Cell Stem Cell
Review

Metabolic Reprogramming of Stem Cell Epigenetics

James G. Ryall,[1,*] Tim Cliff,[2] Stephen Dalton,[2] and Vittorio Sartorelli[3,*]
[1]Stem Cell Metabolism and Regenerative Medicine Group, Basic & Clinical Myology Laboratory, Department of Physiology, The University of Melbourne, Melbourne, VIC 3010, Australia
[2]Department of Biochemistry and Molecular Biology, Paul D. Coverdell Center for Biomedical and Health Sciences, The University of Georgia, Athens, GA 30602, USA
[3]Laboratory of Muscle Stem Cells and Gene Regulation, National Institute of Arthritis, and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, MD 20829, USA
*Correspondence: jgryall@gmail.com (J.G.R.), sartorev@mail.nih.gov (V.S.)
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For many years, stem cell metabolism was viewed as a byproduct of cell fate status rather than an active regulatory mechanism; however, there is now a growing appreciation that metabolic pathways influence epigenetic changes associated with lineage commitment, specification, and self-renewal. Here we review how metabolites generated during glycolytic and oxidative processes are utilized in enzymatic reactions leading to epigenetic modifications and transcriptional regulation. We discuss how "metabolic reprogramming" contributes to global epigenetic changes in the context of naive and primed pluripotent states, somatic reprogramming, and hematopoietic and skeletal muscle tissue stem cells, and we discuss the implications for regenerative medicine.

Introduction

Both successful organismal development and healthy tissue maintenance are reliant on the activity of stem cells. During development, the earliest totipotent stem cells rapidly give rise to the blastocyst, from which pluripotent embryonic stem cells (ESCs) arise. These ESCs in turn commit to specific somatic cell lineages to eventually differentiate and form the numerous tissues and organs of the body. Importantly, in many fully differentiated tissues of the adult, a subset of stem cells persists which has often lost the ability to differentiate into more than just a select few cell types. In contrast to the highly proliferative state of ESCs, tissue-specific adult stem cells (ASCs) often exist in a quiescent state (a state termed G_0) and only re-enter the cell cycle to maintain tissue homeostasis or in response to tissue damage (Arai et al., 2004; Buczacki et al., 2013; Cheung and Rando, 2013; Pastrana et al., 2009; Tumbar et al., 2004).

An important role for metabolism in regulating stem cell biology derives from studies documenting the rapid and dynamic changes in substrate utilization observed during early embryogenesis (Leese, 2012). In the pre-implantation stage of mammalian development, cellular energy in the form of adenosine triphosphate (ATP) is generated primarily through the oxidation of carbon sources such as lactate, pyruvate, amino acids, and fatty acids, which allow the generation of reducing equivalents that drive the electron transport chain (ETC) and oxidative phosphorylation (Oxphos) (Brinster and Troike, 1979; Jansen et al., 2008; Martin and Leese, 1995). In contrast, implantation leads to a reduced oxygen availability and energy production becomes more dependent on anaerobic glycolysis. In this latter situation, the ETC and Oxphos become less important to satisfy energy needs (Houghton et al., 1996; Leese, 2012; Leese and Barton, 1984). Due to the changing environments experienced by stem cells as they progress from pluripotency through differentiation—including oxygen and substrate (carbohydrates, fatty acids, and amino acids) availability—it is perhaps not surprising that the metabolism of ESCs differs quite considerably from that of differentiated tissues. Similarly, ASCs often exist in specialized cellular locations termed "niches," which exhibit a broad array of oxygen and substrate availabilities, indicating that they too may differ in their metabolic state.

While the better part of the 20th century focused on the importance of cellular metabolism for the generation of energy, recent work has uncovered an essential role for metabolism in the generation of the building blocks (nucleotides, phospholipids, and amino acids) required by rapidly dividing cells (Lunt and Vander Heiden, 2011). Additionally, the metabolite balance of both stem and differentiated cells has been found to directly influence the epigenome through post-translational modifications of histones, DNA, and transcription factors (Carey et al., 2015; Moussaieff et al., 2015a; Ryall et al., 2015; Shiraki et al., 2014; Wellen et al., 2009). These findings indicate that cellular metabolism is not a passive player in the process of stem cell lineage commitment, but rather, they suggest that changes in metabolism regulate many of the important cell fate decisions made by stem cells. This role for metabolism in regulating cell fate has been termed "metabolic reprogramming" and represents a rapidly growing field of research.

The last decade has witnessed significant advances in our understanding of the transcriptional regulation of the pluripotent state in ESCs and the self-renewing capacity of tissue-specific ASCs. A better understanding of the link between metabolism and cell identity will likely lead to improvements in nuclear reprogramming (such as that used in the development of inducible pluripotent stem cells, iPSCs), transdifferentiation, and ex vivo expansion of stem cells for transplant therapies. In this Review, we aim to describe the current state of knowledge regarding stem cell metabolic reprogramming in ESCs, iPSCs, and two types of ASCs, hematopoietic stem cells (HSCs) and skeletal muscle stem cells (MuSCs, also termed satellite cells).

The Role of Metabolites in Epigenetic Regulation of Transcription

From the Greek word μεταβολή (*metabolē*), "to change," metabolism defines the series of reactions necessary for the

