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Metabolism in physiological cell proliferation and differentiation

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Stem and progenitor cells proliferate and give rise to other types of cells through differentiation. Deregulation of this process can lead to many diseases including cancer. Recent evidence suggests that an extensive metabolic reconfiguration of cancer cells allows them to sustain pathological growth by providing anabolic intermediates for biosynthesis. This raises the question of the physiological role of metabolic pathways during normal cell growth and differentiation. Metabolism changes with differentiation, and metabolic pathways may be controlled by the same signals that control cell proliferation and differentiation. However, metabolism could also reciprocally influence these signals. The role of metabolic regulation may extend beyond the provision of intermediates for the biosynthetic needs of proliferation, to affect cell differentiation. Here we bring together a large number of recent studies that support this suggestion and illustrate some of the mechanisms by which metabolism is linked to cell proliferation and differentiation.

The relationship between metabolism and cell fate

Stem and progenitor cells divide and produce different types of cells, a process that requires extensive rearrangements in cellular function. Metabolic demands vary in different cell types, and therefore the activity of pathways that import and allocate nutrients is expected to change during differentiation. How this happens and whether it might be important has not been studied in detail. Indications that metabolic regulation plays major roles during development and in stem cells have emerged, however, from recent findings on the metabolism of cancer cells. Overwhelming evidence now shows that widespread rewiring of cellular metabolism underlies the ability of cancer cells to grow uncontrollably. This rewiring includes the generation of some ATP through aerobic glycolysis, where glucose is converted to lactate at high rates even in the presence of oxygen, a phenomenon known as the Warburg effect [1], and changes in lipid synthesis, redox balance, the tricarboxylic acid (TCA) cycle, and amino acid metabolism, compared to healthy adult tissues [2]. Many of these metabolic changes result in the rerouting of metabolites from energy-producing catabolic pathways to biosynthetic pathways, and a major function of metabolic changes in cancers might be to serve the anabolic needs of cell growth and proliferation [3]. The corollary of this is that normal proliferating cells, but not postmitotic cells (which constitute the majority of healthy adult tissues), will be metabolically similar to tumor cells [3,4]. In support of this hypothesis, normal lymphocytes and thymocytes *in vitro* show a Warburg-like metabolism when stimulated to divide [5,6], and fast-dividing pluripotent stem cells are generally considered to be highly glycolytic [7,8]. By contrast, because mutated oncogenes and tumor suppressors confer new attributes to cancer cells, or lock them in a cellular state that in normal proliferating cells is more flexible, aspects of tumor metabolism may be present only under very specific circumstances during normal growth.

Metabolism could relate to a changing cell fate in three ways [9] (Figure 1). First, metabolites may generally not be limiting for cellular function, and changes in metabolite demand during differentiation could lead to corresponding adjustments in metabolism, through local mechanisms such as feedback control, but without the need for wholesale metabolic rewiring (Figure 1A). Second, differentiation may require a fundamental shift in the metabolic landscape of the cell, for example by regulation of metabolic enzyme expression (Figure 1B). Last, and perhaps most intriguingly, metabolic changes could not only be necessary for differentiation, but may also control it by regulating signaling pathways and gene expression (Figure 1C). These schemes are not mutually exclusive and probably cooperate to varying degrees in different tissues. The role of metabolism in cancer cell proliferation has been discussed in several recent reviews [1,2], and in this review we focus our attention on the metabolism of normal growth in development and stem cells. We describe how metabolism changes with cell fate and how metabolic function is linked to other mechanisms regulating cell proliferation and differentiation. We also propose some hypotheses about the function of metabolic adaptations during differentiation, and suggest directions for future research.

