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The Paradox of Metabolism in Quiescent Stem Cells

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Abstract

The shift between a proliferating and a nonproliferating state is associated with significant changes in metabolic needs. Proliferating cells tend to have higher metabolic rates, and their metabolic profiles facilitate biosynthesis, as compared to those of non-dividing cells of the same sort. Recent studies have elucidated specific molecules that control metabolic changes while cells shift between proliferation and quiescence. Embryonic stem cells, which are rapidly proliferating, tend to have metabolic patterns that are similar to those of non-stem cells in a proliferative state. Moreover, although adult stem cells tend to be quiescent, their metabolic profiles have been reported in multiple organs to more closely resemble those of proliferating than those of nondividing cells in some respects. The findings raise questions about whether there are metabolic profiles that are required for stemness, and whether these profiles relate to the metabolic properties that may be required for quiescence. Here, we review the literature on how metabolism changes upon commitment to proliferation, and compare the proliferating and nonproliferating metabolic states of differentiated cells, and embryonic and adult stem cells.

Metabolism and the cell cycle are linked

Several lines of evidence support coordination of metabolism and the cell cycle. Early studies in yeast discovered that there is a metabolic cycle layered upon the cell cycle [1]. Specific metabolic functions were shown to be temporally compartmentalized in coordination with cell division [1]. In synchronized yeast, short bursts of high levels of oxygen consumption were found to occur periodically; in between these bursts, the cells consumed less oxygen [2]. DNA synthesis was found to occur in synchrony with this cycle and during a phase when oxygen consumption was low [1]. This study raised the interesting possibility that cells protect their DNA during replication when it is single-stranded and nucleotides are exposed from the reactive oxygen species expected to be produced by the electron transport chain by temporally separating mitochondrial activity and DNA replication. The findings were important for demonstrating an important functional connection between metabolism and cell cycle.

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Shift in metabolism with the transition between quiescence and proliferation

Further support for a relationship between metabolism and the cell cycle was developed based on the demonstration that in mouse fibroblasts, there is a shift in metabolism between cells that are actively proliferating compared with cells that have exited the proliferative cell cycle [3, 4]. Such a shift might be expected: proliferating cells have biosynthetic requirements to double in size, and must synthesize DNA, proteins and lipids to create a new cell. Indeed, as early as 1959, studies of mouse fibroblasts revealed that the rates of glucose uptake and lactate production were highest during the early logarithmic growth period as compared with fibroblasts that were not actively proliferating [3]. Subsequent studies revealed that mitogen stimulation of human lymphocytes [5], mouse lymphocytes [6], and rat thymocytes [7, 8] all result in both increased glucose uptake and more excretion of lactate. Further, consistent with results in yeast defining a metabolic cycle, lactate excretion in mitogen-stimulated mouse lymphocytes changed throughout the cell cycle and peaked in S phase [6], when mitochondrial activity would be expected to be reduced.

To understand the relationship between metabolism and the cell cycle, detailed studies were performed in mouse hematopoietic cells comparing cells that were quiescent, that is, reversibly exited the proliferative cell cycle, with cells that were proliferating [9]. These studies revealed that stimulating quiescent mouse T cells induces a substantial increase in glucose uptake, which supports the increased proliferation of activated mouse T cells in the presence of its cognate antigen [10, 11]. When mouse hematopoietic cells or lymphocytes were not dividing, they exhibited little glucose uptake, performed reduced amounts of glycolysis, secreted less lactate, and instead, relied on oxidative phosphorylation as their major source of energy [9]. When stimulated to divide in response to growth factors or cytokines, mouse hematopoietic cells and lymphocytes exhibited a surprisingly strong shift to increased glucose consumption and elevated rate of glycolysis [9].

The increased reliance on glycolysis in cells that are actively dividing compared with non-dividing cells makes intuitive sense. Glycolysis can provide ATP needed for the energy-consuming task of synthesizing biomass for new cells [4]. Though glycolysis produces only 2 ATP molecules per molecule of glucose, because it is rapid, it can provide glucose at a faster rate than oxidative phosphorylation [4, 12]. Glycolysis also provides metabolic intermediates [13]. The glycolytic intermediate 3-phosphoglycerate can be used to generate amino acids; dihydroxyacetone phosphate and acetyl-CoA can be used for lipid synthesis; glucose-6-phosphate can be used to generate nucleotides [14]. Thus, glycolysis can help to provide metabolites used for the major biosynthetic pathways required for the generation of a new cell.

Growth factors promote both cell proliferation and glycolysis

These findings suggest that it is important to link cell proliferation with glycolysis. While microorganisms evolve to grow as much as possible based on the amount of nutrients available, the situation is more complicated for multicellular organisms [14]. In mammals, most cells do not consume all available nutrients [14]. In mammals, the metabolic rate of an

individual cell is often regulated by the presence of extracellular signaling pathways such as specific growth factors [14]. These pathways signal to a cell whether it should be focused on anabolic processes that produce macromolecules, or instead, if it is the correct time for catabolic processes that break down larger macromolecules to provide energy [14]. Shifting between proliferation and quiescence is often associated with a change in these signaling pathways and the balance between anabolism and catabolism [14].

For many cells, stimulation of glucose uptake occurs in response to growth factor signaling [14]. Growth factors, such as epidermal growth factor and vascular endothelial growth factor, serve as cues for cells to take up glucose [14–16]. These factors interact with their cognate receptor tyrosine kinases. Phosphorylation of the cytoplasmic tails of these receptors leads to activation of downstream signaling pathways [14, 17]. In mouse T cells, for instance, antigen stimulation leads to activation of the T cell receptor, which becomes phosphorylated and triggers downstream signaling pathways [10, 14, 17]. In mammalian muscle and liver, which respond to insulin, extracellular glucose that is absorbed by the cell is largely stored as glycogen [14]. In mammalian adipocytes, glucose uptake is also insulin-responsive and is stored largely as triglycerides (fat) [14]. In most other cell types in mammals, stimulation with growth factors results in an accumulation of biomass [14, 17]. When primary human fibroblasts were induced into quiescence by contact inhibition [18, 19], glucose consumption and lactate secretion declined, approximately two-fold [19], a more modest decrease than that observed in activated versus quiescent lymphocytes [9]. The results suggest that for some cell types such as fibroblasts, the cell’s biosynthetic requirements during quiescence require significant energy consumption [9].

Receptor tyrosine kinase activation increases glucose uptake

When receptor tyrosine kinases are activated in the presence of their ligands, they will not only stimulate pathways like the MAP kinase pathway that will lead to DNA synthesis. Receptor tyrosine kinase activation by mitogens can also activate the phosphatidyl inositol 3-kinase (PI3K) and AKT pathways. In mouse T cells, phosphorylation of the cytoplasmic tail of the activating CD28 receptor will activate signaling cascades including the PI3K-AKT axis [10, 14]. Activation of AKT kinase promotes expression [20] and plasma membrane localization [17, 21] of the glucose transporter GLUT1 [14]. This results in increased glucose uptake in response to receptor tyrosine kinase activity. AKT kinase also increases the activity of the key glycolytic enzyme hexokinase that ensures that glucose is passed into the glycolytic pathway rather than being shunted to alternative metabolic pathways [14, 17]. Some studies have demonstrated the importance of these metabolic changes for survival and proliferation. As one example, when growth factors are withdrawn, mouse T cells undergo apoptosis [14, 17, 22]. Adding back GLUT1 and hexokinase rescues T cell survival in the absence of growth factors [14, 17, 22].

Molecules that mediate the shift to higher glycolysis with proliferation

In addition to AKT activation, several other molecules have been identified that are important for the shift to a metabolic pattern more consistent with proliferation when cells divide. One molecule that has been identified as regulating higher glycolytic flux in

proliferating cells is 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase isoenzyme 3 (PFKFB3). PFKFB3 produces fructose-2,6-bisphosphate, a strong positive regulator of the rate-limiting glycolytic enzyme phosphofructokinase 1. PFKFB3 is an isoform that has a high kinase to phosphatase activity and consequently mostly promotes glycolysis [23–25]. In human endothelial cells, there is an increase in glycolytic flux prior to cell cycle commitment [13, 26, 27]. Overexpression of the glycolytic enzyme PFKFB3 was sufficient to promote cell cycle entry of endothelial cells [13, 26, 27], while knockdown of PFKFB3 impairs angiogenesis, thus demonstrating the importance of regulation of glycolytic rate as a determinant of proliferation rate.