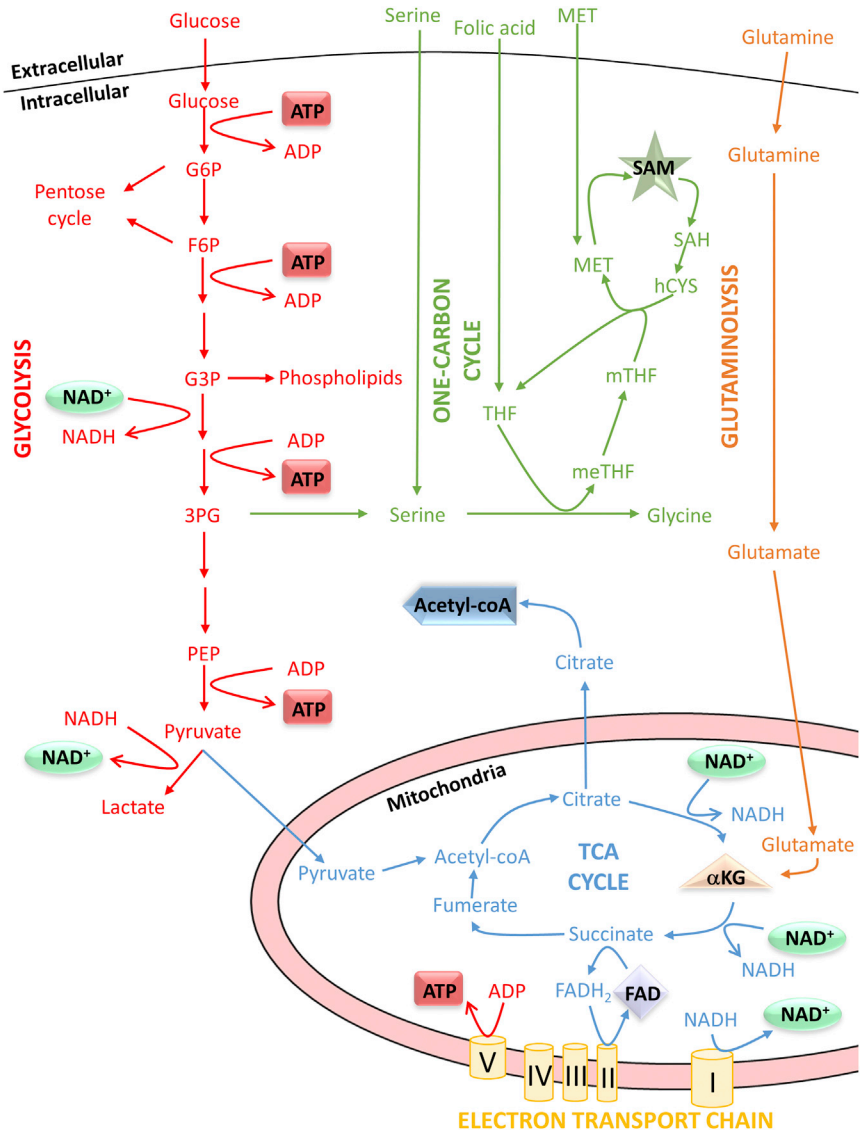

Figure 1. Cellular Metabolism and the Production of Metabolic Cofactors for Acetylation and Methylation Reactions

A schematic depicting the major metabolic pathways involved in the production of metabolites that act as cofactors for histone deacetylation and acetylation and histone/DNA demethylation and methylation. Note that several intermediate steps have been excluded for clarity.

generation of ATP required for life. Metabolism can be broadly categorized as either oxidative (completed in the mitochondria) or non-oxidative (completed in the cytoplasm), and it performs at least three essential functions in stem cells: (1) the generation of ATP necessary for energy-consuming processes; (2) the production of glycolytic intermediates essential for anabolic reactions during cell division; and (3) the release of metabolites employed in enzymatic reactions, including those involved in mediating epigenetic modifications (Figure 1).

One of the major sources of cellular ATP is the carbohydrate glucose. Glucose is primarily transported into the cell by a family of specialized glucose transporters (GLUT family proteins) (Mueckler and Thorens, 2013), where it undergoes a series of reductive reactions to produce pyruvate and two molecules of ATP (Figure 1) (Koopman et al., 2014; Lunt and Vander Heiden, 2011). In the presence of oxygen, pyruvate can be transported into the mitochondria by a monocarboxylate transporter (MCT) for Oxphos and production of a further 32–36 molecules of ATP (Lin et al., 1998). In the absence of oxygen, pyruvate is converted to lactate, which restores the reductive power of glycolysis by converting oxidized nicotinamide (NAM) adenine dinucleotide (NAD^+) to reduced NAM adenine dinucleotide (NADH). In addition to ATP, the catalytic reactions of glycolysis provide several intermediates that are essential for the production of new biomass, in the form of de novo nucleotides, phospholipids, and amino acids (Lunt and Vander Heiden, 2011).

Recent progress has led to a significant advance in our understanding of the transcriptional networks that regulate different stem cell states, while improvements in genome/transcriptome sequencing have greatly enhanced our comprehension of the role played by epigenetic regulators in this process. However, work over the last several years has identified an essential role for metabolites in the regulation of epigenetics and transcription, including S-adenosyl methionine (SAM) produced via the one-carbon cycle, acetyl-CoA from glycolysis, α-ketoglutarate (αKG) and flavin adenine dinucleotide (FAD) from the TCA cycle, and NAD^+ from the integration of glycolysis and Oxphos.

Glycolysis, Biomass Generation, and Protein Acetylation

In addition to its well-documented role in generating ATP, glycolysis has been found to serve several other essential roles that are of particular relevance to the current discussion, including the production of glycolytic intermediates necessary for the production of new biomass and metabolites required for epigenetic changes in transcription (Wellen et al., 2009).

The first evidence for an important role of glycolysis in cell proliferation was provided by the seminal work of Professor Otto Warburg (Warburg, 1956). Warburg found that highly proliferative tumor cells preferentially utilized the glycolytic pathway, even in the presence of saturating levels of oxygen. This process has since been termed "aerobic glycolysis" or the Warburg effect. Since Warburg's original studies, aerobic glycolysis has been found to be essential for the production of glycolytic intermediates, including glucose-6-phosphate (G6P), fructose-6-phosphate

(F6P), and glyceraldehyde-3-phosphate (G3P) for the generation of nucleotides (via the pentose phosphatase pathway, PPP). G3P has also been found to contribute to the production of new phospholipids, while 3-phosphoglycerate (3PG) is necessary for the generation of the non-essential amino acids serine and glycine (Figure 1). Lactate, previously considered to be a byproduct of glycolysis, has also been found to contribute to anabolic and ATP-producing processes (Sonveaux et al., 2008; Tohyama et al., 2013).

In addition to being able to convert to lactate, pyruvate can be transported into the mitochondria where it is converted to acetyl-CoA in a reaction catalyzed by pyruvate dehydrogenase (PDH). The activity of PDH is inhibited following phosphorylation via pyruvate dehydrogenase kinases (PDK1–4); thus, the glycolytic production of acetyl-CoA is regulated both by the availability of pyruvate and the activity of PDH and PDK enzymes (Takubo et al., 2013). While increased PDK activity leads to a decrease in the conversion of pyruvate to acetyl-CoA, the activity of PDK is itself regulated via the inhibitory actions of pyruvate and NAD^+, forming a negative feedback loop between pyruvate, PDH, and PDK (Sugden and Holness, 2003). Importantly, acetyl-CoA can contribute to several cellular processes including the initiation of the TCA cycle, the de novo synthesis of lipids, and acetylation of specific amino acid residues (predominantly lysine, K) on both histone and non-histone proteins (Figure 2A) (Koopman et al., 2014; Lunt and Vander Heiden, 2011).

Wellen and colleagues found that mammalian cells rely on ATP citrate lyase (ACL) to convert glucose-derived mitochondrial citrate to nuclear acetyl-CoA, which was then found to serve as the substrate for histone acetylation (Figure 2A). Interestingly, while fatty acid oxidation (FAO) is also known to contribute to the production of mitochondrial acetyl-CoA (and citrate), it does not contribute to histone acetylation in the absence of glucose (Wellen et al., 2009). Similar links between glycolysis and histone acetylation have been observed in ESCs (Moussaieff et al., 2015b), MuSCs (Ryall et al., 2015), tumor cells (Liu et al., 2015), and yeast (Friis et al., 2009).