Cell differentiation involves metabolic changes

A major way to control tissue growth during embryonic development and tissue renewal in adults is through cell differentiation. Terminally differentiated cells often lose their ability to divide and, even among proliferating cells, only a subpopulation of undifferentiated stem cells can undergo long-term self-renewal [10]. Cancer has been proposed to be a disease of differentiation [11], and cancer

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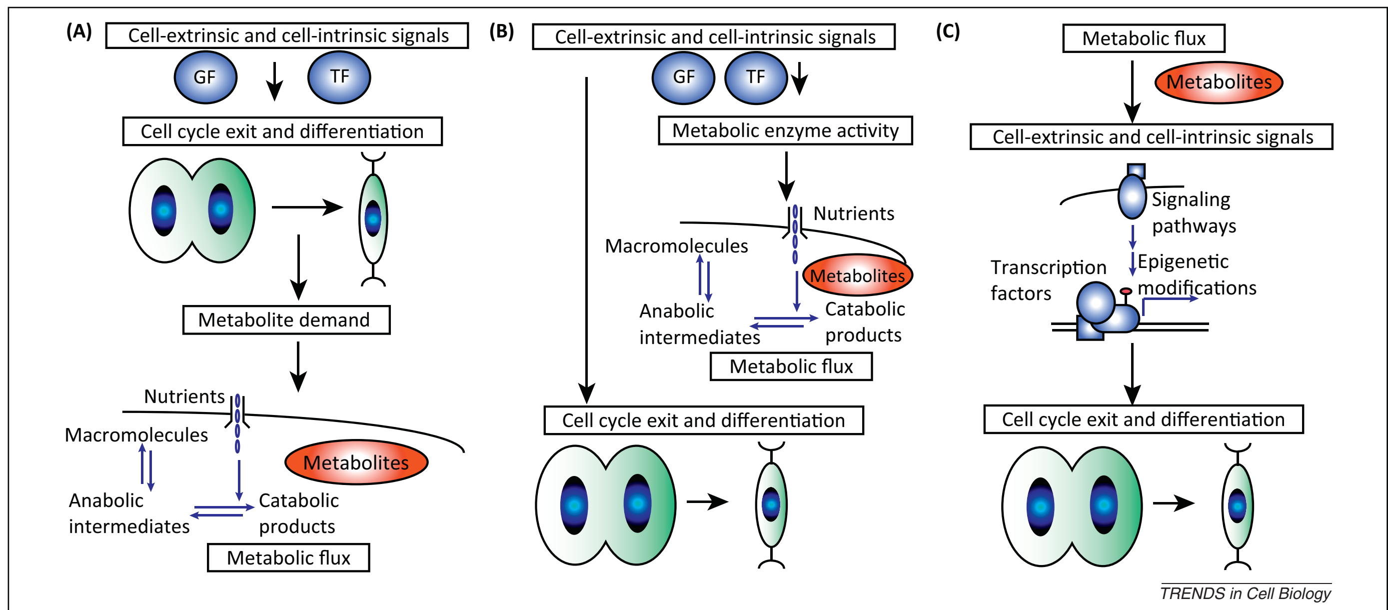

Figure 1. The ways metabolic changes may be linked to cell differentiation. **(A)** Growth factors (GF), transcription factors (TF), and other cell signaling proteins (blue) direct cells to stop proliferating and differentiate. Cell cycle exit changes demand for metabolites (red), for example by reducing the rate of nucleotide and amino acid incorporation into macromolecules. By altering local equilibrium, or through allosteric effects on metabolic enzymes, this change in demand redirects metabolic flux. **(B)** Cellular signaling directs cells to differentiate and in parallel regulates metabolic enzyme activity. The resulting rewiring of metabolic pathways serves the altered demands of the differentiated cells, for example through reduced rates of nucleotide and amino acid production. **(C)** Changes in metabolic flux alter the concentration of specific metabolites, which regulate cellular signaling proteins, and consequently influence cell differentiation. The mechanisms by which this might take place are summarized in Figure 2.

cells may employ the same transcriptional mechanisms used by stem cells, which are characterized not by increased proliferation compared to progenitor cells – indeed they are often less proliferative – but by a distinctive undifferentiated state [10]. This raises the possibility that metabolic changes observed in cancer, or during normal growth, are related not only to the anabolic needs of proliferation but also to the maintenance of an undifferentiated state.