The protein degradation-inducing E3 ubiquitin ligase APC/C-CDH1 is active in cells that are not dividing and helps to ensure that cells do not inappropriately enter the cell cycle [23]. Loss of APC/C-CDH1 in neoplastic human neuroblastoma cells and human embryonic kidney 293 cells results in both cell proliferation and increased glycolysis [23, 28]. PFKFB3 is a target of APC/C-CDH1 [28, 29]. PFKFB3 is detectable in rat astrocytes where APC/C-CDH1 activity low, and undetectable in rat cortical neurons where APC/C-CDH1 activity is high [29]. Introducing PFKFB3 with a mutation that made it resistant to degradation by APC/C-CDH1 increased glycolysis, but not proliferation [28]. PFKFB3 is an important regulator of glycolytic flux and can help to coordinate proliferation with metabolism [28].

The E2F transcription factor is an important regulator of cell cycle entry [30], and also serves to coordinate proliferation with metabolism in mammalian cells [13]. One mechanism by which E2F regulates metabolism is by upregulating pyruvate dehydrogenase kinase, an enzyme that phosphorylates and inhibits pyruvate dehydrogenase, the enzyme that converts pyruvate to lactate [31]. By regulating this enzyme, E2F promotes an anaerobic glycolysis metabolism in which pyruvate is not fed into the TCA cycle or the electron transport chain in the mitochondria, but rather is converted to lactate, which is then secreted [31]. Thus, as demonstrated in cultured mouse myoblasts and human fibroblasts, activation of E2F by growth factors represents another mechanism whereby proliferating cells adapt an anaerobic glycolysis metabolic profile.

Finally, cyclin-dependent kinases are the key kinases that mediate cell cycle entry and transitions between cell cycle phases [32]. Metabolic enzymes have been identified as targets of cyclin-dependent kinases. In particular, the metabolic enzyme PKM2 isoform of pyruvate kinase has been discovered to be phosphorylated by the same cyclin-dependent kinases that drive the cell cycle [33]. Cyclin D3/CDK6 has been shown to phosphorylate PKM2, an enzyme that catalyzes the final step in the glycolytic pathway, the dephosphorylation of phosphoenolpyruvate to pyruvate [34]. In proliferating human T-cell acute lymphoblastic leukemia cells, phosphorylation of PKM2 reduces flux through glycolysis and increases flux through the pentose phosphate pathway, which is required for nucleotides for DNA synthesis as described further below [34].

Metabolism can drive the cell cycle

There is even evidence that in some cases, metabolic pathways not only shift with proliferation, but can be an regulators of cell proliferation [23]. In mammalian cells,

nutrients are usually plentiful and cell division decisions are based on signaling pathways. However, in certain circumstances, nutrient availability can become an overriding consideration for cell cycle commitment. For example, ATP/AMP ratios can serve as an input for decision making about the commitment to proliferation versus quiescence [35]. As demonstrated in a human hepatocellular carcinoma cell line and mouse embryonic fibroblasts, if glucose levels are low and ATP production stalls, there will be high levels of AMP that activate 5'-AMP-activated protein kinase (AMPK) [36, 37]. AMPK will then phosphorylate the transcription factor and tumor suppressor p53 [23]. p53 phosphorylation, in certain contexts, can result in G1-S cell cycle arrest, which would halt cell division under conditions in which nutrients are truly limiting [37]. In these conditions of glucose and energy deprivation, cells might not have sufficient energy to complete a cell cycle [23]. Conversely, in pancreatic β-cells, glucose uptake has been reported to promote cell proliferation. In mouse pancreatic β-cells, glucose consumption activates PI3K and AKT, which activate forkhead box O transcription factors [23, 38]. These transcription factors reduce expression of cyclin D2 repressor BCL-6 [38]. Increased cyclin D2 transcription promotes β-cell division, thereby representing a mechanism whereby the availability of glucose can promote the proliferation of insulin-secreting cells within the pancreas [38].

Metabolism in embryonic stem cells

Metabolism has been recently recognized as an important driver of pluripotency versus differentiation of embryonic stem cells. Pluripotent stem cells in the mammalian preimplantation epiblast of embryos are in a naïve state [39]. These stem cells become primed during development after implantation [40]. Naïve rodent preimplantation epiblasts can be cultured using media containing LIF, GSK3β and MEK inhibitors [41]. Mouse epiblast stem cells and human embryonic stem cells are highly glycolytic, while mouse embryonic stem cells switch from glycolysis to mitochondrial respiration as needed [39, 42, 43]. Primed mouse pluripotent stem cells express high levels of glucose transporters [42, 44]. These findings in culture may reflect the situation *in vivo*; prior to implantation, mouse embryos preferentially use mitochondrial oxidative phosphorylation and they shift to anaerobic glycolysis once implanted into the uterine wall [39, 45, 46]. It is also possible that the conditions used to culture naïve cells contribute to their use of oxidative phosphorylation. LIF can induce Stat3, which promotes transcription of mitochondrial genes [47]. The high rates of glycolysis in the primed pluripotent stem cells may help to support their rapid expansion [39]. Again, for these studies, the precise conditions for culturing the different types of stem cells, including levels of glucose, glutamine, could affect the measurements of glucose uptake and glycolysis rates [39]. Nevertheless, the findings suggest that rapidly proliferating mouse embryonic stem cells heavily utilize aerobic glycolysis. Consistent with these findings, when mouse induced pluripotent stem cells are formed from differentiated cells through reprogramming, there is a switch from mitochondrial oxidative phosphorylation to glycolysis [39, 48].

Human pluripotent stem cells exhibits the opposite trend of the mouse stem cells. Human naïve pluripotent stem cells use mainly aerobic glycolysis to produce ATP rather than mitochondrial oxidative phosphorylation [49–51]. Human naïve pluripotent stem cells have higher glycolysis than primed pluripotent stem cells [49–53]. Human ESCs depend on

glycolysis and convert 70–80% of the glucose consumed to lactate [54, 55]. The higher glycolysis in naïve human PSCs results in more glucose carbons entering lactate, nucleotides and serine [53], and is associated with high nuclear N-MYC and C-MYC [53]. Reducing glycolysis decreased self-renewal in naïve, but not primed, human embryonic stem cells [53]. It is important to note that culture conditions play a role here as well, as the presence of feeders or feeder-secreted factors affects glucose dependence [53]. In both human and mouse models, when embryonic stem cells differentiate and slow their division rate, they reduce the rate of glycolysis and transition to oxidative phosphorylation and greater use of mitochondrial respiratory capacity.

Adult stem cells are quiescent and highly glycolytic

In adult mammals, tissue homeostasis and regeneration after injury are controlled by the proliferation of adult tissue-specific stem cells [56]. Adult quiescent stem cells are found as satellite cells in muscle, neural stem cells in the brain subventricular zone, hematopoietic stem cells and mesenchymal stromal cells in the bone marrow, and epithelial cells in the stem cell bulge in hair follicles [39]. These cells can be induced to proliferate and then differentiate into specific lineages. When they proliferate, these adult stem cells can divide asymmetrically to generate another stem cell and a cell that will proliferate and differentiate [57]. The ability of adult stem cells to remain in a quiescent state allows for their long-term viability to support tissue repair throughout the life of an organism [58, 59]. Defects in maintaining quiescence can lead to stem cell depletion over life [56, 60, 61]. Adult stem cells in many tissues are mostly maintained in a specified physical location or niche in a quiescent state.

Somewhat surprisingly, many adult stem cells exhibit the opposite metabolic profile from that described for differentiated cells such as T cells, endothelial cells and fibroblasts above. In the case of adult stem cells, the overall pattern is that they are highly glycolytic as stem cells, even though they are quiescent and not dividing. This metabolic pattern seems to contradict the pattern and principles developed for fibroblasts, endothelial cells and lymphocytes in which increased glycolysis is associated with more proliferation.