The removal of the acetyl group from histone proteins, termed histone deacetylation, is regulated by a family of histone deacetylases (HDACs). HDACs are classified into one of four major classes (I–IV). Class I, II, and IV include HDAC1–11 and are Zn^{2+} dependent. These proteins remove the acetyl group from histones and convert it to acetate. In contrast, class III HDAC proteins are dependent on NAD^+ for their deacetylase activity and are known as Sirtuins (SIRT1–7). Sirtuins convert NAD^+ and the acetyl group to NAM and 2′-O-Acetyl-ADP-Ribose during the process of deacetylation (Figure 2A) (Cantó et al., 2015; Ryall, 2012). Due to their requirement for NAD^+, Sirtuins are often thought of as energy or redox sensors. Shifts in metabolism from aerobic to anaerobic metabolism (or vice-versa) have been linked to changes in the NAD^+/NADH ratio and Sirtuin activity. Specifically, increased glycolysis results in decreased NAD^+ availability and SIRT1-mediated deacetylation of its target histone, H4 lysine 16 (H4K16) (Ryall et al., 2015). However, due to the role of NAD^+/NADH in glycolysis, the TCA cycle, and Oxphos, there is likely a complex interplay between metabolism, Sirtuins, and histone and non-histone protein deacetylation (Dölle et al., 2013; Stein and Imai, 2012).

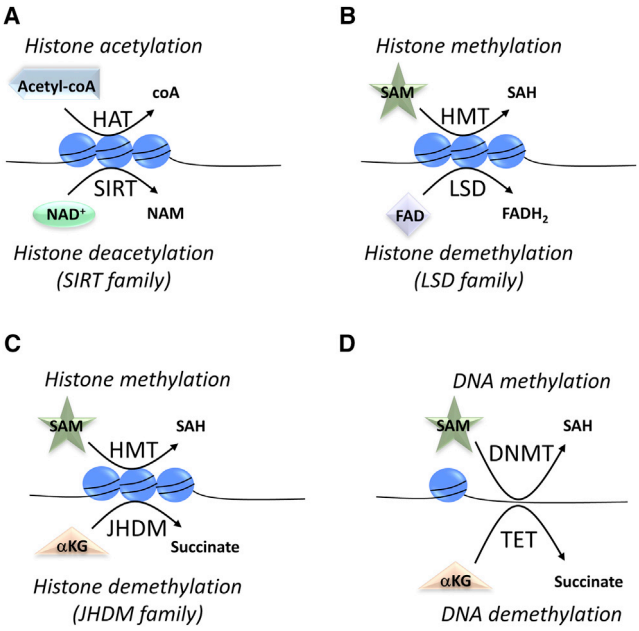

Figure 2. Metabolites as Essential Cofactors in the Epigenetic Regulation of Transcription

(A) Acetylation of histones is achieved via the actions of histone acetyltransferases (HATs), which attach an acetyl group to lysine residues in a reaction that releases co-enzyme A. In contrast, histone deactylation is regulated via HDAC proteins, including the Sirtuin family. Sirtuins are dependent on a readily available pool of NAD^+ for their deacetylase activity and produce NAM and 2'-O-Acetyl-ADP-Ribose.

(B–D) Methylation of either histone proteins or DNA is achieved via the attachment of a methyl group (from SAM) to either lysine or arginine residues in a reaction mediated via HMTs (B and C) or DNMT (D). Histone demethylation can occur via the actions of (B) the LSD family of demethylases, which require FAD as a cofactor, or (C) the JHDM family, which require αKG as a cofactor. The TET family of DNA demethylases similarly require αKG for their activity (D).

The One-Carbon Cycle and DNA/Histone Methylation

One-carbon metabolism describes a series of complex cyclical reactions involving the transfer of a single carbon unit, and it includes three pathways: the folate cycle, the methionine (MET) cycle, and the *trans*-sulphuration cycle (Figure 1) (Locasale, 2013). The combination of these cycles generates carbon units essential for the production of phospholipids and nucleotides during cell proliferation (Labuschagne et al., 2014), and it may also regulate cellular redox status through both the oxidation of NADPH and the generation of glutathione (via the *trans*-sulphuration pathway).

The one-carbon cycle begins when tetrahydrofolate (THF, a reduced form of folate) is converted to 5,10-methylene THF (meTHF) and then reduced to 5-methyl THF (mTHF), which can then in turn donate a carbon atom to homocysteine (hCYS) to generate MET. The subsequent adenylation of MET leads to the production of SAM, which functions as the methyl donor for histone and DNA methylation reactions (Locasale, 2013). While the most well-studied carbon donor is serine, both glycine and threonine-derived glycine have also been found to donate a carbon atom to initiate the cycle (Shyh-Chang et al., 2013a; Zhang et al., 2012).

The processes of histone and DNA methylation are carefully regulated through the targeted actions of histone

methyltransferases (HMTs) and DNA methyltransferases (DNMTs), respectively. In each case, a methyl group is transferred from SAM to a lysine or arginine amino acid, producing S-adenosyl homocysteine (SAH) as a byproduct. SAH can then be "recharged" with a methyl group via conversion to hCYS and re-entry into the MET cycle (Figure 1, Figures 2B–2D). Interestingly, acute changes in intracellular SAM can have direct effects on the level of histone methylation (particularly those associated with active gene transcription), suggesting a dynamic role for one-carbon metabolism in this process (Zee et al., 2010).

The mechanisms regulating the transport of metabolites (including SAM) between intracellular compartments have yet to be fully elucidated; however, several groups have found that the nucleus contains key metabolic enzymes, including those required for the conversion of MET to SAM. In a study by Katoh et al. (2011), the SAM-generating enzyme MET adenosyltransferase II (MATIIα) was found to localize to the nucleus and serve as a transcriptional corepressor of the transcription factor MafK by interacting with the chromatin modifier complexes Swi/Snf and NuRD and supplying SAM for methyltransferases.

The TCA Cycle and DNA/Histone Demethylation

Typically, mitochondrial acetyl-CoA (from glycolytic or fatty-acid-derived acetyl-CoA) enters the TCA cycle where it undergoes a process of oxidative decarboxylation to produce large amounts of NADH and $FADH_2$ to drive the ETC and production of ATP (Figure 1). Several TCA and ETC intermediates are exported out of the mitochondria and contribute to amino-acid synthesis and epigenetic regulation of transcription via histone acetylation (citrate) and demethylation of both DNA (αKG) and histones (αKG and FAD).