The metabolism of embryonic (ESCs) and induced pluripotent (iPSCs) stem cells, which can be studied in large quantities *in vitro*, has been looked at closely [7,8]. Several studies revealed high levels of glycolysis in pluripotent cells compared to more differentiated cells including cardiomyocytes and fibroblasts, suggesting metabolic similarities of ESCs to cancer cells. Unlike cancer cells, whose respiratory capacity is generally not damaged, it has been proposed that pluripotent cells are glycolytic due to an immature mitochondrial network, characterized by low numbers of mitochondria and reduced oxidative capacity [12,13]. A recent study, however, argued that pluripotent cells have intact respiratory capacity but favor glycolysis, possibly by restricting the amount of substrate reaching the mitochondria [14]. In agreement with the idea that pluripotent cells are glycolytic, ESC differentiation to cardiomyocytes involves upregulation of oxidative phosphorylation and downregulation of glycolysis [15]. The reverse route, reprogramming fibroblasts to iPSCs, is accompanied by upregulated glycolysis [16,17]. However, differentiation of human ESCs to proliferating neural stem cells (NSCs) results in the opposite pattern, with a decline in oxidative phosphorylation and a slight increase in glycolysis, which is inhibited only when NSCs are terminally differentiated to neurons [18]. It is perhaps the case that the changes in energy metabolism that accompany ESC differentiation are dependent on the cell types into which ESCs differentiate. Pluripotent cells might not all be metabolically similar because human ESCs and the corresponding mouse epiblast SCs are highly glycolytic compared to the more immature mouse ESCs, due to higher HIF-1α activity and defects in the electron transport chain [19].

The most prevalent explanation for aerobic glycolysis in tumors suggests that it is not a strategy to deal with hypoxia or impaired respiratory capacity, but instead it better serves the anabolic demands of proliferation [4]. Consequently one would expect to find aerobic glycolysis associated not only with cancer but with normal cell proliferation, and to be extinguished upon differentiation to a postmitotic cell. This is particularly important to examine in physiological conditions because *in vitro* conditions influence metabolic flux, which may be different *in vivo* [20]. In the embryonic retina of *Xenopus laevis*, glycolysis contributes substantially to ATP production in proliferating progenitors, but when these cells differentiate into neurons they become reliant on oxidative phosphorylation. Similarly to cancer cells, retinal progenitors are not obligatorily glycolytic due to restricted oxygen availability or respiratory defects. Cell-intrinsic regulation of energy production might be a fundamental aspect of differentiation during normal growth, at least in this tissue [21,22].

Although in some cases stem and progenitor cells are glycolytic irrespective of their environment, in other tissues undifferentiated cells have been proposed to be glycolytic due to the restrictions imposed by a hypoxic environment. Culture in 2–5% O_2 enhances the survival and proliferation of stem cells *in vitro* compared to atmospheric 21% O_2, perhaps because this is a better approximation of *in vivo* normoxia [23]. O_2 levels *in vivo* are

variable [23], and might fall significantly even within a few cell diameters from the O_2 source, suggesting that O_2 concentration might have regulatory properties for cell proliferation. Hematopoietic stem cells (HSCs) reside mostly next to sinusoidal capillaries in the bone marrow [24], and have been proposed to be hypoxic based on low diffusion of Hoechst dye from the capillaries and staining with the hypoxia dye pimonidazole hydrochloride [25] which, at O_2 partial pressures less than about 1.3%, forms protein adducts in cells and can be detected by antibodies. However, a recent thorough analysis found that hematopoietic stem and progenitor cells stain strongly for pimonidazole and HIF-1α irrespectively of their localization, even compared to neighboring cells in the bone marrow, suggesting these attributes are due to cell-intrinsic reasons and not low environmental oxygen [26]. In the brain, cells in the dentate gyrus of the hippocampus also stain with pimonidazole, and may be served by a lower-density capillary network compared to differentiated areas of the hippocampus [27]. Even so, it is also the case that the other major neurogenic niche, the subventricular zone, is rich in blood vessels, with NSCs lying close to them [28,29]. Therefore a reduced blood supply is probably not a feature of all neurogenic niches, and the relationship between capillary density and oxygen tension in stem-cell niches remains to be determined. Direct measurements of oxygen tension were performed *in vivo* in developing *Xenopus* embryos, and did not reveal gross differences between proliferating and differentiated retinal cells [21]. More work is required to understand whether in specific tissues proliferative cells, particularly stem cells, are hypoxic compared to their more differentiated progeny, and whether a glycolytic metabolism is due to environmental hypoxia or intrinsic signaling networks.

