-mediated immunity [5]. Defects in this pathway have also been recently linked to the inevitable, age-dependent decline in T cell activation [6].

The conversion of serine to glycine is catalyzed by serine hydroxymethyltransferase (SHMT) enzymes, and SHMT1 is localized to the cytosol whereas SHMT2 resides in the mitochondria. Enzymatic activation of these enzymes requires binding of the active form of vitamin B6, pyridoxal-5′-phosphate (PLP) and the formation of a tetramer [7]. Of note, in addition to their role in metabolism, SHMT proteins are found in association with BRCC36, a deubiquitinating enzyme (DUB) that regulates immune signaling [8]. BRCC36 is part of a multiprotein complex termed BRISC (the BRCC36 isopeptidase complex) which regulates the ubiquitination and hence the levels of interferon receptors [8]. A recent cryo-electron microscopic structure of the BRISC–SHMT2 complex has been established [9]. This demonstrated that inactive dimers of SHMT2 bind to and inactivate BRISC whereas active tetramers do not. Levels of PLP regulate the interaction of SHMT2 and BRISC, and this metabolite in turn regulates inflammatory signaling [9]. As such, SHMT adds to a growing list of metabolic enzymes including pyruvate kinase M2, aldolase, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) that appear to have additional, non-metabolic and non-canonical functions [10]. These additional functions include metabolic enzymes moonlighting to regulate transcription, cytoskeletal dynamics, and, as in the case of SHMT, intracellular signaling. It is likely that additional dual-functioning enzymes will be discovered because they provide a structural mechanism to directly link metabolism to energy-consuming intracellular functions, much as SHMT ties one-carbon metabolism to interferon signaling. Interestingly, this is unlikely to be the only metabolic control over this pathway because a recent report demonstrated that oligomerization of the prion-like mitochondrial antiviral signaling protein (MAVS) is modulated by intracellular lactate, and this in turn modulates interferon production [11].

Recent work has greatly expanded on the myriad roles that metabolism plays in modulating T cell function. For instance, with regard to the mitochondria, complex III of the electron transport chain appears to play an essential role in regulatory T cell (T_{reg}) suppressive function [12]. Moreover,

in CD4 T cells, a distinct role for complex I activity versus complex II activity has recently been delineated [13]. Disruption of complex II activity through a conditional deletion of succinate dehydrogenase complex subunit C resulted in type 1 T helper (T_H1) cells that proliferated more than control cells but appeared to be less capable of differentiation and effector function, as evidenced by a reduction in interferon γ (IFN-γ) production (Figure 1). The metabolic regulation of IFN-γ has also been examined in the setting of T cells engineered to lack lactate dehydrogenase A (LDHA) [14]. These T cells are unable to reduce pyruvate to lactate, a reaction that also serves to regenerate NAD^+. The downstream metabolic consequences of LDHA deficiency appear to culminate