The existence of histone demethylase proteins remained controversial until Shi et al. (2004) identified and characterized the actions of the protein lysine-specific demethylase 1 (LSD1, also known as KDM1a), and found that it specifically targeted mono- and di-methylated H3K4 and H3K9 (Shi et al., 2004, 2005). To catalyze the demethylation of its histone targets, LSD1 has been found to require FAD, which is reduced to $FADH_2$ as a result of this process (Figure 2B) (Forneris et al., 2005). Due to the close link of FAD to several metabolic processes, this requirement of FAD for histone demethylation is suggestive of a potential link to the metabolic status of the cell.

Intracellular FAD is produced from riboflavins (vitamin B2) and ATP and has been found in the cytoplasm, the mitochondria, and the nucleus (Giancaspero et al., 2013). FAD is reduced to $FADH_2$ during the conversion of acyl-CoA to 2-enoyl-CoA in FAO and by complex II (succinate dehydrogenase) in the mitochondria. The reverse reaction, oxidation of $FADH_2$, occurs in the ETC, leading to renewal of FAD and the release of two electrons. Therefore, LSD1 demethylase activity may be dependent on both de novo FAD synthesis from riboflavins and the relative activity of FAO, the TCA cycle, and the ETC. Interestingly, LSD1-mediated histone demethylation has been found to inhibit the transcription of many key metabolic enzymes and transcription factors, including *Ppargc1a* and *Pdk4* (Hino et al., 2012). These results suggest an intricate relationship between cellular metabolism, LSD1-mediated histone demethylation, and the transcription of metabolic regulators. Of particular relevance to the current discussion are recent findings indicating that LSD1 can promote demethylation of H3K4me1 at the enhancer region of Oct4/Nanog/Sox target genes during ESC differentiation (Whyte et al., 2012).

Following the initial discovery of LSD1-mediated histone demethylation, a second family of demethylases was identified, with each member found to contain a characteristic Jumonji C (JmjC) domain (Tsukada et al., 2006). The JmjC family of proteins can target a wide range of lysine and arginine residues for demethylation and, in contrast to LSD1, require αKG (and Fe^{2+}) to induce demethylation in an oxidative dicarboxylic reaction that converts αKG to succinate (Figure 2C) (Tsukada et al., 2006). The TET family of DNA demethylases similarly requires αKG (and Fe^{2+}) for demethylase activity (Figure 2D) (Tahiliani et al., 2009; Yang et al., 2014). αKG is a key component of the mitochondrial TCA cycle. It is produced from D-isocitrate in a reaction catalyzed by isocitrate dehydrogenase 2 (Idh2). In order to be utilized by either JmjC or TET demethylation reactions, αKG is first exported out of the mitochondria, where it can also be converted to citrate (for the production of acetyl-CoA) or one of several non-essential amino acids. Intracellular αKG levels are maintained through a series of anaplerotic reactions, most notably the breakdown of glutamine (Lunt and Vander Heiden, 2011).

Together, the above discussion strongly links metabolism and intermediate metabolites to the processes of DNA and histone methylation and demethylation and histone acetylation and deacetylation. The remainder of this discussion will focus on how metabolism changes in stem cells and how this may deregulate the global epigenetic signature of these cells during lineage commitment and specification.

Metabolic Reprogramming in Pluripotent Stem Cells

Pluripotency is not an exact cell state but instead relates more to the ability of a cell to generate the three embryonic germ layers during development. Two broad classes of pluripotent stem cells (PSCs) have been isolated and cultured from embryonic sources: a "naive" or "ground-state" and a "primed" state where PSCs are poised to differentiate. In both cases, these cells have been found to have a reduced mitochondrial content and a lower inner mitochondrial membrane potential than their differentiated counterparts (Prigione and Adjaye, 2010; Van Blerkom, 2009) (Figure 3A). Interestingly, naive and primed PSC states from human and murine origins exhibit a significantly different metabolic signature. Human embryonic stem cells (hESCs) are frequently referred to as being in the primed state and closely resemble pluripotent cells of the early post-implantation/epiblast stage embryo (Nichols and Smith, 2009). Soon after hESCs were derived, a similar primed PSC was isolated and characterized from early-post-implantation mouse embryos, with these cells commonly being referred to as "epiblast-like stem cells" (EpiSCs) (Brons et al., 2007; Tesar et al., 2007). Primed PSCs are rather unusual in that they generate most of their ATP from glycolysis rather than the more familiar mode of energy generation through Oxphos as observed in naive PSCs (Figure 3A) (Varum et al., 2011).

Primed PSCs Rely on Glycolysis

Elevated rates of extracellular acidification (ECAR, a measure of glycolysis) and lactate production and excretion are hallmarks of human and murine primed PSCs undergoing rapid glycolytic flux (Figure 3A) (Varum et al., 2011; Zhou et al., 2012). Even though

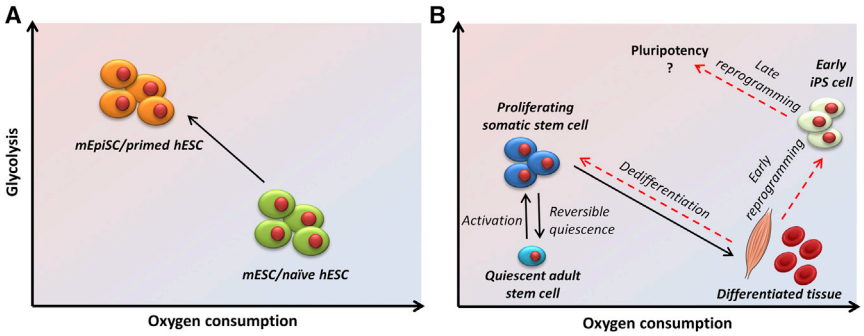

Figure 3. Stem Cell Metabolism during Lineage Commitment and Reprogramming
This diagram attempts to collate our current knowledge regarding metabolism and the processes of lineage commitment and reprogramming in (A) ESCs and (B) ASCs (using MuSCs as an example). The position of each cell type is correct relative to its nearest neighbor; however, the position of reprogrammed pluripotency is speculative.

energy production in primed cells is independent of Oxphos, oxygen is consumed and respiratory complexes in the mitochondria are fully active and serve to maintain membrane potential across the inner membrane (Zhang et al., 2011). Oxygen consumption, however, is uncoupled from ATP generation in this scenario presumably due to low expression of the ETC complex V subunit cytochrome C oxidase (COX) (Zhang et al., 2011). Differences in the expression of COX genes in mouse were confirmed by the comparison of transcript levels across the blastocyst-to-epiblast transition in vivo (Zhang et al., 2011), indicating that the metabolic switch described occurs in normal development. ATP is primarily generated from glycolysis to fuel the cell, with little contribution from the reducing equivalents generated by the TCA cycle. Other consequences of a glycolysis-based energy-generating system include an increase in flux through the PPP to drive ribose production for nucleotide generation, along with the synthesis of enzymatic cofactors coenzyme A and NAD^+/NADH (Figure 1). During the reprogramming of human cells to iPSCs, increased glycolytic activity occurs prior to the establishment of iPSCs, perhaps suggesting that altered metabolism is a prerequisite for establishment of primed pluripotency (Figure 3B) (Hansson et al., 2012; Kida et al., 2015; Panopoulos et al., 2012; Shyh-Chang and Daley, 2013). The utilization of glycolysis as a prime means to generate ATP is reminiscent of the Warburg effect, originally described in tumor cells.