As one example of a type of adult stem cells, hematopoietic stem cells are largely quiescent cells found in stem cell niches in the bone marrow [62]. Quiescent mouse hematopoietic stem cells are found in regions with low levels of oxygen [43]. With low levels of oxygen as a final electron acceptor, mouse hematopoietic stem cells use little electron transport chain activity [63]. Instead, mouse hematopoietic stem cells rely on anaerobic glycolysis for ATP production [59, 63]. Quiescent mouse hematopoietic stem cells exhibit an accumulation of glycolytic metabolite fructose-1,6-bisphosphate and high enzymatic activity of glycolytic enzyme pyruvate kinase, consistent with a high glycolytic flux [62, 64]. Several mechanisms for the high levels of glycolysis in hematopoietic stem cells have been reported. Quiescent mouse hematopoietic stem cells contain the glycolysis-activating transcription factor MEIS1, which transcriptionally induces HIF-1α [39, 63]. Other studies have focused on high levels of pyruvate dehydrogenase kinases PDK1 and PDK3 in the mouse hematopoietic stem and progenitor cells compared with committed myeloid progenitors. Pyruvate dehydrogenase kinases will inhibit pyruvate dehydrogenase and cause a shunting of pyruvate

to lactate rather than the TCA cycle [39, 65]. For quiescent hematopoietic stem cells, a reliance on anaerobic glycolysis in a hypoxic environment is thought to provide the benefit of shielding them from reactive oxygen species. When levels of reactive oxygen species increase, hematopoietic stem cells are stimulated to proliferate and then differentiate or apoptose [39, 66]. In another study, loss of PDK2 or PDK4 in mouse hematopoietic stem cells did not affect colony forming ability of hematopoietic stem and progenitor cells, but did reduce the ability of hematopoietic stem cells to repopulate peripheral blood after transplantation [64], demonstrating the importance of shifting pyruvate to lactate for this process [64]. Further studies showed that $Pdk4^{-/-};Pdk2^{-/-}$ murine blood progenitors exhibit slow G0/G1 progression to limit blood repopulation [64]. Thus, the pattern observed for mouse hematopoietic stem cells is the opposite of the trend observed in proliferating versus quiescent differentiated cells where the more proliferative cells consume more glucose. In this case, the driving principle seems to be a protection from the production of reactive oxygen species.

This pattern observed in mouse hematopoietic stem cells is also observed in other adult stem cells as well. New neurons are generated in the hippocampus within the subgranular zone of the mouse dentate gyrus [67–69]. Neural stem cells within this region are largely quiescent, but can proliferate and generate new neurons that integrate into neural circuits. Signaling from pathways including notch and bone morphogenetic proteins control the transition between mouse neural stem cells and their progenitors [70–73]. Some studies have reported that neural stem cells or neural progenitor cells generated from human fibroblasts rely more on glycolysis and switch to increased oxidative phosphorylation upon differentiation [74, 75]. In one study, expression of hexokinase 2 and lactate dehydrogenase declined as neural progenitor cells differentiated [74]. Hexokinase 2 and lactate dehydrogenase regulators c-MYC and N-MYC also declined with differentiation [74]. During differentiation, a switch in pyruvate kinase splicing from PKM2 to PKM1 occurred [74]. In this study, depletion of HK2 or LDHA slowed the growth of neural progenitor cells [74]. Constitutive expression of HK2 and LDHA during differentiation resulted in cell death specifically in the differentiated neurons, which demonstrates that downregulation of aerobic glycolysis is required for proper differentiation [74]. Similarly, another study found that differentiation of human neural stem cells into post-mitotic motor neurons is associated with decreased glycolytic flux and increased mitochondrial biogenesis [75]. As described below, however, another study of neural stem cell metabolism came to a different conclusion and determined that neural stem cells rely on fatty acid oxidation rather than glycolysis to survive [76].

Mouse muscle stem cells, called satellite cells because of their location on the periphery of the muscle, are mostly quiescent yet also rely heavily on glycolysis and have few mitochondria [77]. Mouse muscle stem cells, like stem cells from other tissues, are reported to have high levels of glycolytic activity [78, 79]. Upon differentiation, mitochondrial density increases dramatically and newly formed mouse muscle cells rely on oxidative phosphorylation to provide energy for contraction [78]. Phosphorylated p38α/β MAPK pathway is induced upon mouse muscle stem cell activation [80]. In addition, the mTORC1 signaling pathway described above has been reported to be activated upon muscle activation [56, 81]. It has been reported that mTORC1 is activated in damaged muscle in response to hepatocyte growth factor/scatter factor stored in the extracellular matrix [81]. Upon release,

hepatocyte growth factor (HGF) induces a signaling cascade through PI3K-AKT pathway resulting in activation of mTORC1 that promotes muscle proliferation [81]. Further, a mouse with muscle stem cell-specific ablation of cMET, a receptor that triggers PI3K signaling pathway, or RAPTOR, in the mTOR pathway, prevented repair of skeletal muscle because the cMET-null contralateral satellite cells failed to enter the cell cycle [81]. It is important to note that another study came to different conclusions about metabolism in muscle stem cells, as described more below [82].

Other quiescent adult stem cells have also been discovered to follow a similar pattern to the hematopoietic stem cells, muscle stem cells and neural stem cells. Human mesenchymal stem cells derived from the bone marrow also preferentially use glycolysis while quiescent [83]. Like mouse hematopoietic stem cells, human mesenchymal stem cells express higher levels of glycolytic enzymes and lower levels of oxidative phosphorylation proteins compared to their differentiated counterparts, osteoblasts [83]. As another example, mouse hair follicle stem cells were found to use glycolytic metabolism and produce more lactate than other cells in the epidermis [84]. Depleting lactate dehydrogenase prevented hair follicle cell activation [84]. Most follicles lacking lactate dehydrogenase remained in telogen, demonstrating that the quiescent hair follicle cells were unable to initiate a new hair follicle cycle in the absence of lactate dehydrogenase activity [84]. In contrast, genetic mouse models that induce lactate dehydrogenase activity in hair follicle stem cells, but not other epidermal cells, promoted hair follicle growth [84]. The skin entered the anagen phase of the hair follicle cycle and produced hair follicles with normal histology [84]. The findings demonstrate that anaerobic glycolysis is important for the ability of hair follicle stem cells to enter the proliferative cell cycle and generate new hair follicles [84].

While this general pattern tends to hold true for multiple types of stem cells, there are adult stem cells that do not follow this pattern. Intestinal stem cells, on the other hand, have different proliferative properties and display a different metabolic pattern [85]. The mouse intestine renews itself every four to five days and intestinal stem cells are more proliferative so they can contribute to the cell replacement needed for this to occur [86]. Lgr5+ mouse intestinal stem cells are found at the bottom of the intestinal crypt and are surrounded by differentiated Paneth cells [85, 87]. Mouse intestinal stem cells were found to have high mitochondrial activity, while the differentiated Paneth cells were discovered to have high rates of glycolysis. In intestinal organoid culture, inhibiting oxidative phosphorylation in the stem cells affected their ability to form organoids, while inhibiting glycolysis in the Paneth cells reduced their organoid-forming ability [85].

As another counter example, adult male rat germ cells, spermatogonia, use lactate provided by supporting Sertoli cells as their most important energy source [88–90]. Rat spermatogonia, when cultured, have little capacity to metabolize glucose [88, 89, 91]. The lactate they consume helps to protect them from apoptosis [88, 89]. This may reflect the fact that spermatogonia are located outside the blood brain barrier, as more mature germ cells, spermatocytes and spermatids, are found within the blood brain barrier and can use glucose as an energy source [88, 89]. Thus, while there are exceptions, many types of quiescent, adult stem cells with access to the vasculature use glycolysis as an energy source.

Oxidative phosphorylation

As mouse hematopoietic stem cells transition from quiescence to proliferation and then differentiation, there is an increase in the number of mitochondria and the levels of oxidative phosphorylation [51, 64, 92]. Human and mouse hematopoietic stem cells, mesenchymal stem cells and neural stem cells reside in hypoxic niches [93–97]. Low oxygen tension in these niches is thought to contribute to maintenance of an undifferentiated state [59, 93, 98]. Mouse hematopoietic stem cells rely on anaerobic glycolysis to protect them from reactive oxygen species (ROS) that would be generated through use of the electron transport chain [39, 62]. In the presence of excessive ROS, mouse hematopoietic stem cells are induced to proliferate and then differentiate or apoptose [39, 59, 99]. For mouse hematopoietic cells, the activation to a proliferative and then lineage-committed state may occur when they migrate out of their hypoxic niche to a more oxygen-rich microenvironment [62].