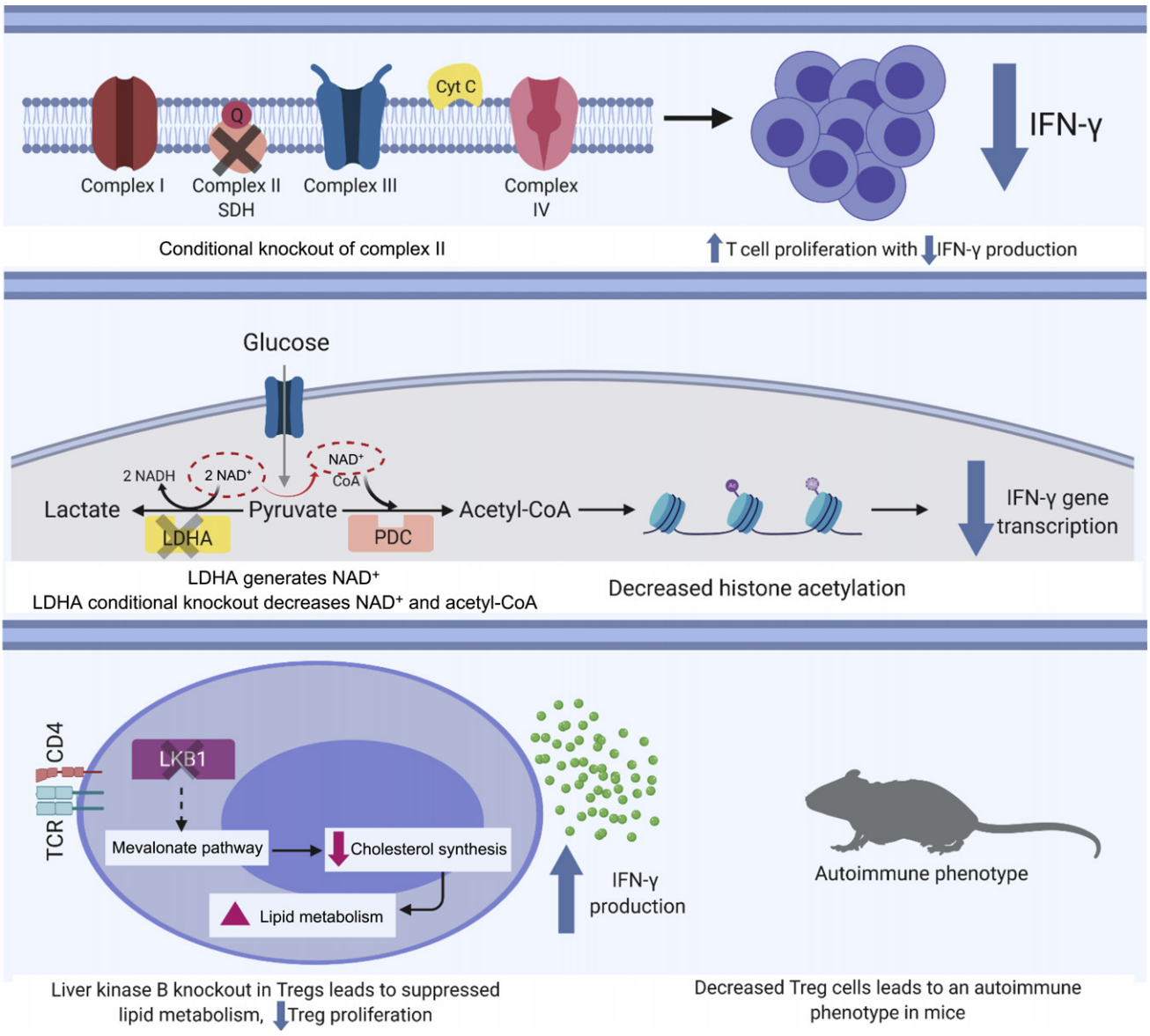

Figure 1. Metabolic Regulation of T Cell Function. Manipulation of diverse metabolic pathways can impact on T cell activation, as assessed by IFN-γ production. This includes genetic disruption of complex II of the mitochondrial electron transport chain (top), altering cytosolic glycolysis by deletion of LDHA (middle), or altering intracellular lipid metabolism (bottom). Abbreviation: Treg, regulatory T cell.

in a reduction in acetyl-CoA, which in turn leads to reduced histone acetylation and a subsequent reduction of IFN-γ transcription [14]. Finally, in T_{reg} cells, a role for lipid oxidation appears to be important in modulating IFN-γ production [15]. In particular, deletion of liver kinase B (LKB) in T_{reg} cells suppressed lipid metabolism and resulted in augmented IFN-γ production, thereby triggering an autoimmune type pathology in these mice [15]. Thus, as the above discussion illustrates, a range of metabolic perturbations, from disrupting mitochondrial electron transport, to manipulating cytosolic glycolysis, to modulating fatty acid oxidation, can either positively and negatively affect T cell function and IFN-γ production (Figure 1).

Metabolic Modulation of the Tumor Microenvironment

In all *in vitro* cell culture models, cells are normally exposed to a vast excess of nutrients. As such, neither oxygen nor metabolic substrates are ever truly limiting. This is not, however, the case *in vivo* where there is often fierce competition for a limited nutrient supply. This is perhaps best exemplified in the violent, competitive landscape of the tumor microenvironment. Growing evidence suggests that metabolic competition can alter cell fate and function. Much of this work has been spurred by the desire to better understand why immune cells often fail to effectively neutralize seemingly immunogenic tumors. Although the answer to this question is complex, there is growing evidence at the cellular level that the tumor microenvironment represents a true microcosm of Darwinian survival (Figure 2). This sometimes involves the expression within the tumor of enzymes such as indoleamine 2,3-dioxygenase that avidly consumes tryptophan, thereby depriving surrounding cells of this essential amino acid [16]. Other times, the hypermetabolic state of the tumor can produce high levels of byproducts such as lactate, which can modulate the activity and fate of infiltrating T cells [17] and macrophages [18]. This metabolic milieu appears to have important physiological consequences. For instance, high glucose consumption by the tumor appears to limit T cell effector function, with a decrease in IFN-γ production and evidence for tumor progression [19]. Interestingly, antibodies against checkpoint proteins such as CTLA-4, PD-1, and PD-L1 appear to function, in part, by directly altering tumor metabolism, restoring glucose availability to the niche, and thereby rejuvenating infiltrating T cell function [19]. Other studies have demonstrated that the high glucose consumption of the tumor results in decreased glycolytic flux by infiltrating immune cells, and that the absence of the specific glycolytic metabolite phosphoenolypyruvate (PEP) acts as a T cell metabolic checkpoint [20]. The consumption of glucose or tryptophan by the tumor is not, however, the only way that the harsh environment of the tumor microenvironment influences the immune response. Within the rapidly expanding tumor, high rates of cell death in turn lead to high levels of extracellular potassium. Emerging evidence suggests that this extracellular potassium dramatically restricts the ability of T cells to take up what limited nutrients are available [21]. This nutrient deprivation in turn modulates acetyl-CoA levels in T cells, their levels of histone acetylation, and ultimately their function [21].