The majority of mammalian cells (including primed PSCs) require glutamine for proliferation as a source of nitrogen and anaplerosis (the maintenance of TCA cycle intermediates). In primed PSCs the absence of glutamine has been found to lead to depleted αKG levels, which in turn reduces the activity of histone demethylases and results in increased H3K9, H3K27, H3K36, and H4K20 trimethylation and subsequent differentiation (Carey et al., 2015). Interestingly, results from the same study showed that naive PSCs could proliferate and maintain histone demethylation in the absence of glutamine through the utilization of glucose to maintain αKG levels (Figure 1) (Carey et al., 2015).

The most frequent argument to explain why primed PSCs use glycolysis and not Oxphos is that the early post-implantation-stage embryo is not vascularized by capillaries and grows at a rapid rate in a low-oxygen environment. Although this is a logical assertion, there are several aspects of this metabolic program that do not seem very efficient. For example, why dispose of large quantities of carbon skeleton in the form of lactate? Lactate secretion may provide some form of beneficial effect and may serve as a carbon source for surrounding cells that support development of the embryo. The rate at which glycolysis produces pyruvate may exceed the rate at which it can be further oxidized through the TCA cycle; the resulting excess can be eliminated from the cell by its conversion and secretion as lactate. Additionally, the conversation of pyruvate to lactate can recycle the NADH generated by GAPDH in glycolysis back to NAD^+, allowing the cell to modulate its NAD^+/NADH ratio and thus Sirtuin-mediated histone deacetylation. Production of lactate could therefore be an unnecessary consequence of elevated glycolytic flux. Uncoupling Oxphos from energy production also reduces the accumulation of reactive oxygen species (ROS) that have genotoxic effects. It is obviously beneficial to limit ROS levels in stem cell populations so as to maintain genomic integrity (Suda et al., 2011). Primed PSCs are now frequently cultured under reduced oxygen conditions (~2%) as this has been reported to suppresses spontaneous differentiation (Ezashi et al., 2005).

Naive PSCs Predominantly Utilize Oxphos

The naive PSC state represents an earlier stage of pluripotency than primed cells, and these cells more closely resemble PSCs of the inner cell mass (ICM). For the purpose of this discussion, murine ESCs (mESCs) cultured under traditional conditions with leukemia inhibitory factor (LIF) and fetal calf serum (FCS) will be considered alongside ground-state cells as they more closely resemble ICM-derived PSCs. Unlike primed PSCs, naive cells are less reliant on glycolysis for energy generation, release less lactate, and utilize Oxphos for energy generation (Figure 3A) (Takashima et al., 2014; Zhou et al., 2012). This metabolic switch coincides with increased COX gene activity and an overall reconfiguration of mitochondrial activity. In the preceding discussion, reasons were posed to explain why primed PSCs use primarily glycolysis for their energetic needs. Although the rationale used to argue that glycolysis-based metabolism is beneficial for primed PSCs is sound, it is unclear why the same factors do not seem necessary for naive cells where genomic integrity is equally important. Cell multiplication rates are comparable so biomass considerations are unlikely to be the explanation. So too is the argument that glycolysis supports anabolic metabolism. Reports that oxygen consumption in primed cells remains elevated is surprising as oxygen tension would be a potential cause. The rationale for why naive and primed PSCs utilize a remarkably different mode of metabolism is therefore unclear.

Dramatic changes in metabolic patterns emphasize the concept that metabolism is developmentally regulated and has

the potential to be highly dynamic during different stages of embryogenesis. It will be interesting then to determine how changes in metabolic pathways are regulated and to establish how they are hard-wired into developmental programs. One interesting possibility is that differing enzymatic isoforms, which utilize different reaction rates, substrate affinities, and modes of regulation, can alter metabolic modes and are regulated at the transcriptional level by developmental transcription factors that orchestrate cell fate decisions.

Shiraki and colleagues (2014) have reported that hPSCs consume MET at elevated levels relative to their differentiated counterparts. When MET is depleted from media, cells undergo increased rates of apoptosis and sporadic differentiation. Elevated MET fluxes increase levels of SAM that impact the epigenetic status of a cell (Figure 1). For example, SAM seems to be required for maintenance of normal H3K4 trimethylation and DNA methylation in hPSCs (Shiraki et al., 2014). MET deprivation is rescued by SAM supplementation, indicating that MET's critical role is in epigenetic regulation. Whether this is specifically part of a PSC program is unclear, however, as these pathways are clearly important in other cell types.

Similarly to hESCs, SAM appears critical for the maintenance of pluripotency in mESCs. However, in contrast to humans, mice have an active copy of the gene encoding threonine dehydrogenase (*Tdh*) and rely more heavily on threonine as the main source of SAM (Shyh-Chang and Daley, 2013). The availability of key amino acids therefore seems to be an important factor in determining the epigenetic state in PSCs. Other examples include L-proline, which destabilizes self-renewing mESCs and promotes differentiation (Washington et al., 2010). In this case, the L-proline-induced transition of mESCs to a mesenchymal state is linked to changes in H3K36me2 and H3K9me3 status (Comes et al., 2013). Moreover, ascorbate has been implicated as a cofactor in epigenetic regulation of mESCs by regulating JmjC histone demethylation enzymes (Wang et al., 2011; Young et al., 2015) and TET enzymes (Blaschke et al., 2013; Gao et al., 2013; Minor et al., 2013) that catalyze the conversion of 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC). Regulation of histone acetylation by cytoplasmic acetyl-CoA also seems to be an important determinant of the PSC state. High glycolytic rates drive this process and have recently been shown to be critical for stem cell maintenance (Moussaieff et al., 2015a). Consistent with this, glycolytic rates, acetyl-CoA levels, and global histone acetylation decline during early differentiation.

