Cell Stem Cell
Perspective

Metabolic Regulation in Pluripotent Stem Cells during Reprogramming and Self-Renewal

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Small, rapidly dividing pluripotent stem cells (PSCs) have unique energetic and biosynthetic demands compared with typically larger, quiescent differentiated cells. Shifts between glycolysis and oxidative phosphorylation with PSC differentiation or reprogramming to pluripotency are accompanied by changes in cell cycle, biomass, metabolite levels, and redox state. PSC and cancer cell metabolism are overtly similar, with metabolite levels influencing epigenetic/genetic programs. Here, we discuss the emerging roles for metabolism in PSC self-renewal, differentiation, and reprogramming.

Introduction

Pluripotent stem cells (PSCs), including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), have an unlimited capacity for self-renewal and can differentiate into every cell type in our bodies, which holds significant promise for applications in regenerative medicine. Cellular features of "stemness" and underlying genetic and epigenetic mechanisms that control self-renewal, differentiation, and reprogramming are being intensely studied (Boyer et al., 2005; Orkin and Hochedlinger, 2011). Rapidly increasing attention is also being directed toward the roles for metabolism in PSCs. In contrast to differentiated cells, PSCs have a short G1 phase of the cell cycle, during which most biomass accumulation and differentiation occurs, limiting PSC growth and differentiation potential (Singh and Dalton, 2009; Wang et al., 2008). To facilitate rapid cell duplication, PSCs must balance energetic with biosynthetic demands, a feature shared with highly proliferative cancer cells. In general, ATP is produced by glycolysis and oxidative phosphorylation (OXPHOS), while the synthesis of lipids, nucleotides, and proteins requires nutrient uptake, processing, and internal metabolite precursor entry into multiple anabolic pathways (DeBerardinis et al., 2008). Key differences in metabolism between PSCs and differentiated cells exist, in contrast to striking similarities in metabolism between PSCs and cancer cells. Metabolism in highly proliferative cancer cells directly influences chromatin organization and transcription (Dang, 2012; Ward and Thompson, 2012), which likely also occurs in PSCs to control physiology and fate. Here, to accompany the Perspective in this issue of *Cell Stem Cell* by Folmes et al. (2012), we provide a perspective on the current state of PSC metabolism, which includes consideration of energetics, multiple nutrient and carbon sources, and oxidation-reduction (redox) states in the context of early mammalian development, adult-type stem cells, and cancer. We also examine emerging links between selected signal transduction pathways, PSC metabolism, and genetic and epigenetic regulatory networks. Of note, the modest available data from studies of metabolism in PSCs contrasts with extensive studies in cancer, which has led to gap-filling assumptions for PSCs based on similar studies in cancer that should be interpreted carefully.

Energetics of Pluripotency

OXPHOS can theoretically generate up to 38 mol ATP per mol glucose (depending on NADH shuttling into mitochondria and electron transport chain [ETC] coupling efficiency), whereas glycolysis generates only 2 mol ATP per mol glucose. Yet, numerous studies show that mouse and human ESCs and iPSCs have an elevated dependence on glycolysis under aerobic conditions compared to highly respiring (e.g., cardiomyocytes) or lowly respiring (e.g., fibroblasts) differentiated cell types (Chung et al., 2007; Folmes et al., 2011; Panopoulos et al., 2012; Prigione et al., 2010; Varum et al., 2011; Zhang et al., 2011). In cancer, a high glycolytic flux provides sufficient ATP and anabolic precursors for rapid proliferation, with the pentose phosphate pathway generating ribose-5-phosphate for nucleotides and NADPH-reducing power for nucleotide and lipid biosynthesis (DeBerardinis et al., 2008; Locasale and Cantley, 2011). Human PSCs also have a high glycolytic flux (Prigione et al., 2010) and mouse ESCs require increased pentose phosphate pathway activity for survival during oxidative stress and