The tumor milieu can also induce metabolically driven stress signaling in infiltrating immune cells. A recent report demonstrated that malignant ascites fluid derived from ovarian cancer patients can suppress the expression of glucose transporters such as GLUT1 on the surface of $CD4^+$ T cells [22]. This metabolic stress, in turn, activated the endoplasmic reticulum (ER) stress response, leading to increased activity of the XBP1 arm of the unfolded protein response (UPR) [22]. Previous work has clearly indicated that ER stress can be induced when nutrients are overabundant [23]; however, these recent results could suggest that either an excess or deficit of nutrients could trigger the UPR. The induction of the UPR in T cells exposed to ovarian ascites fluid resulted in reduced expression of multiple glutamine transporters, leading to decreased glutamine influx and hence a decline in overall mitochondrial function. These glutamine-poor T cells appeared to have reduced IFN-γ expression and impaired antitumor function [22].

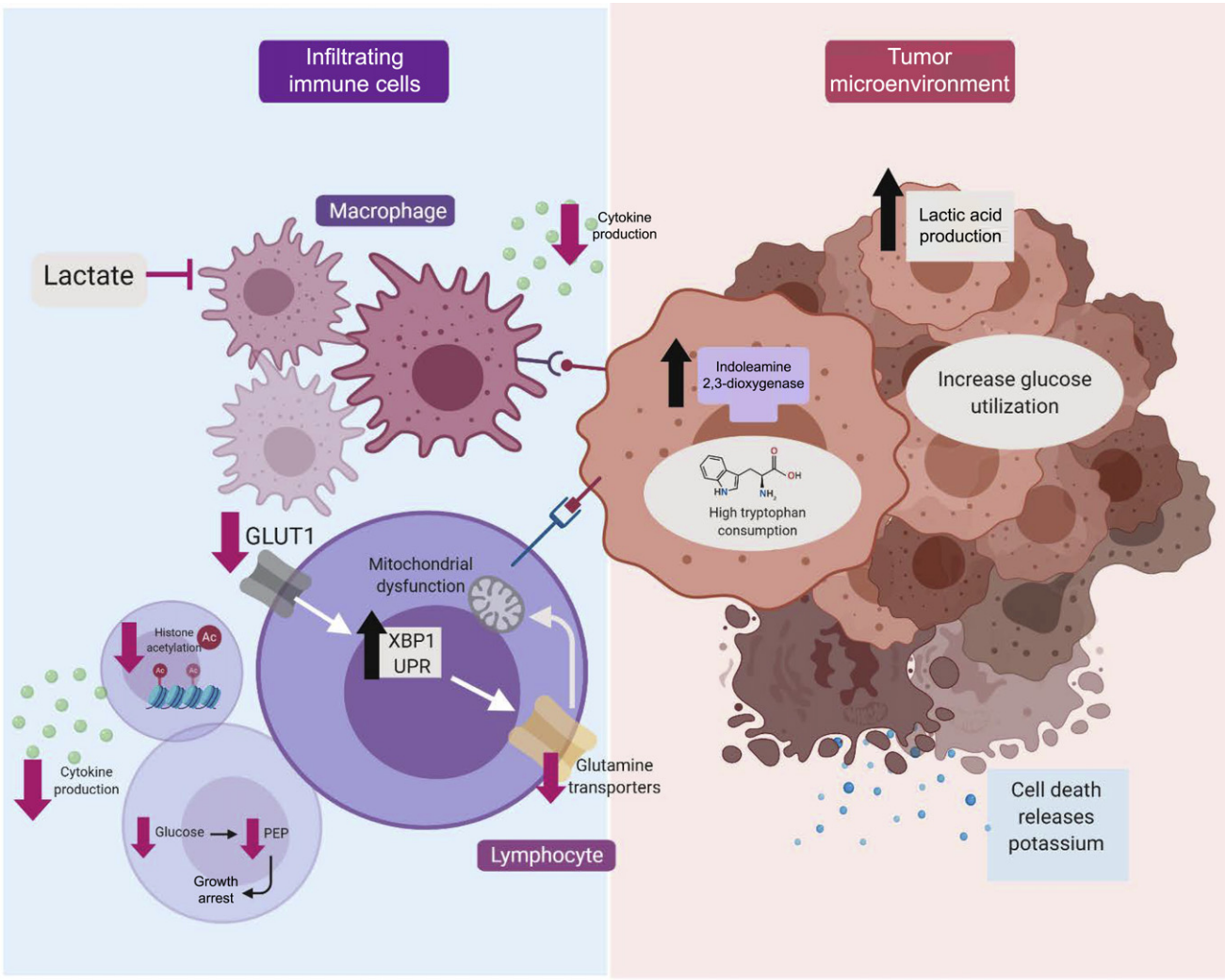

Figure 2. Metabolic Influences in the Tumor Microenvironment. Numerous metabolites appear to play a role in the tumor microenvironment, and help to govern the growth of the tumor and the corresponding strength of the immune response. High rates of tumor glucose and tryptophan consumption can limit the availability of these metabolites for invading immune cells. Moreover, tumor production and the subsequent excretion of lactate can have a paracrine influence on a wide range of immune cells. Recent evidence also suggests that nutrient deprivation created by the tumor can trigger signaling pathways within immune cells such as activation of the unfolded protein response (e.g., XBP1) or directly trigger growth arrest by reducing the levels of key nutrients (e.g., phosphoenol pyruvate, PEP).