Modulation of intracellular O-GlcNAc levels also appears to be important in PSCs. O-GlcNAc is a carbohydrate modification primarily associated with post-translational modification of intracellular proteins on serine and threonine residues and is proposed to function as a nutrient sensor. O-GlcNAc impacts a wide range of targets that affect epigenetic status, including histones, RNA polymerase II, and histone deacetylases, and seems to be a key point of regulation that links nutritional status to gene regulation and cell identity (Hanover et al., 2012). One particularly interesting role of O-GlcNAc modification is its impact on DNA methylation in mESCs. Here, the TET1 and TET2 enzymes, which convert 5-mC to 5-hmC, are activated by O-GlcNAc modification in mESCs (Shi et al., 2013; Vella et al., 2013). In this context, O-GlcNacylation of TET1 would demethylate target genes, thereby contributing to the pluripotent state perhaps by regulating CpG islands. The key metabolic regulator and oncogene MYC is another target of O-GlcNAc transferase (OGT) (Chou et al., 1995). MYC is especially interesting in the context of stem cell biology because it is a well-established regulator of pluripotency and self-renewal (Cartwright et al., 2005; Smith et al., 2010). Other members of the core pluripotency network such as Oct4 and Sox2 are also OGT-modified in PSCs (Jang et al., 2012). Mutation of OCT4 that prevents it from being modified by OGT reduces the functionality of Oct4 including its ability to sustain pluripotency. Presumably, high glycolytic fluxes in PSCs maintain O-GlcNAc availability and support the pluripotent state by targeting critical epigenetic modifiers.

Metabolism, Pluripotency, and Reprogramming

Increasing evidence linking glycolytic metabolism and pluripotency raises a question about the role of metabolism during nuclear reprogramming. Small molecule activators of glycolysis and inhibitors of Oxphos have been shown to increase reprograming efficiencies with the Yamanaka factors; correspondingly, inhibition of glycolysis through small molecules greatly diminishes reprogramming abilities (Folmes et al., 2011; Prigione et al., 2014; Zhu et al., 2010). Research into the understanding of the minimal requirements for cellular reprograming has led to the identification of metabolic modulators as MYC targets. It has been shown that many MYC targets lie outside the canonical Oct4/Sox2/Nanog regulatory network (Cao et al., 2015; Chen et al., 2008; Kim et al., 2008; Ng and Surani, 2011). MYC, as with the other Yamanaka factors, has been shown to be replaceable in numerous reprograming protocols when these replacements are metabolic modulators (Chen et al., 2011; Li et al., 2005; Shyh-Chang and Daley, 2013). For example, inactivation of mTOR via rapamycin or other compounds induces glycolytic gene expression during reprograming in the absence of MYC (Chen et al., 2011; Zhu et al., 2010). Modulation of miRNAs, such as miR-290/371 and the lin28/let-7 pathway, which promotes glycolytic gene expression, can also be used to replace MYC as a reprograming factor (Cao et al., 2015; Shyh-Chang and Daley, 2013). Induced expression of key metabolic enzymes (*Ldha* and *Pkm2*) can also replace MYC as reprograming factors, while their repression blocks iPSC formation (Cao et al., 2015). Finally, upregulation of genes associated with a glycolytic-based metabolism occurs before expression of pluripotency genes during iPSC formation (Cao et al., 2015; Folmes et al., 2011; Prigione et al., 2014; Zhu et al., 2010).

Recently, Kida and colleagues have investigated the metabolic changes that occur during the establishment of pluripotency in iPSCs (Kida et al., 2015). In this study, the authors identified the estrogen-related nuclear receptor (ERRα and ERRγ), PGC1α, and PGC1β as key switches in the regulation of Oxphos during early iPSC reprogramming and demonstrated a significant increase in both oxygen consumption and extracellular acidification (an indirect measure of glycolysis) during the first 3–4 days of reprogramming in mouse embryonic fibroblasts (MEFs, Figure 3B). This "metabolic burst" was then followed by a significant decrease in oxygen consumption and a further increase in extracellular acidification such that successfully reprogrammed iPSCs had a 2-fold increase in extracellular acidification compared to the MEF cells from which they were

derived (Kida et al., 2015). Notably, these significant changes in cellular metabolism occurred during a period marked by minimal changes in gene expression (Polo et al., 2012), suggesting that metabolic reprogramming is a prerequisite for successful induction of pluripotency. Further evidence for a link between metabolism and reprogramming efficiency is provided by a recent study demonstrating that the oocyte-enriched factor Tcl1 improved iPSC reprogramming efficiency by suppressing the mitochondrial polynucleotide phosphorylase (PnPase). By preventing the mitochondrial localization of PnPases, Tcl1 inhibited mitochondrial biogenesis and oxygen consumption and elevated the ECAR. Interestingly, these changes were associated with an enrichment of several glycolytic intermediates, including fructose 1,6-biphosphate and phosphoenolpyruvate (Khaw et al., 2015).

Metabolic Reprogramming in ASCs

Many bodily tissues and organs have been found to contain their own population of stem cells, with examples including hematopoietic, neural, stomach (crypt), intestine, and MuSCs. In contrast to the pluripotent state exhibited by ESCs, many ASCs are tissue-specific and capable of asymmetric division leading to a committed daughter cell and a stem cell for self-renewal (Goulas et al., 2012; Kuang et al., 2007; Rocheteau et al., 2012; Troy et al., 2012; Zimdahl et al., 2014).

Tissue-specific stem cells have been found to vary dramatically in their turnover rates, with some populations undergoing constant cell division and others existing in a quiescent state outside of the cell cycle, only undergoing cell division in response to tissue damage. In addition, the local niche environment of ASCs can vary in substrate and oxygen availability and pH, among other parameters. There is currently a dearth of knowledge regarding the metabolic status of these tissue-specific stem cell populations and how the local metabolic milieu may act to regulate aspects of stem cell biology. Of particular interest is the metabolic status of those stem cell populations that exist in a quiescent state, such as adult HSCs and MuSCs. While an increased reliance on glycolysis appears common for most ASC populations during phases of proliferation, the metabolic status of quiescence is only just beginning to receive attention.

HSCs

HSCs are typically found in a quiescent state in bone marrow, a location originally believed to be highly anaerobic (Passegué et al., 2005; Simsek et al., 2010; Suda et al., 2011). Computational modeling based on data obtained by BrdU and histone H2B-GFP label-retaining assays suggests that a population of dormant mouse HSCs (d-HSCs) divides every 149–153 days and harbors the vast majority of multilineage, long-term self-renewing cells induced by hematopoietic stress. This population of HSCs is poised for rapid entry into the cell cycle in response to an emergency situation (e.g., significant blood loss) and gives rise to all hematological progenitors including red blood cells, granulocytes, platelets, T cells, and B cells. A second, active HSC (a-HSC) subset generates the progenitors and mature cells involved in the day-to-day maintenance of hematopoietic homeostasis and cycles every 28–36 days (Foudi et al., 2009; van der Wath et al., 2009; Wilson et al., 2008).