When quiescent mouse hematopoietic stem cells commit to a specific lineage, the mitochondria, an important source of ROS, become more abundant in the differentiated cells than they are in the quiescent stem cells [62, 100]. Indeed, when mouse bone marrow aspirates are isolated, the adult hematopoietic stem cells can be identified prospectively based in part on low mitochondrial membrane potential [39, 63]. Culturing mouse hematopoietic stem cells in hypoxic conditions improved the maintenance of hematopoietic stem cells and improved repopulation efficiency [99]. While mitochondria are less abundant in mouse hematopoietic stem cells, it is nevertheless important that they are of high quality for stem cell maintenance. Mitophagy, a quality control process for mitochondria, is essential for self-renewal of mouse and human hematopoietic stem cells [101]. Activating mitophagy results in mouse and human hematopoietic stem cell expansion [101]. Mitofusin 2, a protein that mediates mitochondrial fusion and tethering to the endoplasmic reticulum, is also important for the maintenance of mouse hematopoietic stem cells with lymphoid potential [102]. Excessive mitochondrial stress occurs in hematopoietic stem cells derived from old mice as a result of inactivation of sirtuins, cytoplasmic deacetylases and stress resistance genes [103, 104]. SIRT2 inactivation results in depletion of the hematopoietic stem cells through pyroptosis, a caspase 1-dependent form of cell death, myeloid-based differentiation, and a reduced capacity to repopulate blood cells in donor mice [103]. Upregulating SIRT3 [104] or SIRT7 [105] in hematopoietic stem cells of old mice improves their regenerative capacity [104].

In mouse hematopoietic stem cells, the commitment to proliferation involves not only more mitochondria, but also activation of the mitochondrial unfolded protein response, UPR^{mt} [105, 106] The UPR^{mt} is a pathway that allows cells experiencing mitochondrial protein folding stress to activate transcription of mitochondrially-localized chaperones and proteases, and repress mitochondrial translation to reduce the stress of extra protein synthesis [105, 107–110]. This protective pathway was shown to enforce a metabolic checkpoint that regulates hematopoietic stem cell division and aging [105]. The UPR^{mt} is activated and functionally required as quiescent mouse hematopoietic stem cells commit to proliferation when mitochondrial number increases [105]. Additional studies have shown that loss of a mitochondrial chaperone activates the UPR^{mit} in the mouse intestine as well.

Intestinal crypts with activation of the UPR^{mit} exhibit loss of stemness and increased cell proliferation [106].

Mitochondrial activity can contribution to differentiation. If the mitochondrial carrier homolog 2 (MTCH2) is deleted specifically in mouse hematopoietic cells, mitochondrial fusion, mitochondrial volume and oxidative phosphorylation increase [111]. The impact of increased mitochondrial activity in mouse hematopoietic stem cells is increased proliferation and differentiation, and they lose their stem-like qualities [39, 111]. In another study, mouse hematopoietic stem cells overexpressing Fbxl10 exhibited increased expression of genes involved in mitochondrial oxidative phosphorylation [112]. Fbxl10 was found by chromatin immunoprecipitation to bind directly to regulatory regions of oxidative phosphorylation genes [112]. Mouse hematopoietic stem cells with Fbxl10 overexpression exhibited an accelerated G0/G1-to-S transition that resulted in expansion of the progenitor pool of cells [112]. Genes involved in mitochondrial oxidative phosphorylation were enriched in Fbxl10-overexpressing hematopoietic stem cells [112].

Finally, in mouse hematopoietic stem cells, like in mouse muscle stem cells, the mTOR signaling pathway plays an important role controlling mitochondrial activity and stem cell self-renewal. mTORC1 can phosphorylate mitochondrial proteins and activate peroxisome proliferator-activated receptor gamma coactivator 1α (PGC-1α) [113–115]. PGC-1α is an upstream regulator of transcription factors involved in mitochondrial biogenesis and oxidative phosphorylation. In this way, mTOR can boost mitochondrial oxidative phosphorylation. In mouse hematopoietic stem cells, deleting TSC1 causes constitutive mTOR activation [116]. TSC1 inactivation in mouse hematopoietic stem cells increases mitochondrial oxidative stress and promotes exit from quiescence and proliferation [117]. Treatment with rapamycin that inhibits mTOR signaling can promote self-renewal of hematopoietic stem cells [117]. For these reasons, low levels of reactive oxygen species are considered important to maintain hematopoietic stem cell quiescence [62].

Similar patterns are observed in other adult stem cells as well. For human mesenchymal stem cells, higher oxygen consumption results in both increased proliferation and more senescence [118]. During the differentiation of human neural stem cells generated from embryonic stem cells or induced pluripotent stem cells into motor neurons, PGC-1α induces increased expression of respiratory subunits, and mitochondrial biogenesis increases [75]. As another example, excessive mTOR signaling can cause hyperproliferation and premature aging of the skin, and exhaustion of epidermal stem cells in adult mice [119].

Thus, in different types of stem cells, glycolysis is used to protect against ROS, and elevated ROS promotes proliferation, differentiation and senescence. The results suggest that stem cells have a very different relationship to metabolic profiles compared with non-stem cells. For non-stem cells, glycolysis levels are higher when the cells are proliferating. Quiescent mouse lymphocytes rely heavily on oxidative phosphorylation and are not triggered to divide.

Control of reactive oxygen species

Low levels of reactive oxygen species in quiescent adult stem cells are achieved not only by maintaining low levels of mitochondrial activity. There are also anti-oxidant pathways that are active in quiescent adult stem cells. In quiescent mouse hematopoietic stem cells, high levels of two different transcription factors, nuclear factor erythroid 2 like 2 (NRF2) and forkhead FoxO, activate transcription of antioxidant enzymes that suppress ROS signaling and protect from the effects of ROS [39, 66]. FoxO3-mutant bone marrow cells had lower levels of anti-oxidant enzymes and higher levels of ROS [120]. Consequently, FoxO-deficient mouse hematopoietic stem cells have higher susceptibility to oxidative stress [66, 121, 122]. Loss of FoxO depletes mouse hematopoietic stem cells as the mice age [66, 121], with a corresponding increase in the myeloid progenitor compartment demonstrating greater commitment of the stem cells to proliferate [120]. Stimulation of FoxO3$^{-/-}$ mouse hematopoietic stem cells with IL3 resulted in hyperphosphorylation of AKT, mTOR and mTOR substrate S6K1 [120]. The effects on mTOR, AKT and progenitor pool size were reversed by treating the mice with anti-oxidants [120]. These studies support a model in which FOXO3 activation in quiescent mouse hematopoietic stem cells maintain low levels of ROS, which prevents AKT/mTOR activation and proliferation [120].

SIRT3 deacetylates two lysine residues on a mitochondrial superoxide and thereby reduces cellular ROS [123, 124]. SIRT3 is highly expressed in hematopoietic stem cells and repressed upon differentiation. By acetylating mitochondrial proteins, SIRT3 reduces mitochondrial ROS levels. As mentioned above, SIRT3 expression in hematopoietic stem cells of old mice can improve their regenerative capacity [104].

Similarly, high levels of ROS induce mouse neural stem cells to proliferate followed by differentiation and apoptosis [125]. To maintain low levels of ROS in quiescent neural stem cells, these stem cells also rely on a FoxO3-driven antioxidant program that is inactivated when they differentiate [125]. If FoxO3 is not present, adult mouse brain neural stem cells are depleted and increased neurogenesis results in the olfactory bulb [39, 126]. FoxO3 inactivation resulted in more oligodendrocytes in the corpus callosum during brain development [39, 126]. The findings suggest that active suppression of ROS prevents mouse neural stem cells from differentiating, while ROS induction promotes neural stem cells to proliferate and differentiate [39, 125].

Amino acid metabolism

Glutamine and glucose can both provide ATP for energy, but glutamine is the most important donor of nitrogen atoms for metabolism [127, 128]. In a three-dimensional model of proliferation and quiescence in human mammary epithelial cells, glutamine metabolism depended greatly on the proliferative state of the cells [127]. In proliferating human mammary epithelial cells, glutamine was absorbed and catabolized to glutamate by the action of transaminases [127]. These transaminases also generated non-essential amino acids that could be used for protein synthesis [127]. In contrast, in quiescent human mammary epithelial cells, glutamine uptake was lower and glutamine that was consumed was catabolized by glutamate dehydrogenases [127]. This resulted in the production of

ammonium rather than non-essential amino acids in cells that were not actively dividing [127]. mTOR activation in proliferating human mammary epithelial cells was found to contribute to the change in glutamine metabolism by suppressing the activity of the GLUD transcript that catabolizes glutamine to form ammonium rather than amino acids [127]. Glutamine breakdown, in addition to glucose consumption, is also important to provide energy for cells that are proliferating [13]. Glutamine breakdown is essential for progression to S phase and for progression from S to G2/M phase in HeLa ovarian cancer cells [13, 129]. To achieve this, the glutaminase enzyme GLS1 is expressed at higher levels at G1 to S and S to G2/M transitions [13, 129].