By contrast, T cells lacking XBP1 were metabolically more robust and appeared to infiltrate tumors better and produce higher levels of IFN-γ. This suggests that, in T cells, there is an important connection between nutrient availability, ER homeostasis, and antitumor activity.

The metabolism of the tumor microenvironment is not only important for the immunological response. Increasingly, the unique metabolism of stromal elements such as cancer-associated fibroblasts (CAFs) is increasingly being dissected. One recent example analyzed 5000 formalin-fixed tumor samples in conjunction with a label-free proteomics platform [24]. This system allowed analysis of the proteome of tumors and their associated stroma as disease progressed from an initial *in situ* lesion all the way to advanced metastatic disease. Interestingly, this analysis suggested that the tumor proteome is relatively stable over this spectrum of disease, although

there were marked differences in the stromal component [24]. Notably, the stroma associated with metastatic disease was enriched for the expression of methyltransferase nicotinamide *N*-methyltransferase (NNMT). This enzyme transfers a methyl group from SAM to nicotinamide. As mentioned previously, SAM is the universal donor for intracellular methylation reactions involving histone and non-histone proteins. Interestingly, NNMT in fibroblasts was associated with the expression of epithelial-to-mesenchymal transition (EMT) markers, a signature known to be associated with metastatic disease [24]. Moreover, increased expression of NNMT in normal fibroblasts promoted cancer cell growth, whereas knockdown of NNMT in CAFs produced the opposite effects, a phenomenon driven by NNMT-dependent alterations in SAM levels [24]. Interestingly, the interplay between stromal metabolism and tumor growth also involves the ability of CAFs to secrete exosomes that appear to supply the tumor with essential nutrients [25]. Finally, the metabolism of the tumor can also modulate the niche. In particular, a recent report demonstrated that breast cancers rely on pyruvate metabolism to remodel the extracellular matrix and thereby drive metastatic growth [26]. Thus, it would appear that the metabolism of the stroma modifies the tumor, and the metabolism of the tumor modulates the niche.

Metabolic Regulation of Fibrosis

Another area where substrate utilization appears to influence and modify cell fate involves the fibrotic response. As we age, alterations in stem and progenitor cell fates take place, and there is both a loss of quiescence and an age-dependent bias in generating differentiated cell types. For instance, older muscle stem cells skew away from generating myofibers and instead preferentially differentiate towards a fibrogenic lineage [27]. The fact that dietary interventions such as caloric restriction appear to mitigate aspects of stem cell aging has prompted intense investigations regarding the connection between nutrients and progenitor cell fate [28]. One interesting recent example involves dissecting the metabolic underpinnings for cell fate decisions by beige adipocyte precursor cells. Both brown and beige adipocytes are of interest for their ability to uncouple mitochondrial respiration from ATP production and thereby potentially counteract the harmful effects of nutritional excess and obesity. Beige adipocytes develop in areas of white adipocyte tissue (WAT) in response to external stimuli such as cold exposure or β3-adrenergic stimulation. As mice and humans age, levels of brown and beige fat decline [29,30]. The zinc-finger transcriptional coregulator PRD1–BF1–RIZ1 homologous domain-containing protein 16 (PRDM16) is a key determinant of the development and maintenance of brown and beige fat. A new study demonstrated that, as mice age, much like muscle stem cells, stimuli that normally induce precursor cells to generate beige fat cells instead appear to result in a fibrogenic response [31]. Interestingly, *Prdm16*$^{+/-}$ mice, which had a 50% reduction in the expression of the coregulator, appeared to mimic the profibrogenic skewing effects of aging, whereas forced expression of PRDM16 in old mice reduced fibrosis and restored beige adipocyte formation [31]. This latter effect was demonstrated to be due to a paracrine effect because PRDM16-overexpressing adipocytes appear to release a factor that suppresses fibrogenesis and stimulates beige adipocyte formation [31]. RNA-seq and metabolomic analyses demonstrated that PRDM16 induces a marked increase in fatty acid oxidation (FAO) because PRDM16-expressing cells incubated with palmitate generated >20-fold higher levels of acetyl-CoA than control cells [31]. Moreover, consistent with this elevated rate of FAO, PRDM16-expressing adipocytes secreted sixfold higher levels of the ketone body β-hydroxybutyrate (BHB) into the medium than control cells [31]. There is growing appreciation that ketone bodies can act as signaling moieties [32], and indeed, in this case, BHB secreted by adipocytes was metabolized in precursor cells and appeared to inhibit the HIF-1α- and TGF-β-driven fibrogenic program [31]. It remains unclear how precisely this occurs; however, the metabolism of BHB presumably elevates acetyl CoA and NAD levels in the precursor cells, which may in turn modulate chromatin and cell fate decisions. These results could have practical implications because this study

demonstrated that dietary BHB supplementation appeared to promote beige fat formation [31]. A similar phenomenon has been previously observed when mice were placed on ketogenic diet [33].