Recent studies have shown that quiescent HSCs specifically associate with rare pericytes ($NG2^+$ cells) and that activation of the HSC cell cycle alters the distribution of HSCs from $NG2^+$ periarteriolar niches to leptin-receptor-positive ($LEPR^+$) perisinusoidal niches. Moreover, depletion of $NG2^+$ cells induces HSC cycling and impairs long-term HSC repopulation in the bone marrow (Kunisaki et al., 2013). Despite bone marrow being a highly vascularized tissue, two-photon microscopy has revealed that the absolute local oxygen tension of the bone marrow is quite low (<32 mm Hg). Interestingly, the lowest oxygen tension (~9.9 mm Hg) is detected in deeper perisinusoidal regions whereas the endosteal region is less hypoxic (Spencer et al., 2014). It will be of interest to determine whether these low-oxygen-tension perisinusoidal regions correspond to the $LEPR^+$ perisinusoidal niches and whether decreased oxygen tension is sufficient to induce HSC activation.

The hypoxic niche hypothesis for the maintenance of quiescence in HSCs is, in part, supported by findings that the transcription factor hypoxia-inducible factor 1α (HIF1α) is stabilized within these cells (Takubo et al., 2010). HIF1α can form heterodimers with the constitutively expressed HIF1β and induce transcription of several genes, including key glycolytic enzymes. The depletion of HIF1α has been found to coax HSCs toward a more oxidative metabolism and leads to depletion of the HSC pool (Takubo et al., 2013). While the importance of HIF1α in the maintenance of HSCs is clear, its stabilization can occur through hypoxia independent mechanisms (Lu et al., 2002), so its activity alone cannot be used to define HSC metabolism. The vast majority of studies have proposed that quiescent HSCs rely on anaerobic glycolysis, a result supported by findings of elevated levels of PKM2 and PDK1–4. However, Nombela-Arrieta et al. (2013) have employed laser scanning cytometry to demonstrate that the bone marrow niche has an extensive vascular network, and Takubo et al. (2013) have used a Seahorse XF Bioanalyzer to demonstrate that quiescent HSCs still consume oxygen (~50% of that consumed by actively proliferating HSCs). These results suggest that despite the PDK-mediated inhibition of the conversion of pyruvate to acetyl-CoA, the TCA cycle and ETC remain active in quiescent HSCs. One possible explanation for these discrepant results is a reliance on FAO, which circumvents the need for glucose-derived acetyl-CoA. Clearly, the roles of vascularization and oxygen tension in the regulation of HSC biology remain a fertile and controversial area of investigation.

Long-term HSCs (LT-HSCs) contain fewer mitochondria than more committed progenitors, in terms of both mitotracker incorporation (Simsek et al., 2010) and morphology (Kim et al., 1998), and utilize glycolysis instead of Oxphos via a transcriptional regulatory network comprising Meis1 and HIF1α (Simsek et al., 2010). Takubo and colleagues have published a metabolic profile of glycolytic metabolism in HSCs and their progenitors, with the results suggesting an elevated level of several important metabolites in HSCs, including F1, 6BP, and pyruvate (Takubo et al., 2013). Interestingly, several TCA cycle intermediates were present at detectable levels in all cells examined, suggesting that while glycolysis may predominate in HSCs, the mitochondria remains active in these cells (Takubo et al., 2013).

Similarly to ESCs, the reliance of quiescent HSCs on glycolysis has been proposed as a method to minimize the buildup of damaging ROS (Suda et al., 2011). Utilizing conditional deletion strategies in mice, Wang and colleagues have found that PKM2 plays an important role in the inhibition of Oxphos activity and

HSC proliferation. In contrast, these authors also found that LDHA is essential for HSC self-renewal (Wang et al., 2014). As LDHA plays an important role in the regeneration of NAD^+ to allow glycolysis to proceed, it is possible that SIRT1 deacetylase activity is altered under these conditions. Indeed, SIRT1 activity has previously been linked to the maintenance of the HSC pool (Rimmelé et al., 2014). However, whether this is related to the changing metabolic state of HSCs awaits further investigation.

An additional mechanism important for the inhibition of Oxphos activity in quiescent HSCs is the elevated expression of PDK1–4. Takubo et al. have demonstrated that increased PDK activity is essential to prevent conversion of glucose-derived pyruvate to acetyl-CoA in the mitochondria and that PDK activity is directly linked to the self-renewal capacity of HSCs (Takubo et al., 2013). In this study, the authors isolated HSCs from $PDK2^{-/-}PDK4^{-/-}$ double knockout mice and found that they had a reduced capacity to successfully repopulate an irradiated recipient mouse. Furthermore, HSCs from $PDK2^{-/-}PDK4^{-/-}$ mice exhibited increased Oxphos activity and expression of the senescent marker p16 (Takubo et al., 2013). These results differ from those obtained in a more recent study where ESCs cultured in the presence of dichloroacetic acid (DCA, an inhibitor of PDKs) were better able to maintain pluripotency (Moussaieff et al., 2015a). Thus, the process of metabolic reprogramming is likely different in different populations of stem cells.

Ito et al. (2012) have identified an essential role for FAO in the maintenance of quiescence and self-renewal in HSCs. In this study the authors found that treatment of quiescent HSCs with the FAO inhibitor etomoxir led to an initial expansion of cell numbers (suggestive of an exit from quiescence), followed by an exhaustion of the HSC pool. These authors also found that targeted deletion of *Ppard* (a transcription factor intricately linked to the process of FAO) in HSCs led to precocious exit from quiescence (Ito et al., 2012). Therefore, it is essential that future studies investigate the role of FAO-derived acetyl-CoA and Oxphos in the maintenance of the HSC pool, particularly as acetyl-CoA has been linked to pluripotency in ESCs (Moussaieff et al., 2015a).

Many of the enzymes regulating DNA methylation and histone modifications described in the preceding paragraphs have important functions in HSC maintenance and differentiation (Cullen et al., 2014), thus suggesting an intertwined role of metabolism and epigenetics in HSCs biology.

MuSCs

MuSCs sustain early post-natal muscle growth and are responsible for the high regenerative capacity of skeletal muscle in response to trauma or injury and during the compensatory phases of muscle degeneration characteristic of several muscle dystrophies (Brack and Rando, 2012; Ryall, 2013; Yin et al., 2013). Like HSCs, MuSCs exist in a quiescent state; however, the rate of cellular turnover is significantly less in MuSCs, with a recent study estimating the average age of nuclei in human adult muscle to be >15 years (Spalding et al., 2005). Furthermore, while HSCs are poised for activation, the process of MuSC activation (measured as the time to first division) has been found to be upward of 30 hr (Rocheteau et al., 2012; Rodgers et al., 2014). Based on H2B-GFP intensity, at least two MuSC populations have been identified: MuSCs retaining (LRC) or losing (nonLRC) the H2B-GFP label. Both LRCs and nonLRCs are formed at birth and remain during post-natal growth and adult muscle repair. Adult LRCs give rise to both LRCs and nonLRCs, with the former self-renewing and the latter dedicated to differentiation (Chakkalakal et al., 2012, 2014). MuSC LRCs may be the equivalent of d-HSCs, whereas the behavior of MuSC nonLRCs is reminiscent of that of a-HSCs. Similar to HSCs, quiescent MuSCs have been found to closely colocalize with capillaries, suggestive of an aerobic niche (Christov et al., 2007; Ryall, 2013). Interestingly, skeletal muscles that exhibit greater capillarization (so called "oxidative" or "slow-twitch" muscles) have also been observed to contain a higher density of MuSCs (Christov et al., 2007). Thus, while both quiescent HSCs and MuSCs are located in close proximity of vasculature, the actual local oxygen tension to which these cells are exposed can change depending on their anatomical location (perisinusoidal versus endosteal regions for HSCs). The local oxygen tension of quiescent MuSCs remains to be determined.