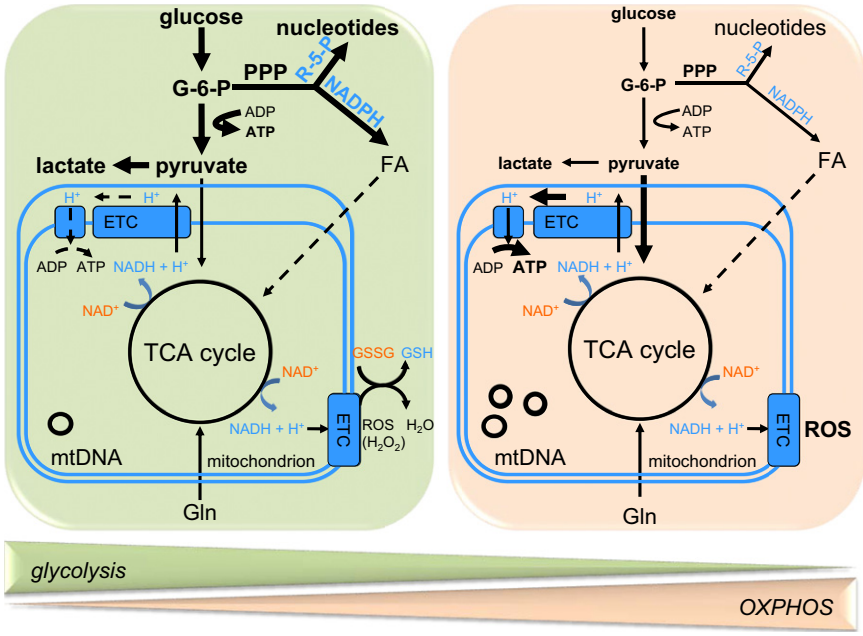

Figure 1. Key Differences in Metabolism between PSCs and Differentiated Cells

Energy metabolism shifts from glycolysis to OXPHOS with differentiation, or from OXPHOS to glycolysis with reprogramming to pluripotency. Glycolytic flux is elevated in PSCs (left panel) to provide ATP and intermediate metabolites through the pentose phosphate pathway for nucleotide and lipid biosynthesis. Glycolysis regulating enzymes, such as lactate dehydrogenase and hexokinase, are highly expressed in PSCs, which also consume oxygen through a functional electron transport chain. PSCs rely more on glycolysis for energy because respiration is lower and less coupled to energy production than in differentiated cells. Pyruvate entry into mitochondria is limited by an inactive pyruvate dehydrogenase complex or by high expression of uncoupling protein 2. The mitochondrial membrane potential is partially maintained by hydrolysis of ATP in the complex V ATP synthase. Less reactive oxygen species is present in PSCs from lower respiration and elevated antioxidant enzymes. These features establish a reduced cellular redox environment (blue text) that becomes more oxidative (orange text) with differentiation. The tricarboxylic acid cycle in PSCs provides intermediate metabolites such as citrate and α-ketoglutarate that are siphoned for lipid and amino acid biosynthesis. ETC, electron transport chain; FA, fatty acid; G-6-P: glucose-6-phosphate; GSH; glutathione; GSSG, glutathione disulfide; mtDNA, mitochondrial DNA; NAD: nicotinamide adenine dinucleotide; NADP: nicotinamide adenine dinucleotide phosphate; PPP, pentose phosphate pathway; R-5-P, ribose-5-phosphate; ROS, reactive oxygen species; TCA, tricarboxylic acid cycle. Elevated metabolic pathways are indicated with bold letters and arrows.

to control cell fate (Filosa et al., 2003; Manganelli et al., 2012). Carbon tracing studies show that human ESCs obtain ~70% of their ribose from added glucose, with genes in the pentose phosphate and lipid biosynthesis pathways highly expressed before ESC differentiation (Varum et al., 2011; Zhang et al., 2011). Thus, aerobic glycolysis is a common feature of PSC and cancer cell metabolism in culture (Figure 1) (Dang, 2012; Warburg, 1956; Ward and Thompson, 2012).