The link between FAO and fibrogenesis was also observed in another context, this time involving the conversion of mature endothelial cells into a fibroblast-like state, a process known as endothelial-to-mesenchymal transition (EndoMT). EndoMT is very closely related to the well-studied phenomenon of epithelial-to-mesenchymal transition (EMT), as briefly discussed above, and is known to be stimulated by TGF-β signaling [34]. Endothelial cells stimulated to undergo EndoMT with TGF-β developed a marked decrease in FAO, which in turn led to a marked decline in acetyl-CoA levels [35]. This TGF-β-induced reduction in FAO has been observed in other contexts, including disease states such as chronic kidney disease that are characterized by significant fibrosis [36]. Mitochondrial FAO requires the transport of long-chain fatty acids across the outer mitochondrial membrane, and again across the inner mitochondrial membrane. This transport is catalyzed by the action of two sequential enzymes, namely CPT1 (on the outer mitochondrial membrane) and CPT2 (on the inner mitochondrial membrane). In culture, endothelial knockdown of either CPT1 or CPT2 augmented EndoMT [35]. This phenomenon could, however, be reversed by supplementing the culture medium with acetate, a cell-permeable metabolite that can be converted into acetyl-CoA by the action of acetyl-CoA synthetase 2 (ACSS2). Interestingly, in previous studies it was demonstrated that in neurons ACSS2 can be found in the nucleus, where it appears to generate a local reservoir of acetyl-CoA that is required for histone acetylation and for crucial functions such as memory formation [37]. Outside the mitochondria, the generation of acetyl-CoA can also occur by the metabolism of mitochondria-exported citrate and the subsequent action of the enzyme ATP-citrate lyase (ACLY). Again, the activity of ACLY appears to be crucial for the maintenance of histone acetylation [38], as well as for other functions including DNA repair [39]. Interestingly, in endothelial cells, inhibiting ACLY activity stimulated EndoMT induction, suggesting that non-mitochondrial endothelial acetyl-CoA levels are crucial determinants of this cell fate change [35]. The maintenance of acetyl-CoA levels might be cell type-specific, and there is evidence in endothelial cells suggesting it requires FAO, whereas in other cases it might derive primarily from pyruvate metabolism [40]. It is also important to note that the pool of acetyl-CoA in the mitochondria is not directly exchangeable or in equilibrium with the pool of acetyl-CoA in the cytosol and nucleus (Figure 3). Metabolic compartmentalization occurs for other metabolites such as NAD/NADH and complicates the interpretation of total cellular metabolomics data. Better tools, including ratiometric fluorescent reporters, will be necessary to allow real-time and subcellular resolution of metabolites. Some such tools are emerging [41]. Finally, although the above results highlight a role for endothelial FAO in regulating EndoMT, other reports have shown that endothelial FAO maintains acetyl-CoA levels and is thereby essential for *de novo* nucleotide synthesis [42] and for maintaining redox homeostasis [43].

Interestingly, although the above discussion suggests that a decrease in FAO may push endothelial cells towards a fibroblast lineage, recent reports suggest that the phenotype of mature fibroblasts may be modulated by the balance between FAO and glycolysis [44,45]. In particular, as in many other cell types, glycolysis appears to fuel the generation of biosynthetic mass, which in the case of fibroblasts largely involves the production of extracellular matrix (ECM). Earlier studies demonstrated that human skin maintains a high rate of anaerobic glycolysis, and nearly 70% of consumed glucose is diverted towards lactate production [46]. By contrast, a more recent study suggests that stimulating FAO in fibroblasts appears to stimulate these cells to engage in ECM degradation [47]. Because FAO can often be stimulated by small molecules that activate the peroxisome proliferator-activated receptor (PPAR) pathway, this hints at a potential