By combining lineage-tracing models and fluorescence-activated cell sorting (FACS), several authors have identified a process of mitochondrial biogenesis during the shift from quiescence to proliferation of MuSCs (Rocheteau et al., 2012; Rodgers et al., 2014). Despite mitochondrial biogenesis having been observed to increase in activated MuSCs (Ryall et al., 2015; Tang and Rando, 2014), the rate of oxygen consumption does not seem to change between quiescent, early (3 hr in growth media), and late (20 hr in growth media) cultured activated MuSCs, with the rate of glycolysis increasing 3-fold within the first 3 hr of activation (Figure 3B) (Ryall et al., 2015). These results match the metabolic gene signature obtained via RNA sequencing and microarray, with an enrichment of FAO genes in quiescent MuSCs and glycolytic genes in activated MuSCs (Liu et al., 2013; Pallafacchina et al., 2010; Ryall et al., 2015). This increase in glycolysis is likely a result of the augmented demand for glycolytic intermediates essential for the generation of new biomass required by dividing cells (Lunt and Vander Heiden, 2011). The lack of increase in oxygen consumption in activated MuSCs, despite mitochondrial biogenesis, is reminiscent of that observed during the shift from the naive/ground-state of ESCs to the primed state of EpiSCs (Varum et al., 2011). Whether the mechanisms underlying this disconnect between mitochondrial density and oxygen consumption are similar between ESCs and MuSCs deserves further investigation.

The metabolic signature of quiescent MuSCs is linked to an elevated NAD^+/NADH ratio and high activity of the deacetylase Sirtuin SIRT1 (as inferred by low acetylation levels of the SIRT1 target histone H4 lysine 16, H4K16ac) (Ryall et al., 2015). In contrast, the increase in glycolysis observed in activated MuSCs was linked to a decrease in the NAD^+/NADH ratio and a reduction in SIRT1 deacetylase activity (10- to 20-fold increase in H4K16ac). Importantly, these changes in histone acetylation were linked to the transcription of several important myogenic regulatory genes (Ryall et al., 2015). These results provide an example of how metabolic modifications can be linked, via epigenetics, to changes in transcription and stem cell state.

Several groups have identified changes in both global and site-specific histone methylation as MuSCs leave quiescence and enter the cell cycle and again during differentiation (Juan et al., 2011; Liu et al., 2013). Liu and colleagues have previously used freshly isolated and cultured single muscle fibers to

demonstrate that quiescent MuSCs exhibit a high level of global H3K4me3. Further analysis of site-specific H3K4me3 enrichment using chromatin-immunoprecipitation sequencing (ChIP-seq) demonstrated that the number of genes marked by H3K4me3 did not change following MuSC activation (Liu et al., 2013). In contrast, both global and site-specific H3K27me3 have been found to be enriched following MuSC activation, likely a result of elevated levels of the HMT *Ezh2* and a reduction in the expression of the histone demthylase *Jmjd3* (Juan et al., 2011; Liu et al., 2013). What role the one-carbon cycle and FAD/αKG availability may play in these changes has yet to be investigated. However, it is interesting to note that several genes encoding key serine biosynthesis enzymes (*Phgdh*, *Psat1*, and *Psph*) are >10-fold elevated following MuSC activation, suggesting a possible link to histone/DNA methylation (see the supplemental RNA-seq and microarray data files in Liu et al., 2013; Ryall et al., 2015).

In addition to stem cells utilizing available substrates, recent work has suggested that MuSCs (and indeed other stem cell populations) may use protein degradation as a novel method to generate new substrates. The autophagic pathway, leading to protein degradation, is typically associated with the maintenance of homeostasis due to its role in removal of damaged or misfolded proteins (Das et al., 2012). However, autophagy has recently been found to play an important role in stem cell biology. Tang and Rando (2014) have found that the processes of MuSC activation and proliferation are associated with an increase in autophagic activity. While this increase may be due to the bioenergetic demand for ATP, it is interesting to postulate an additional role for autophagy in the production of metabolites essential for the extensive chromatin remodeling that occurs during MuSC activation and specification (Tang and Rando, 2014).

Therapeutic Potential of Stem Cell Metabolic Reprogramming

The results presented in this discussion link metabolism to changes in the epigenetic state of both embryonic cells and ASCs. Accordingly, several groups have now demonstrated the efficacy of using targeted metabolic reprogramming strategies to improve nuclear reprogramming or tissue repair following injury (Shyh-Chang et al., 2013b; Zhu et al., 2010). In one such study Shyh-Chang and colleagues have shown that topical application of the glycolytic inhibitor 2-deoxy-D-glucose (2-DG) improves the rate of ear regeneration following pinnal injury; interestingly, this response was associated with an acute decrease in the NADH/NAD ratio (Shyh-Chang et al., 2013b).

While this Review has focused on the ability of metabolism to regulate transcription, it is also important to recognize that transcription factors, RNA-binding proteins, and chromatin remodeling machineries involved in either cell reprogramming or influencing cell fate determination also exert profound effects on the metabolic state. Small molecules modulating metabolic pathways and transcriptional outcomes influence stem cell fate and function (Li et al., 2013), and corrected PSCs from patients with mitochondrial DNA mutations display normal metabolic function compared to mutant cells (Ma et al., 2015).

Conclusions

In this Review we have presented an extensive discussion of how metabolism and substrate utilization can regulate transcription via metabolites acting as cofactors for epigenetic regulators. In addition, we have described the current state of knowledge regarding the changes in metabolism that occur during stem cell specification, differentiation, and reprogramming. However, it is our hope that this Review has also highlighted the gaps in our understanding of the process of metabolic reprogramming in stem cells. Future studies investigating epigenetic changes in stem cell populations will likely include analyses of intracellular metabolites and cellular metabolism, as an increased understanding of the process of metabolic reprogramming in stem cells will lead to significant advances in the fields of stem cell transplantation and regenerative medicine.

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