Important metabolic changes might occur not only when a proliferating cell terminally differentiates but also during gradual changes in differentiation state. During mesodermal differentiation from immature somites to muscle progenitors in zebrafish, the expression of genes involved in oxidative metabolism is associated with more differentiated cells [30]. Isolated perinatal hearts are more glycolytic compared to older hearts [31]. The rewiring of metabolism during differentiation could conceivably extend to subcellular compartmentalization. For example, a differentiating retinal ganglion cell extending an axon could have very different biosynthetic needs at the growing axon compared to its cell body. At the axon tip or in the dendrites of a neuron, requirements for protein synthesis [32] are reminiscent of those during cell division. Therefore different areas within a cell might be metabolically diverse.

Signaling pathways that control normal proliferation and differentiation also control metabolism

Multiple signaling pathways affecting both proliferation and differentiation are also implicated in the control of cellular metabolism (Figure 1B and Table 1). The PI3K/AKT/mTOR pathway is a major pathway induced by growth factors, which promotes anabolic pathways and cell proliferation in multiple contexts. It stimulates glucose import and glycolysis through regulation of glucose transporters and glycolytic enzymes, promotes fatty acid synthesis through SREBPs, and protein synthesis through 4EBP and S6K [2,3]. At the same time, this pathway controls stem and progenitor cell fate. Loss of PTEN, a negative regulator of the PI3K/mTOR pathway, leads to increased proliferation of NSCs [33] but depletes HSCs [34]. Deletion of *Tsc*, a negative regulator of mTOR, also depletes HSCs [35]. The TOR pathway promotes neuronal differentiation in the *Drosophila* eye [36] and in the chick neural tube [37]. The Ras pathway, which is activated by growth factors, stimulates glucose import and glycolysis in colorectal and pancreatic cancers [38,39], and has been implicated in the control of differentiation in many tissues. The Lkb1/AMPK pathway is activated by energy stress, it suppresses anabolic and activates catabolic pathways (Table 1) and inhibits proliferation [40]. *Lkb1* deletion causes a temporary increase in HSC proliferation, followed by cell death, although this is probably not mediated by AMPK [41]. The Hedgehog pathway, which controls proliferation and differentiation in multiple tissues [42], was recently suggested to activate aerobic glycolysis in adipocytes through non-canonical signaling from its receptor Smoothened [43]. These pathways therefore regulate both metabolism and cell differentiation, and interact with each other to coordinate cellular state and metabolism. Metabolism also interacts with the cell cycle (Box 1), suggesting the presence of abundant reciprocal regulation between signaling pathways, cell cycle factors, metabolic enzymes, and cell fate determinants.

Table 1. Effects of signals that control cell proliferation and differentiation on metabolism[a]

Signal	Metabolic pathways regulated
PI3K	Stimulates glucose uptake, glycolysis, fatty acid synthesis
mTOR	Stimulates glycolysis, lipid synthesis, protein synthesis, pyrimidine synthesis
Ras	Stimulates glucose uptake, pentose phosphate pathway; regulates glutaminolysis
Hedgehog	Stimulates glycolysis
HIF	Redirects energy metabolism from oxidative phosphorylation to glycolysis
Myc	Stimulates glycolysis, glutaminolysis, nucleotide synthesis
AMPK	Inhibits glycolysis and fatty acid synthesis; promotes fatty acid oxidation
Sirtuins	Regulate TCA cycle, glycolysis, fatty acid oxidation

[a]PI3K, mTOR, Ras, Hedgehog, HIF, and Myc in general favor anabolism, whereas AMPK and the Sirtuins promote catabolism. The effects of these pathways on metabolism and on cell proliferation and differentiation depend on the tissue and context.