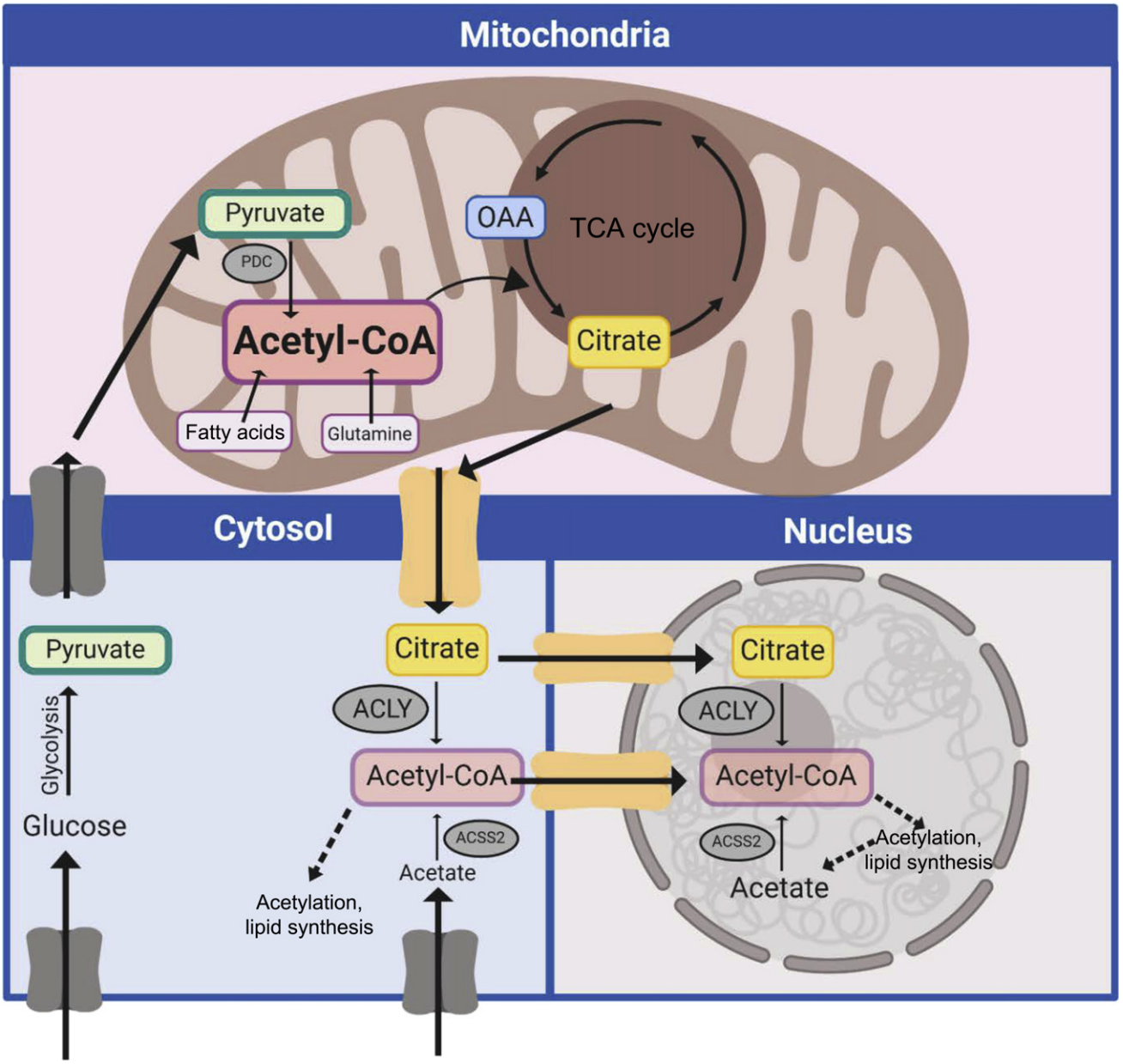

Figure 3. The Compartmentalization of Acetyl-CoA. Acetyl-CoA can be generated in different cellular compartments including the mitochondria, cytosol, and nucleus. The pool of mitochondrial acetyl-CoA is higher than, and is not in equilibrium with, the cytosolic–nuclear pool. Citrate exported from the mitochondria can be reconverted to acetyl-CoA by the action of the enzyme ACLY. Moreover, acetate, taken up from the extracellular milieu or produced intracellularly, can also generate acetyl-CoA by the action of the enzyme ACSS2. ACLY and ACSS2 are found in the cytosol and nucleus. In the nucleus, these enzymes are believed to generate locally high concentrations of acetyl-CoA, presumably near sites of active chromatin acetylation. Abbreviations: OAA, oxaloacetate; TCA cycle, tricarboxylic acid cycle.

metabolic-based therapeutic strategy to modulate excessive fibrosis. Namely, by stimulating fibroblast FAO one might potentially impact on the excess fibrosis that occurs in several skin conditions including scleroderma, graft-versus-host disease, or as an unintended consequence of radiation therapy [44].

Metabolic Regulation of Stem and Progenitor Cells

As discussed in part above, there is emerging appreciation of the role of metabolism in regulating stem and progenitor cell function. Most of this work has focused on neural stem and progenitor cells [48] or in the well-studied hematopoietic stem cell system [49]. However, the metabolic control of stem and progenitor function extends to other niches. In the gut, intestinal stem cells reside adjacent to differentiated Paneth cells at the bottom of the intestinal crypt. Metabolic analysis has demonstrated that, in comparison to Paneth cells, intestinal stem cells demonstrate higher levels of mitochondrial activity [50]. This increase mitochondrial activity appears to drive the production of reactive oxygen species (ROS), which function as signaling molecules to activate a redox-dependent, p38 MAPK pathway that drives crypt formation [50]. The beneficial role of ROS here is not unique. For instance, although there are certainly examples where the sustained production of ROS inhibits stem cell function [51,52], there are also contrary examples where ROS production appears to provide a necessary signaling function [53]. In the case of intestinal stem cells (ISCs), neighboring Paneth cells metabolize glucose and hence secrete lactate that can diffuse out of the Paneth cell and be taken up by the neighboring stem cell. This lactate is then converted into pyruvate within the intestinal stem cell, and is used as a mitochondrial substrate in the tricarboxylic acid (TCA) cycle. This additional example of metabolic compartmentalization, where Paneth cells support the mitochondrial activity of neighboring stem cells, suggests an anatomical-metabolic requirement for optimal stem cell function. In many ways this is also similar to our previous discussion of metabolic coupling between mature adipocytes and adipocyte precursor cells [31]. Interestingly, a similar paradigm exists in the mature brain where astrocytes break down stored glycogen, producing lactate that can be used by adjacent neurons. This process is essential for diverse neuronal functions including memory formation [54] as well as excitability and plasticity [55].