Growth factor signaling not only controls glucose uptake, it also regulates amino acid uptake and utilization [14]. Amino acid uptake, like glucose uptake, is controlled by the mTORC1 kinase [130]. In response to growth factor signaling, phosphorylation of tuberous sclerosis complex (TSC) subunit 2 in proliferating HeLa cells and 293 cells leads to dissociation of the TSC complex from the lysosome [131–133]. Dissociation of the TSC complex results in RAS homologue enriched in brain (RHEB) activation which, in turn, activates mTORC1 kinase [134]. mTORC1 then phosphorylates p70S6 kinase 1 (S6K1) and eIF4E binding protein 1(4EBP1), which increases translation [130]. AKT and mTORC1 activation also increases cell surface expression of amino acid transporters [135]. Thus, proliferating cultured human cells that are stimulated with growth factor receptors and have activated AKT and mTORC1 signaling are expected to express higher levels of amino acid transporters and take up more amino acids.

Glutamine is also essential for human and mouse embryonic stem cells. Naïve mouse embryonic stem cells rely on oxidative metabolism [42, 136], while mouse epiblast stem cells and human embryonic stem cells rely mostly on not only glycolysis, as described above, but also glutaminolysis for energy [42, 136]. In cultured mouse embryonic stem cells, even in the presence of glucose, most glutamate, αKG and malate in the TCA are derived from glutamine [137].

Mouse pluripotent stem cells also upregulate the threonine catabolizing enzyme Thr dehydrogenase (Tdh) [138], which converts threonine to glycine. Mouse pluripotent stem cells are sensitive to deprivation of threonine compared with differentiated cells [138, 139]. Pluripotent mouse stem cells, but not differentiated, proliferating cells, are specifically sensitive to threonine deprivation, suggesting threonine degradation may be important for pluripotent stem cell self-renewal [138]. Threonine was found to provide glycine and acetyl coenzyme A needed for S-adenosylmethionine (SAM) metabolism. Depletion of threonine reduced the accumulation of SAM and decreased histone H3 lysine 4 trimethyl mark, which resulted in more differentiation [138]. In humans, Tdh is a pseudogene and this same pathway is not important for human pluripotent stem cells. For human pluripotent stem cells, methionine plays this role [140]. Methionine deprivation of human stem cells results in a rapid decrease in SAM, loss of H3K4me3 and differentiation into the three embryonic layers [140]. This may reflect that without H3K4me3, there is a reduction in the expression of pluripotency factor NANOG [140]. SAM levels have also been shown to affect histone H3K27me3 marks in naïve human pluripotent stem cells and to be critical for preventing differentiation [136].

Skeletal stem cells are a source of osteoblasts [141]. Glutamine consumption increased during mouse osteoblast differentiation when skeletal stem cells became more proliferative [141]. Glutamine metabolism has been demonstrated to play a key role in the differentiation of skeletal stem cells [141]. Providing glutamine induced mouse skeletal stem cells to undergo differentiation, while glutamine withdrawal reduced mouse skeletal stem cell differentiation into the osteoblast lineage, and increased differentiation to adipocytes [141]. The enzyme glutaminase GLS deaminates glutamine to form glutamine as the first step in glutamine catabolism. Loss of glutaminase GLS specifically in mouse skeletal stem cells reduced the amount of osteoblasts [141]. Glutaminase and transaminase-dependent glutamate conversion to α-ketoglutarate were found to be required in mouse skeletal stem cells to proliferate and differentiate into osteoblasts [141]. Thus, glutamine consumption increases in proliferating, differentiated cells and in activated, proliferating skeletal stem cells.

Nucleotide metabolism

A large difference is expected in nucleotide synthesis between proliferating cells that replicate DNA during S phase, and may synthesize more rRNA and mRNA, compared with quiescent cells. Indeed, production of nucleotides is particularly important for proliferating cells because the synthesis of nucleotides is usually a more important contributor to the nucleotide pool than scavenging from the extracellular environment [14]. In order to synthesize nucleotides, proliferating cells from all species need to generate the ribose base sugar, and this molecule is generally produced by the oxidative phase of the pentose phosphate pathway [14]. To feed metabolites into the pentose phosphate pathway, glucose-6-phosphate dehydrogenase can direct glucose 6-phosphate toward the pentose phosphate pathway and away from glycolysis [14].

In comparing proliferating and quiescent human dermal fibroblasts, proliferating fibroblasts used the pentose phosphate pathway to generate ribose for nucleotides [19]. Quiescent human dermal fibroblasts, somewhat surprisingly, also shunted glucose toward the pentose phosphate pathway at rates similar to proliferating fibroblasts, but the quiescent fibroblasts recycled ribose back to glycolysis rather than generating nucleotides [19]. In quiescent, human dermal fibroblasts, the NADPH generated from the pentose phosphate pathway oxidative phase could be used to protect against reactive oxygen species [19].

In neuronal stem cells versus neurons, the more differentiated cells appear to be more dependent on the anti-oxidant activities of the pentose phosphate pathway. As described above, APC/C-CDH1 ubiquitinates and degrades PFKFB3. This activity is high in nondividing, terminally-differentiated rat neurons, which contain undetectable levels of PFKFB3 as a result of its continuous ubiquitination by high levels of APC/C-CDH1 [29]. By lowering levels of glycolysis in rat neurons, the low levels of PFKFB3 result in more glucose 6-phosphate available for pentose phosphate activity [23, 29]. This results in NADPH in post-mitotic, terminally-differentiated neurons that can contribute to antioxidant defense [29].

In mouse embryonic stem cells, a different pattern is observed with high pentose phosphate pathway activity in highly proliferative cells. Inactivation of pentose phosphate pathway enzyme glucose 6-phosphate dehydrogenase in mouse embryonic stem cells resulted in differentiation into endodermal precursors, which may reflect a lack of nucleotides for continued proliferation [142].

Nucleotide synthesis requires not just the sugar but also the nucleotides themselves. Both pyrimidine and purine nucleotide synthesis have been found to be regulated by mTORC1 kinase. Pyrimidine nucleotide synthesis is initiated by the action of three different enzymes that are synthesized together in the trifunctional protein carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase (CAD) [14]. CAD is regulated by growth factor signaling, which results in a connection between proliferation and pyrimidine synthesis. In rat liver epithelial cells [143] and baby hamster kidney cells [144], MAPK signaling, which regulates cell proliferation, can activate CAD [143, 144]. In addition, in cultured mouse embryonic fibroblasts and human cancer cell lines, mTORC1 phosphorylates CAD via S6K1 and increases its activity, resulting in more pyrimidine nucleotides for ribosomal biogenesis and DNA replication [145, 146] under conditions when mTORC1 is active.

Purine metabolism requires contributions from nitrogens in glycine and methyl groups from the folate cycle [14]. Purine production is coordinated with nutrient status and mTORC1 signaling so that proliferating cells generate more purines in human and mouse cell lines [14, 147]. mTORC1 promotes purine synthesis in proliferating cells in part by regulating enzymes in serine biosynthesis and folate metabolism pathways [147].

Fatty acid metabolism

Proliferating, but not quiescent, cells require lipids for the plasma membrane and membranes surrounding organelles in newly synthesized cells. As a result, the expectation is that fatty acid metabolism will be quite different between proliferating and quiescent cells. In most cell types, many of the fatty acids required for membranes can be acquired from the circulation [14, 148]. As needed, carbons consumed as glucose can also contribute to fatty acid synthesis, and increased glucose uptake can produce fatty acids needed for biosynthesis [14]. When glucose enters the TCA cycle, it can be diverted as the metabolite citrate. Citrate can be transported to the cytoplasm, where ATP citrate lyase (ACL) can convert citrate into acetyl-CoA that can be used for fatty acid chain elongation [14]. Fatty acid synthase (FASN) then catalyzes the elongation of fatty acids.

Changes in fatty acid synthesis have been reported in proliferating versus quiescent non-stem cells. Citrate utilization varies dramatically in proliferating versus contact-inhibited, quiescent human dermal fibroblasts. While citrate is created at similar rates after ^{13}C-labeled glucose addition in proliferating and quiescent states in human fibroblasts, its fate is quite different [19]. In quiescent, contact-inhibited fibroblasts, labeled citrate is largely transferred to α-ketoglutarate, succinate and malate through the TCA cycle [19]. In contrast, in proliferating human fibroblasts, much of the labeled citrate is lost from the TCA cycle and the formation of α-ketoglutarate by isocitrate dehydrogenase is barely observed [19].