The role of metabolic adaptations in cellular differentiation

The function of metabolic rewiring, particularly of aerobic glycolysis, in enhancing biosynthesis to satisfy the needs of proliferation has gathered widespread supporting evidence [1]. Nevertheless, a variety of recent results suggest that there might be additional functions of aerobic glycolysis in the regulation of differentiation. Inhibition of glycolysis attenuates the reprogramming of dividing somatic cells to induced pluripotent stem cells without affecting cell proliferation [16,44]. Energy metabolism is involved in immune cell activation and the choice between proinflammatory or anti-inflammatory immune cells [45]. A switch

Box 1. The relationship between cell cycle and metabolism

Metabolic function determines the rates at which cells grow. Cell growth in most contexts is not merely a passive consequence of increased cell cycle activity and must be regulated concurrently with the cell cycle to achieve appropriate levels of tissue growth [96]. This is nicely illustrated by a series of genetic manipulations in the fly wing disc. Overexpression of cell cycle activators or inhibitors changes cell number but also causes the reverse changes in cell size such that the total compartment area does not change [97]. Similarly, in the *Xenopus* nervous system pharmacological inhibition of progenitor proliferation results in fewer but bigger neurons [98]. The mechanisms that link the cell cycle with the metabolic activity that determines cell growth are beginning to be investigated. Yeast cells go through metabolic cycles in synchrony with cell cycle phases [99], and acetyl-CoA production affects cell cycle progression [77]. In mammalian cell lines, PFKFB3, which phosphorylates fructose-6-phosphate to fructose-1,6-bisphosphate in glycolysis, and GLS1, which provides TCA cycle intermediates from glutamine, are degraded by APC/C-CDH1, the same ubiquitin ligase complex that controls cyclin levels. Therefore, glycolysis and glutaminolysis might be activated in late G_1 and S phase in periods of rapid growth [100]. Conversely, metabolism can affect the cell cycle, for example mitochondrial dysfunction causes cell cycle arrest through signaling to cyclin E and the cdk inhibitor dacapo [101].

from oxidative to glycolytic metabolism is necessary for dendritic cell maturation downstream of Toll-like receptor signaling, irrespectively of changes in proliferation [46]. Glycolysis favors T_H17 over T_{reg} differentiation [47], and conversely lipid oxidation has the opposite effect [48]. Importantly, deletion of HIF-1α, which promotes glycolysis in T_H17 cells, results in increased T_{reg} versus T_H17 differentiation without affecting proliferation [47,49]. In the frog retina *in vivo*, shifting energy production from aerobic glycolysis to oxidative phosphorylation does not impair progenitor proliferation or survival, although a complete glycolytic block leads to biosynthetic and cell cycle arrest and apoptosis [21]. The ability of some glycolytic enzymes to promote tumor growth has been taken as strong evidence for a biosynthetic role for aerobic glycolysis; however, it is now understood that these enzymes also have nonmetabolic effects that contribute to their proliferative function. For example, the pyruvate kinase isoform PKM2 can translocate to the nucleus and cooperate with β-catenin to activate the expression of cell cycle genes including *MYC* and *Cyclin D1*, as well as other glycolytic genes, and this nuclear function is necessary for the Warburg effect and for tumor growth [50]. Similarly, aldolase might promote proliferation independently of its glycolytic role, through effects on the cytoskeleton [51]. Although it is clear that glycolysis is crucial for biosynthesis and proliferation, we speculate that in some cases aerobic glycolysis might also regulate differentiation. The molecular mechanisms by which this could operate are not clear. A recent study suggested that the Warburg effect might regulate gene expression in colon cancer cells by causing butyrate accumulation and consequent inhibition of histone deacetylases [52]. Much evidence also places aspects of metabolism upstream of cellular signaling, a theme we will return to later.