Other more recent studies have expanded on the role of metabolism in intestinal stem cell biology. Given the presumed benefits of reducing caloric intake on overall health, there is substantial interest in understanding how dietary manipulations regulate intestinal stem cell function. In that regard, a recent report demonstrated that acute fasting markedly stimulated the *ex vivo* organoid-forming capacity of mouse crypts, a measure of ISC function [56]. Interestingly, this fasting-induced stimulation of ISC function appears to be mediated by an increase in FAO in these cells. In particular, deletion of CPT1A in the ISCs resulted in abrogation of the fasting-induced stimulation of organoid formation [56]. The potential molecular basis of fad diets such as the ketogenic diet has also been recently studied. Interestingly, when compared to their more differentiated progenitor cells, ISCs express significantly higher levels of 3-hydroxy-3-methylglutaryl-CoA synthetase 2 (HMGCS2), the rate-limiting step in ketone body production [57]. Deletion of HMGCS2 in ISCs impaired their stem cell regenerative capacity and appeared to cause premature differentiation of these stem cells towards the secretory lineage [57]. This effect appeared to be mediated through the ability of ketone bodies, such as β-hydroxybutyrate, to inhibit histone deacetylases. This study further demonstrated that ketogenic diets could enhance ISC number and function, whereas a glucose-supplemented diet had the opposite effect [57]. Although the above studies have primarily dealt with cell-autonomous metabolism, there is also growing appreciation that metabolites produced by the gut microbiome can also dramatically affect ISC function [58].

There are additional examples where stem cell fate appears to be determined or at least influenced by metabolic determinants. A recent example centers on bone marrow mesenchymal stromal cells (BMSCs). These are normally quiescent, but upon injury can differentiate into either adipocytes, osteoblasts, or chondrocytes. As we age, the differentiation potential of BMSCs skews away from osteoblast formation and towards adipogenesis [59]. This cell fate skewing is

believed to contribute to age-related osteoporosis. Interestingly, disruption of glutamine metabolism within BMSCs resulted in a recapitulation of this age-dependent differentiation bias. In particular, conditional deletion of glutaminase (GLS), the rate-limiting enzyme in glutamine