Similar findings were observed when human fibroblasts were fed ^{13}C-labeled glutamine rather than ^{13}C-labeled glucose [19]. The reason for the loss between citrate and α-ketoglutarate in proliferating, but not quiescent, human fibroblasts is not clear. One possibility is that the citrate lost from the TCA cycle in proliferating cells is channeled toward fatty acid synthesis, the condition where new membrane is required for the production of daughter cells.

Proliferating neural stem cells, like proliferating fibroblasts, require the build-up of lipids through de novo lipogenesis [149]. Conditional deletion of FASN in mouse neural stem cells impaired the formation of new neurons [149]. FASN deletion in the mouse resulted in a reduction in the number of mouse neural stem cells with Sox2 and GFAP expression, demonstrating that lipid metabolism is required for these cells [149]. In terms of a mechanism, Spot14 was found to be expressed in mouse neural stem cells with low levels of proliferation [149]. Spot14 reduces the availability of malonyl-CoA for lipogenesis and could explain the low levels of lipid synthesis in quiescent mouse neural stem cells [149]. Thus, lipid metabolism is a regulator of mouse neural stem cell proliferation [149]. The results are consistent with proliferating, differentiated cells and progenitor cells exhibiting increased lipid production, as expected given the requirements for increased fatty acids for membranes in cells that are dividing.

Fatty acid oxidation is the catabolism of fatty acid molecules to generate acetyl CoA. When fatty acid oxidation occurs in the mitochondria, the acetyl CoA produced can enter the TCA cycle, and NADH and $FADH_2$ produced can be used for the electron transport chain [39]. Changes in fatty acid degradation have been associated with the cell cycle. In cultured human lung fibroblasts and a rat myogenic cell line, inhibiting the synthesis of fatty acids or cholesterol results in a block in the G1 phase of the cell cycle with little effect on passage through G2/M phase [150]. Quantitative modeling of metabolic fluxes in human dermal fibroblasts revealed increased fatty acid breakdown in quiescent than proliferating fibroblasts [19].

Hematopoietic stem cells have been shown to functionally rely on fatty acid oxidation for maintaining stemness. Inhibiting fatty acid oxidation resulted in mouse hematopoietic stem cells leaving their quiescent state and dividing into two differentiated cells, rather than a stem cell and a differentiated cell [151]. Activating fatty acid oxidation in mouse stem hematopoietic stem cells, in contrast, resulted in more asymmetric divisions that create a stem cell and a progenitor cell [151].

Quiescent mouse neural stem cells rely on fatty acid oxidation [76]. Quiescent mouse neural stem cells have high levels of carnitine palmitoyltransferase 1a (Cpt1a)-dependent fatty acid oxidation, which is downregulated in proliferating neural progenitor cells [152]. In one study, cultured mouse neural stem cells died when deprived of fatty acid oxidation [152]. Pharmacological inhibition and conditional deletion of Cpt1a *in vitro* and *in vivo* show that Cpt1a-dependent fatty acid oxidation is required for stem cell maintenance and neurogenesis [152]. Manipulating the levels of malonyl-CoA, a metabolite generated by fatty acid oxidation, induced exit from quiescence and increased mouse neural stem and progenitor cell proliferation [152]. When treated with fatty acid oxidation inhibitor etomoxir, mouse

neural stem cells have decreased oxygen consumption, and reduced neural stem cell self-renewal [153]. Lineage tracing experiments showed that fatty acid oxidation prevents mouse neural stem cells from committing to two differentiated progenitors upon cell division [76] and promotes asymmetric cell divisions that replace the dividing stem cell. In this study, surprisingly, the authors found that mouse neural stem cells do not rely on glucose for oxygen consumption [76]. They note that the fetal brain oxidizes primarily free fatty acids and hypothesized that stem cells in the adult brain would share this phenotype [76]. The authors concluded that undifferentiated mouse neural stem cells rely on free fatty acids for proliferation, while their differentiated progeny are able to metabolize glucose or fatty acids depending on availability [76].

Fatty acid oxidation is important for the long-term viability of intestinal stem cells [154]. Inactivation of Cpt1a, the rate limiting enzyme for fatty acid oxidation, from mouse intestinal stem cells reduced regeneration in crypts after radiation and abrogated the pro-regenerative effects of fasting [154]. Fatty acid oxidation is also important for mouse skeletal muscle stem cells. Indeed, some studies have concluded that during their exit from quiescence, mouse muscle stem cells deactivate fatty acid oxidation in favor of glucose catabolism [39, 82]. Further, fatty acid oxidation is required for mouse muscle stem cells to maintain a quiescent state [39]. Metabolic inhibition of fatty acid oxidation in peroxisomes, but not mitochondria, resulted in loss of quiescent and premature differentiation of cultured mouse satellite muscle cells [155]. Thus, fatty acid oxidation is high in quiescent differentiated and stem cells, and is important for the viability of quiescent mouse cells and for maintenance of mouse stem cells in multiple tissues.

Taken together, the existing data support a model in which the metabolism of adult stem cells resembles that of other quiescent cells in multiple ways, such as reduced glutamine consumption and nucleotide and lipid biosynthesis, and increased ROS detoxification and fatty acid oxidation. But the increased reliance on glycolysis in many adult stem cells is not typical of quiescent cells. The findings raise the question why adult stem cells utilize glycolysis.

Histone modifications in proliferation versus quiescence

One possible explanation for the high levels of glycolysis in adult stem cells might be that this specific metabolic state facilitates a chromatin state needed for quiescent cells with stem cell properties. Histone marks that are characteristic of stem cells have been identified. Mouse embryonic stem cells contain “bivalent” domains that consist of large regions of histone H3 lysine 27 methylation with smaller regions of histone H3 lysine 4 methylation embedded within them [156]. Overlap of activating and repressive marks is unusual and is considered likely to reflect the “poised” state of stem cells. Because metabolites are required to generate histone modifications, there is a close relationship between metabolism and epigenetic modifications to histones.

This connection between metabolite levels and epigenetic changes was supported by studies of metabolic enzymes during development. One recent study focused on proliferating pluripotent cells at the two-cell stage of mouse development during zygotic genome

activation, a time during development when pyruvate is essential for continued development [157]. The authors found that some, but not all, of the enzymes that constitute the TCA cycle normally localized to mitochondria in adult cells are partially localized within the nucleus [157]. In human embryos as well, they discovered that the enzyme pyruvate dehydrogenase is transiently localized to the nucleus at the time of human embryonic genome activation [157]. These TCA enzymes can provide acetyl-CoA and α-ketoglutarate that can modify epigenetic marks in the nucleus, contribute to zygotic activation and allow continued embryo development [157]. The findings reinforce the close association between metabolic intermediates and epigenetic modifications, suggesting a partial TCA cycle may actually occur within the nucleus at the very beginning of an organism's life.

A close tie between metabolism and epigenetics is further supported by observations that in proliferating human oocytes [158], mouse ESCs [159] and human ESCs [48], mitochondria exhibit a perinuclear localization, which is also seen in cultured human cancer cell lines [160, 161]. Three to seven days after initiation of mouse embryonic differentiation, mitochondria move from the perinuclear space to a more dispersed localization [159]. Reprogramming mouse embryonic fibroblasts to a proliferative, stem cell-like state is associated with movement of mitochondria to the region around the nucleus [48]. The proximity of mitochondria to nuclei in proliferative conditions supports a model in which the availability of metabolites is important for epigenetic modifications.

Cell cycle entry has been associated with changes in epigenetics and histone marks. Acetylation of lysines on histone tails can provide binding sites for proteins containing bromodomains, and neutralize histone positive charges that would result in a more "open" chromatin conformation [162–164]. This open state may help to maintain the pluripotent epigenetic state and self-renewal of mouse and human pluripotent stem cells [165–167]. Consistent with this model, human and mouse pluripotent stem cells produce cytosolic acetyl-CoA through glycolysis and pyruvate-derived citrate flux via ACL [168, 169]. The acetyl-CoA produced can be used to acetylate histone tails. Upon differentiation, this pathway is reduced in human and mouse embryonic stem cells [168]. Pharmacological inhibition of cytosolic acetyl-CoA production revealed that this pathway is required to inhibit differentiation. Further, chemical inhibition of histone decacetylases promotes reprogramming of fibroblasts into pluripotent stem cells in mouse [170] and human models [171].