Aside from changes in aerobic glycolysis, other metabolic manipulations can also affect differentiation. Knockdown of three enzymes of central carbon metabolism, phosphoglycerate kinase, hexose-6-phosphate dehydrogenase, and ATP-citrate lyase (ACL), promoted myoblast differentiation *in vitro* [53]. ESCs show an abundance of unsaturated metabolites compared to differentiated neurons or cardiomyocytes, and inhibition of the eicosanoid pathway, which oxidizes unsaturated metabolites, can delay differentiation and promote a pluripotent state, consistent with the idea that metabolic oxidation is related to differentiation [54].

If there is a metabolic shift upon differentiation, it may be accompanied by changes in the metabolic sources of cellular damage and the mechanisms to protect against it. Stem cells are thought to possess high aldehyde dehydrogenase (Aldh) activity [55]. Combined inactivation of *Aldh2* and of the Fanconi anemia (FA) DNA repair pathway in mice causes leukemia, and a pronounced and specific depletion of hematopoietic stem and progenitor cells due to aldehyde toxicity. Therefore, HSCs have metabolic defenses to withstand aldehyde damage that may be different to those of their progeny, and their override could account for bone marrow failure in FA patients [56,57]. At the other end of the spectrum, differentiation into cells performing a specific function probably also requires extensive metabolic rearrangements to avoid damage. For example, photoreceptors require large amounts of ATP for neural activity, but they also have elevated biosynthetic needs for continuous renewal of their outer segments, and are exposed to high levels of ultraviolet light and oxygen tension, which may contribute to photoreceptor degeneration in retinitis pigmentosa [58]. Strikingly, familial homozygous mutations in *IDH3β*, causing a severe reduction in the activity of the NAD^+-dependent isocitrate dehydrogenase, which produces α-ketoglutarate in the TCA cycle, cause retinitis pigmentosa but no other health problems [59]. Thus, even something as fundamental as disruption of a core TCA cycle enzyme can have cell type-specific effects.

A major source of metabolic damage to cells is the generation of reactive oxygen species (ROS), in part through oxidative phosphorylation. Some normal [6] and cancer cells [60] use aerobic glycolysis and the pentose phosphate pathway to minimize ROS. High ROS are thought to be deleterious to HSCs [61]. Deletion of multiple genes, including FOXO family members [62], *Prdm16* [63], and *Tsc1* [35], causes ROS accumulation which contributes to HSC or NSC dysfunction. However, ROS might not simply be mediators of oxidative stress and damage but also have signaling roles. ROS generation from NADPH oxidase (NOX) can promote NSC self-renewal and neurogenesis, an effect mediated by increased PI3K/AKT activity [64]. Developing cardiomyocytes have an open mitochondrial permeability transition pore and high ROS, and pharmacological closure of the pore or treatment with an antioxidant enhance myocyte differentiation [65]. By contrast, differentiation of human mesenchymal stem cells to adipocytes requires increased oxygen consumption and ROS generation from mitochondrial complex III [66]. Similarly, high endogenous ROS levels in *Drosophila* prime hematopoietic progenitors for differentiation [67]. ROS production after tail amputation in *Xenopus* is necessary for regeneration, possibly through regulation of Wnt activity and proliferation [68]. Therefore, depending on the

tissue and the ROS source, ROS might either promote or antagonize differentiation, and regulation of their levels could be a mechanism for metabolism to regulate cellular differentiation.