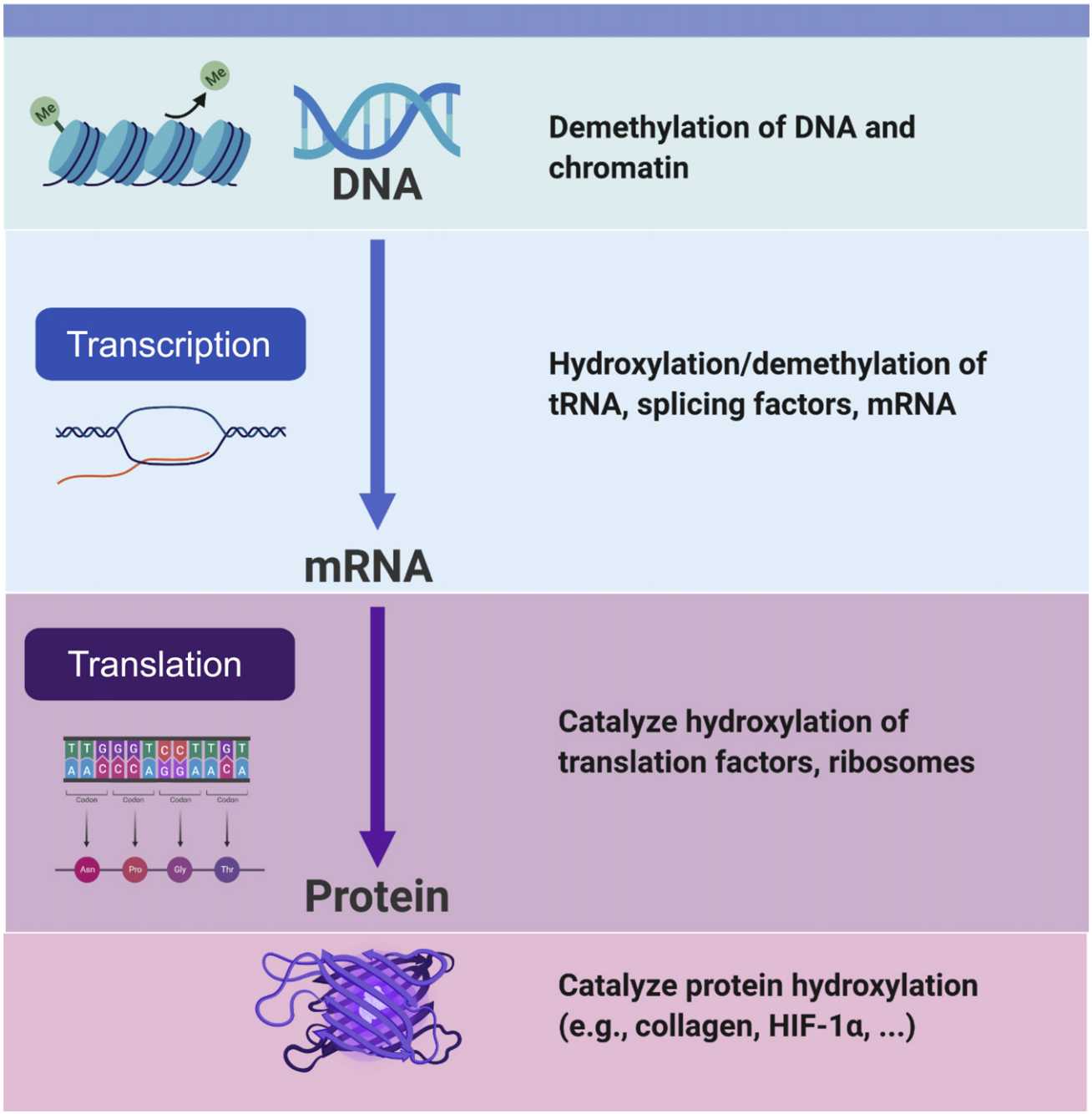

Figure 4. The Role of α-Ketoglutarate (αKG)-Dependent Enzymes. This family of enzymes, that all require the tricarboxylic acid (TCA) cycle metabolite αKG, exert influences on all aspects of the central dogma of biology, from DNA to RNA to protein modification. Multiple different enzymes and substrates are involved, and reactions include αKG-dependent demethylation and αKG-dependent hydroxylation.

metabolism, resulted in skeletal stem cells (SSCs) that had increased adipogenic potential and a decreased ability to form osteoblasts [60]. Lack of GLS expression in mesenchymal progenitor cells resulted in mice with decreased bone mass and increased marrow adiposity, although the latter phenotype, for unclear reasons, was more evident in female mice [60]. The precise mechanisms underlying for these effects remain elusive but, interestingly, some of the defects in GLS-deficient BMSCs could be rescued by supplying α-ketoglutarate (αKG), a metabolite formed following deamination of glutamate [60]. It is therefore tempting to speculate that some of these effects might be mediated by the Jumonji or TET family of demethylases that use αKG as a cofactor [61]. Indeed, the TCA metabolite αKG (alternatively named 2-oxoglutarate, 2OG) has the potential to impact on cell fate in multiple ways because this large family of αKG-dependent oxygenases can catalyze a range of enzymatic activities including demethylation, hydroxylation, and even halogenation [62]. This range of enzymatic activities is matched by an even greater set of substrates that are regulated by αKG-dependent enzymes (Figure 4). Indeed, the entire central dogma of information, from DNA to RNA to protein, falls under some level of αKG-dependent regulation. This includes regulation of transcription through histone and DNA demethylation, regulation of translation by demethylation and hydroxylation of mRNA, tRNA, and ribosomes, and modulation of protein stability or function through αKG-dependent hydroxylation (e.g., proline hydroxylation for collagen stabilization and in hypoxia signaling). From a stem cell perspective, a large body of evidence has demonstrated that this family of enzymes appears to be essential for the maintenance and acquisition of pluripotency [61].

Concluding Remarks

As the work above testifies, the past several years have seen a rapid expansion of our understanding of the coupling between metabolism and cell fate. Much work is needed and many outstanding questions remain (see Outstanding Questions). These include a need for better tools and increased understanding of the specificity of this coupling. Finally, the seemingly important link to age-related diseases and the therapeutic exploitation of these insights is only beginning. For many years, mitochondrial function and metabolic pathways were thought of in purely industrial terms, as Marx would say, 'the means of production'. In retrospect, this notion of autonomous energy production devoid of any coupling to where that energy is directed and used seems naïve. We are now entering a new phase, one that promises to deliver an ever more sophisticated insight into understanding of how what we eat allows us to become what we are.

Outstanding Questions

Can we develop better tools to easily quantify and dynamically measure the subcellular compartmentalization of metabolites such as acetyl-CoA, NAD^+, and SAM in living cells or tissues?

Why are do chromatin regions preferentially change when metabolic substrates are altered? How is this specificity achieved?

What are the means for regulating the metabolism–cell fate connection? How does the cell sense a change in acetyl-CoA or SAM? What are the molecular sensors? What are the molecular effectors?

Can the pool of carbon metabolites on chromatin (e.g., methyl groups, acetyl groups) be mobilized to help to alleviate nutrient stress? If so, is this an important, perhaps ancient, function for these modifications?

Can cellular substrate utilization be manipulated for therapeutic benefit? Do the changes in cellular metabolism that occur with age act as a primary driver for age-related diseases?

Acknowledgments

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