The availability of acetyl CoA can affect not only differentiation, but also the cell cycle through its role in acetylation of histones [35]. Mitogenic stimulation results in an increase in histone acetylation as mouse fibroblasts exit quiescence and progress through S phase [172]. In immortalized mouse embryonic fibroblasts, acetylation of histone H3 increased as the cells transitioned from quiescence to proliferation after serum stimulation [173]. These acetylation changes require the fatty acid enzyme ATP citrate lyase and extracellular glucose [173]. Supplementing with fatty acids rather than glucose, which generates mitochondrial, but not nucleocytoplasmic, acetyl CoA, did not rescue histone acetylation upon serum stimulation [173]. A similar pattern was observed in apoptosis-deficient mouse hematopoietic cells; growth factor withdrawal resulted in low levels of histone acetylation, and the levels rose when growth factor was added back [173].

Reduced histone acetylation rates in quiescent cells may reflect their altered metabolism. For five different modifications, the incorporation rate of histone acetylations into quiescent human dermal fibroblasts was measured as approximately half of that for proliferating fibroblasts [174]. Once the cells had achieved steady state, a similar steady state level of acetylation was observed in proliferating and quiescent fibroblasts [174, 175].

Changes in histone acetylation have also been observed as quiescent adult stem cells enter the cell cycle. In one study, quiescent mouse muscle stem cells were found to deactivate fatty acid oxidation and activate glucose catabolism as they are activated to proliferate and differentiate [39, 82]. This change in metabolism was found to reduce NAD+ and deactivate the NAD+-dependent histone deacetylase SIRT1 [39, 82]. The resulting decrease in acetylation of histone H4K16 [39, 82] led to activation of myogenic transcription programs and muscle differentiation [39, 82]. In this study, activation of glycolysis was observed as the mouse muscle cells differentiated. Based on these studies, one possibility is that the use of glycolysis in some adult stem cells could protect against an increase in histone acetylation that would induce them to enter the cell cycle or activate lineage-specific genes.

In addition to changes in histone lysine acetylation, changes in histone lysine methylation can also contribute to epigenetic marks. Oxidative phosphorylation in naïve mouse pluripotent stem cells can generate NAD+, and therefore promote TCA cycle activity and α-ketoglutarate production [39]. Consistent with this model, adding α-KG can promote pluripotency of mouse embryonic stem cells, while adding succinate, a competitive inhibitor of the α-KG/Fe^{2+}-dependent dioxygenases, supports differentiation [137]. The α-ketoglutarate pools will be used by Jumonji domain-containing histone demethylases and ten-eleven translocation (TET) methyl cytosine dioxygenases [39]. These α-KG-dependent enzymes remove methylations on histones and chromatin, which may promote pluripotency [39]. Metabolic changes that increase α-KG were also found to reduce the levels of repressive chromatin modifications and promote naïve pluripotency by maintaining highly active and open chromatin in embryonic mouse stem cells [137]. Methylation marks at H3K9me3, H3K27me3, H4K20me3 and DNA were removed in response to changes in nutrient levels [137]. Once the mouse stem cells are primed, in contrast, α-KG promotes stem cell differentiation, also by regulating histone demethylation [39, 176]. The need to protect primed pluripotent mouse stem cells from α-KG to prevent them from differentiating has been suggested as a contributor to the use of oxidative phosphorylation in naïve mouse pluripotent stem cells and differentiating pluripotent mouse stem cells, while primed mouse pluripotent stem cells use glycolysis [39]. It is possible that some adult stem cells use glycolysis and convert pyruvate to lactate to protect themselves from α-KG produced by the TCA cycle that could promote their differentiation because they resemble primed rather than naïve pluripotent stem cells.

Summary: the paradox of metabolism in quiescent stem cells

While the literature has provided substantial insights into the role of metabolism in stem cells, there remain important areas for additional studies. One major technical limitation is our ability to monitor metabolic flux in stem cells as the flow cytometry-based methods used to isolate stem cells from non-stem cells take time and metabolite levels may be altered.

Another important issue is that many studies that knock out a particular gene demonstrate an impact on the generation of progeny from stem cells, but it remains unclear whether the quiescent stem cells lacked viability in the absence of the gene of interest, or whether the quiescent stem cells were unable to enter the cell cycle without the gene of interest. Further, while many studies have identified an important role for a metabolic factor in stem cell viability or proliferation, the downstream effectors are not identified. This has made it more difficult to understand why some metabolic pathways are appear to behave differently in stem versus non-stem cells.

Additional studies will be valuable for us to gain a deeper understanding of the role of metabolism in supporting both quiescent and proliferating states, and the transitions between them. Experiments that carefully characterize the importance of specific metabolic enzymes and pathways in stem and non-stem models will be necessary. The most valuable studies will provide information comparing the functional role of metabolic pathways in stem and non-stem cells in proliferating and quiescent states. Greater insight will be gained from research that establishes the effects of knockout or metabolic flux manipulation on the molecular events downstream, for instance epigenetic marks or changes in the redox state of specific proteins. Of particular interest will be new approaches that allow an analysis of the metabolism of stem cells and its functions for stem cells within their *in vivo* niche [177].

Taken together, the data reported here suggest that the metabolism of proliferating and quiescent cells is designed to address their unique requirements. Cells that are actively dividing may require a boost of energy and metabolites, and increased glycolysis and glutaminolysis can be employed to meet this need. Increased lipid and nucleotide biosynthesis are frequently observed in proliferating cells, consistent with an increased need for DNA and RNA in dividing cells. Metabolic profiles can also be altered to provide for increased antioxidant defense to protect from the destructive or pro-proliferative effects or reactive oxygen species. While quiescent adult stem cells have high levels of fatty acid oxidation and antioxidant defenses, and low levels of glutamine consumption, nucleic acid and fatty acid synthesis, as expected for cells that are not dividing, their reliance on aerobic glycolysis in many, but not all studies, suggests that there may be an important but unexplained role for glycolysis in these cells.

Several specific hypotheses to explain the high levels of aerobic glycolysis in stem cells are raised by the studies described here. One hypothesis is that aerobic glycolysis is used by adult stem cells in order to protect them from epigenetic marks that induce proliferation or lineage commitment. To gain greater insight into this possibility, the effects of metabolic perturbations on histone marks in proliferating and quiescent stem and non-stem cells could be determined. These studies could define whether metabolic pathways are important for maintaining an epigenetic and transcriptional pattern associated with stem cells.

A second hypothesis would be that adult stem cells exhibit high levels of glycolysis because the embryonic stem cells from which they are derived exhibit high levels of glycolysis [84]. This would be similar to the argument put forth to explain the high reliance on fatty acids in adult neural stem cells [76]. In this situation, the hypothesis was that because early embryonic stem cells of the brain required high levels of fatty acids, that neural stem cells of

the adult might retain this fetal metabolic signature [76]. A testable prediction from this model would be that forcing adult stem cells to engage in aerobic glycolysis would not affect their viability, differentiation, self-renewal, proliferation or exhaustion.

A related hypothesis would be there are differences in the signaling pathways that induce differentiated cells versus adult stem cells to proliferate. This possibility could be tested by developing models in which stem and non-stem quiescent cells receive input from the same signaling pathway and the metabolic impacts are compared. While such a hypothesis is possible, based on the literature, the *in vivo* pathways that regulate quiescence and proliferation in stem and non-stem cells appear to overlap. Tyrosine kinase growth factor receptors are important triggers for cell division in differentiated cells and in mouse muscle satellite stem cells [14, 60, 178]. Quiescent muscle stem cells express Sprouty1, a protein that binds to and inhibits the activity of tyrosine kinase receptors [179]. Spry1 levels decline when mouse muscle satellite cells are dividing and deleting Spry1 results in mouse muscle stem cells that do no re-enter quiescence after injury [179]. The MAP Kinase pathway important for regulation of cell division in differentiated cells have also been implicated in mouse muscle satellite stem cell quiescence [56]. In mouse muscle stem cells, MAPK p38α/β activation leads to precocious myoblast differentiation [180, 181]. Similarly, the biosynthesis-inducing PI3K-mTORC1 pathway is also activated as both differentiated cells and adult stem cells from mice are induced to divide [14, 81, 182]. Finally, the notch signaling pathway is important for both promoting quiescence in human fibroblasts [13, 183], and in maintaining quiescence and protecting against differentiation in adult mouse hematopoietic and muscle stem cells [13, 56, 184–190]. Nevertheless, if forcing adult stem cells to alter their metabolic profile resulted in no functional phenotype, then the possibility that their metabolic state reflects different signaling pathways could be considered.