Molecular mechanisms that link metabolism to regulation of differentiation: control of signaling pathways and epigenetic modifications by metabolites

Metabolic networks are controlled by signaling pathways (Figure 1B). However, the converse is also true, and often the same pathways that influence metabolism are themselves regulated by metabolic products (Figures 1C and 2). AMPK is activated by energetic stress through binding of ADP and AMP, and by phosphorylation by LKB1 [40], and promotes catabolic while inhibiting anabolic reactions. In response to glucose restriction in cultured myoblasts, AMPK activation promotes NAD^+ biosynthesis, which results in activation of the NAD^+-dependent histone deacetylase Sirt1 and inhibition of differentiation [69]. AMPK also phosphorylates other components of the epigenetic machinery [40], providing additional mechanisms for influencing differentiation. The mTOR pathway, a negative target of AMPK, is activated by amino acids, promotes anabolic reactions [70], and has several roles in cell differentiation, as discussed above. HIF-1α and HIF-2α are transcriptional activators whose degradation is dependent on O_2 (Figure 2). In hypoxia they are stabilized and activate several targets, most prominently glycolytic genes, switching the energy production of the cell from oxidative phosphorylation to glycolysis, thereby maintaining ATP production and avoiding ROS generation from the electron transport chain [71]. Genetic studies have pointed to multiple developmental roles for HIF factors, including the regulation of differentiation. For example, they inhibit adipocyte differentiation under hypoxia [72], and changes in HIF-1α levels impair HSC function [73]. During hypoxia HIF-1 activates Notch targets and inhibits neural and myogenic differentiation [74], and might even stabilize and activate Notch in a ligand-independent manner during normoxia [75]. Similarly, HIF-1α activates Wnt/β-catenin signaling in embryonic and neural stem cells, and its deletion reduces proliferation and neurogenesis in the dentate gyrus of the hippocampus [27]. The AMPK, TOR, HIF, and Sirtuin pathways reciprocally interact with metabolic pathways and also cross-regulate each other in intricate ways.

Extensive epigenetic remodeling is necessary for differentiation. Histones or DNA are covalently modified by methylation, acetylation, and glycosylation to regulate transcription. Recent evidence suggests that availability of the necessary metabolites affects epigenetic modifications, providing a direct link between nutritional changes, metabolic output, and gene expression [76] (Figure 2). Acetyl-CoA is a central metabolite in energy metabolism and biosynthesis. High levels of acetyl-CoA during periods of exponential growth in yeast promote histone acetylation and the activation of genes involved in cell growth, including ribosomal genes [77,78]. In mammalian cells *in vitro*, ATP-citrate lyase (ACL), which produces cytoplasmic acetyl-CoA for fatty acid synthesis from citrate, mediates changes in histone acetylation downstream of glucose availability or growth factor stimulation. ACL knockdown inhibits expression of glycolytic genes during adipocyte differentiation [79], and in cultured myoblasts it reduces histone acetylation and promotes myogenic differentiation [53].

DNA and histone methylation uses *S*-adenosyl-methionine (SAM), which in the process is converted to *S*-adenosyl-homocysteine (SAH). Carbons to generate SAM are supplied by the amino acids threonine, glycine, and serine through folate metabolism (Figure 2). Mouse ESCs are uniquely dependent on extracellular threonine [80]. Threonine deprivation leads to a decrease in the SAM:SAH ratio and histone H3K4 trimethylation levels, and promotes ESC differentiation [81]. Threonine might also supply mESCs with the acetyl-CoA needed for the high biosynthetic needs of these rapidly proliferating cells [80,81]. Therefore threonine catabolism in mESCs might be important both for the anabolic needs of fast proliferation and for epigenetic regulation of differentiation by providing acetyl and methyl groups to regulate gene expression (Figure 2).

High NAD^+ levels may be an indication of energetic stress, and NAD^+ promotes the activity of the sirtuin family of histone deacetylases. Sirtuins deacetylate several proteins, including histones, therefore regulating gene activity, and have widespread effects on metabolism (Table 1) [82]. Recent evidence implicates sirtuins in differentiation, particularly in relation to Notch signaling. In response to oxidative stress, Sirt1 inhibits neurogenesis by blocking expression of the proneural transcription factor Mash1 [83], although another study found that Sirt1 promotes neural differentiation and represses the Notch target Hes1 [84]. During endothelial cell differentiation, Sirt1 deacetylates the Notch1 intracellular domain, destabilizing and inhibiting Notch [85].

Strong evidence for direct regulation of cell differentiation by metabolites in cancers comes from studies of neomorphic point mutations in the $NADP^+$-dependent isocitrate dehydrogenases IDH1 and IDH2 in gliomas and leukemias. IDH1/2 normally catalyze the conversion of isocitrate to α-ketoglutarate but the mutant enzymes instead produce 2-hydroxyglutarate (2HG) [86]. Overexpression of the mutant IDH1/2, producing 2-hydroxyglutarate, blocks differentiation of hematopoietic cells [87,88], and conversely inhibition of mutant IDH1 promotes astrocytic differentiation in gliomas harboring this mutation [89]. 2HG and the TCA cycle intermediates fumarate and succinate are thought to interfere with several α-ketoglutarate-dependent dioxygenases, including JmjC domain-containing histone demethylases, TET dioxygenases, which convert 5-methylcytosine to 5-hydroxymethylcytosine, and prolyl hydroxylases (PHD), which promote HIF degradation [76]. These enzymes have widespread epigenetic and transcriptional effects. Inhibition of Tet2 affects hematopoietic differentiation and increases HSC self-renewal [90,91], and Tet genes are involved in ESC differentiation [92]. It is therefore possible that fluctuations in the concentration of α-ketoglutarate, succinate, and fumarate in normal cells modulate dioxygenase activity, thereby affecting gene expression and cell fate (Figure 2).

Figure 2. Summary of how metabolism can control cellular signals that regulate cell differentiation. Red circles show metabolites that directly affect signaling, blue circles their target proteins, and red lines their interactions. Metabolic enzymes are in red type. Blue signals affect differentiation, or control metabolism itself, as described in the main text. Abbreviations not mentioned in the text: GLS, glutaminase; HAT, histone acetyltransferases; HDAC, histone deacetylases; IDH*, mutant form of isocitrate dehydrogenase; JmjC KDM, JmjC domain-containing histone demethylases; LDH, lactate dehydrogenase; PDH, pyruvate dehydrogenase; PK, pyruvate kinase.

Nuclear and cytoplasmic proteins are also modified by *O*-linked β-*N*-acetylglucosamine (*O*-GlcNAc) and the donor for this modification, UDP-GlcNAc, is synthesized from glucose and glutamine via the hexosamine biosynthesis pathway [93]. *O*-GlcNAc transferase (OGT) interacts with polycomb group members on chromatin [93] and associates with TET2/3, promoting histone *O*-GlcNAcylation, and regulating transcription [94]. *O*-GlcNAcylation on the promoters of key self-renewal genes such as Oct4 and Sox2 is necessary for ESC maintenance [95]. Metabolic function might therefore regulate epigenetic marks and gene expression through multiple mechanisms.

Concluding remarks

Despite numerous advances in understanding cancer metabolism [2–4], the role of metabolic adaptations

Box 2. Future directions

Investigation of the metabolic changes involved in differentiation should address several key questions. Do the concentrations of metabolites such as acetyl-CoA, SAM, AMP, and others fluctuate enough during differentiation to change signaling and affect cell fate or, despite being necessary for cellular function, are they present in non-limiting concentrations during physiological differentiation? How is metabolic control integrated with the wealth of other mechanisms that we know control the cellular transition from proliferation to differentiation [10]? Given their dense interactions and broad requirements, how are metabolic pathways linked to specific changes in cellular behavior? Can we probe these questions effectively in multicellular organisms *in vivo*?

during normal growth is not well understood. Metabolic changes accompany cell differentiation, and they might be brought about by signals that determine cell fate. Conversely, metabolic pathways can influence these signals. As the fundamental links between metabolism and signaling are being mapped out, the next major challenge will be to establish how these cellular mechanisms operate during tissue growth (Box 2). Apart from understanding how the metabolic factory runs in different types of cells, comparison of physiological and cancer cell metabolism is necessary for the design of targeted metabolic cancer therapies that spare normal proliferating cells.

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