An alternative hypothesis would be that the high levels of glycolysis in the quiescent adult stem cells allows them to be prepared for the metabolic demands of cell cycle re-entry. Indeed, glycolysis would be able to supply ATP rapidly if the cells were induced to proliferate. Models that would allow the stem cells to gain ATP rapidly when called upon to proliferate, but shift the stem cells toward aerobic glycolysis while quiescent, could be used to test this model. If this hypothesis is correct, then simply providing sufficient ATP through any means at the time that stem cells enter the cell cycle would be functionally equivalent to anaerobic glycolysis.

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Table 1:

Summary of Selected References on Metabolism and Cell Cycle/Pluripotency

Topic	Pathway	Model	Finding	Reference
Metabolism and Cell Cycle	Metabolism	Yeast	A metabolism cycle is linked to the cell cycle	[1]
Glycolysis	Glucose uptake	Mouse fibroblasts	Increased glucose uptake in proliferating state	[2]
Glycolysis	Glucose uptake	Mouse lymphocytes	Increased glucose uptake upon activation	[3, 4]
Glycolysis	Glucose uptake	Human fibroblasts	Modest decline in glycolysis with contact inhibition	[3–5]
Glycolysis	Glycolysis	Mouse lymphocytes	Glycolysis promotes survival in the absence of growth factors	[6]
Glycolysis	Glycolysis	Mouse lymphocytes	Receptor tyrosine kinase signaling activates PI3K-AKT that promotes GLUT1 expression and localization	[7, 8]
Glycolysis	Glycolysis	Human endothelial cells	Silencing glycolysis regulator PFKFB3 impairs angiogenesis	[9]
Glycolysis	Glycolysis	Human transformed and untransformed cell lines	A decrease in APC/Cdh1 activates both proliferation and glycolysis	[10]
Glycolysis	Glycolysis	Rat brain neurons and astrocytes	Astrocytes have low APC/C-Cdh1 activity and consequently high PFKFB3 compared to neurons	[11]
Glycolysis	Glycolysis	Cultured mouse myoblasts and human fibroblasts	E2F1 regulates pyruvate dehydrogenase kinase 4 levels	[12]
Glycolysis	Glycolysis	Human T acute lymphoblastic leukemia cells	Cyclin D3/CDK6 phosphorylates PKM2	[13]
Checkpoints	Metabolic checkpoint	Mouse embryonic fibroblasts	AMP-activated protein kinase induces a p53-dependent metabolic checkpoint	[14]
Checkpoints	Metabolic checkpoint	Pancreatic β cells	PI3K and AKT activate cyclin D2 via forkhead box transcription factors	[15]
Glycolysis	Glycolysis and stemness	Human and mouse embryonic stem cells	Glycolysis promoted reprogramming	[16]
Glycolysis	Glycolysis and stemness	Induced mouse embryonic stem cells	Mouse epiblast stem cells and human embryonic stem cells are highly glycolytic	[17]
Glycolysis	Glycolysis and stemness	Mouse hematopoietic stem cells	Mouse hematopoietic stem cells rely on glycolysis	[18]
Glycolysis	Glycolysis and stemness	Mouse hematopoietic stem cells	Loss of pyruvate dehydrogenase kinase reduces quiescence, glycolysis and transplantation ability	[19]
Glycolysis	Glycolysis and stemness	Neural stem cells from reprogramming of human fibroblasts	Aerobic glycolysis in neural progenitor cells transitions to oxidative phosphorylation in neurons	[20]
Glycolysis	Glycolysis and stemness	Mouse muscle stem cells	mTORC1 is sufficient for induction into GAlert	[21]
Glycolysis	Glycolysis and stemness	Human mesenchymal stem cells	Upregulation of aerobic mitochondrial metabolism with differentiation to osteoblasts	[22]
Glycolysis	Glycolysis and stemness	Mouse hair follicle stem cells	Lactate dehydrogenase-deficient hair follicle stem cells cannot initiate a new cycle	[23]
Glycolysis	Glycolysis and stemness	Mouse intestinal stem cells	Stem cells use oxidative phosphorylation while differentiated Paneth cells use glycolysis	[24]
Glycolysis	Glycolysis and stemness	Rat male germ stem cells	Male germ stem cells rely on lactate provided by Sertoli cells for energy	[25, 26]

Topic	Pathway	Model	Finding	Reference
Oxygen metabolism	Oxygen and stemness	Mouse hematopoietic stem cells	Cells maintained in hypoxic conditions had higher marrow repopulation efficiency	[27]
Oxygen metabolism	Oxygen and stemness	Mouse hematopoietic stem cells	Loss of SIRT2 results in mitochondrial activation and pyroptosis	[28]
Oxygen metabolism	Mitochondrial unfolded protein response	Mouse hematopoietic stem cells	The mitochondrial unfolded protein response is required as cells differentiate	[29]
Oxygen metabolism	Mitochondrial unfolded protein response	Mouse intestinal stem cells	Activating the mitochondrial unfolded protein response resulted in proliferation and loss of stemness	[30]
Oxygen metabolism	mTOR pathway	Mouse hematopoietic stem cells	mTOR activation drives mitochondrial biogenesis and proliferation	[31]
Oxygen metabolism	FoxO	Mouse hematopoietic stem cells	FoxO inactivation results in proliferation and differentiation	[32]
Oxygen metabolism	FoxO	Mouse neural stem cells	FoxO inactivation results in proliferation and differentiation	[32]
Amino Acid Metabolism	Glutamine metabolism	Human mammary epithelial cells	Glutamine catabolism uses different pathways in proliferating versus quiescent cells	[33]
Amino Acid Metabolism	Glutamine metabolism	Mouse skeletal stem cells	Glutamine metabolism regulates differentiation and bone formation	[34]
Nucleotide Metabolism	Pentose phosphate pathway	Rat neurons	High glucose 6-phosphate for pentose phosphate pathway activity for redox homeostasis	[11]
Nucleotide Metabolism	Pentose phosphate pathway	Mouse embryonic stem cells	Inactivating the pentose phosphate pathway results in endodermal differentiation	[35]
Nucleotide Metabolism	Pyrimidine metabolism	Mouse embryonic fibroblasts and human cancer	Pyrimidine metabolism is activated by mTOR	[36]
Nucleotide Metabolism	Purine metabolism	Mouse embryonic fibroblasts and human cancer	Purine metabolism is activated by mTOR	[37]
Fatty acid metabolism	Fatty acid synthesis	Mouse neural stem cells	FASN deletion in the mouse reduces neurogenesis	[38]
Fatty acid metabolism	Fatty acid degradation	Human fibroblasts and rat myogenic cell line	Inhibiting fatty acid or cholesterol synthesis results in a G1 arrest	[39]
Fatty acid metabolism	Fatty acid degradation	Mouse hematopoietic stem cells	Inhibiting fatty acid oxidation resulted in loss of quiescence and commitment to differentiated cells	[40]
Fatty acid metabolism	Fatty acid degradation	Mouse neural stem cells	Inhibiting fatty acid oxidation resulted in loss of quiescence and commitment to differentiated cells	[41, 42]
Fatty acid metabolism	Fatty acid degradation	Mouse muscle cells	Inhibiting fatty acid oxidation resulted in loss of quiescence and differentiation	[43, 44]
Epigenetic marks	Metabolites and epigenetics	Mouse two-cell developmental stage	TCA enzymes are in the nucleus	[45]
Epigenetic marks	Metabolites and epigenetics	Human oocytes	Mitochondria are found around the nucleus.	[46]
Epigenetic marks	Histone acetylation and pluripotency	Human and mouse embryonic stem cells	Histone acetylation delays differentiation	[47]
Epigenetic marks	Histone acetylation and pluripotency	Human and mouse embryonic stem cells	Inhibiting histone deaceytylases promotes reprogramming to stem cells	[48, 49]
Epigenetic marks	Histone acetylation and cell cycle	Mouse embryonic fibroblasts	Histone acetylation when induced to proliferate requires glucose-derived acetate	[50]
Epigenetic marks	Histone acetylation and differentiation	Mouse muscle stem cells	Histone acetylation prevented muscle stem cell differentiation	[44]

Topic	Pathway	Model	Finding	Reference
Epigenetic marks	Histone methylation and pluripotency	Mouse embryonic stem cells	Alpha-ketoglutarate promotes self-renewal, succinate promotes differentiation	[51]

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