During PSC differentiation, energy production shifts to the mitochondrion, a double-membrane organelle that regulates cellular levels of ATP and intermediate metabolites, Fe-S cluster and heme biosynthesis, free radical production, Ca^{2+} homeostasis, and apoptosis through the release of proapoptotic and antiapoptotic proteins. Studies of mitochondrial morphology and DNA (mtDNA) levels suggested that ESCs contain fewer and less mature mitochondria than differentiated cells (Prigione et al., 2010). However, PSCs are small and the mitochondrial to total protein mass ratio for PSCs is similar to that measured for fibroblasts (Birket et al., 2011; Zhang et al., 2011). mtDNA encodes for only 13 OXPHOS protein subunits out of the more than ~1,500 proteins imported into mitochondria, and genome copy number alone may not indicate PSC mitochondrial mass or respiratory function (Zhou et al., 2012). ETC complexes I through V are assembled in PSCs and oxygen is consumed at a level similar to that measured for fibroblasts. However, respiration and energy production are less coupled in human PSCs compared to fibroblasts, consistent with a high glycolytic flux for energy generation (Shyh-Chang et al., 2011; Zhang et al., 2011; Zhou et al., 2012). A similar mitochondrial decoupling, or increased expression of uncoupling proteins, has been reported in some cancers as well (Ayyasamy et al., 2011; Samudio et al., 2009). Reduced ETC coupling lessens the production of reactive oxygen species (ROS), which suppresses PSC differentiation and decreases the potential for genome damage (Crespo et al., 2010; Saretzki et al., 2008; Zhang et al., 2011).

In cancer cells and PSCs citrate, α-ketoglutarate, and other intermediate metabolites for lipid and amino acid biosynthesis are siphoned from the TCA cycle to support rapid cell proliferation (DeBerardinis et al., 2008; Locasale and Cantley, 2011; Panopoulos et al., 2012; Dang, 2012; Ward and Thompson, 2012). Substrate oxidation results in transfer of a proton and electrons to NAD^+, generating NADH. To restore the NAD^+ pool and maintain TCA cycle flux requires NADH oxidation through the ETC, with electrons ultimately donated to oxygen (Figure 1). Respiration is at or near maximum capacity for human PSCs, in contrast to fibroblasts, which have a large, untapped respiratory reserve capacity (Folmes et al., 2011; Zhang et al., 2011; Zhou et al., 2012). Low expression of ETC complex IV cytochrome *c* oxidase (COX) subunits, which donate electrons to oxygen, may help limit the maximum human PSC respiration capacity (Zhou et al., 2012), which is a distinguishing feature from cancer cell metabolism (Dang, 2012; Ward and Thompson, 2012).

Molecular mechanisms that regulate energy metabolism in PSCs and the changes that occur during differentiation or reprogramming are just being discovered. Glycolysis-regulating enzymes including hexokinase and lactate dehydrogenase are highly expressed in PSCs and could be controlled by mTOR and PI3K signaling pathways that regulate glycolytic genes in other cell contexts. Glycolytic and OXPHOS pathway gene expression and DNA methylation patterns also change during reprogramming to pluripotency (Panopoulos et al., 2012). Several candidate mechanisms for limiting respiration or ATP production (in addition to low COX subunit expression) in PSC mitochondria have been reported. Pyruvate entry into mitochondria is limited

by an inactive pyruvate dehydrogenase (PDH) complex or by the expression of uncoupling protein 2 (UCP2) (Shyh-Chang et al., 2011; Varum et al., 2011; Zhang et al., 2011). Similar mechanisms have also been reported in cancer. Pyruvate conversion to acetyl-CoA by PDH and entry into mitochondria is blocked in hypoxic cancer cells by hypoxia inducible factor 1α (HIF1α)-induced pyruvate dehydrogenase kinase 1 (PDK1) expression and the inactivation of PDH phosphorylation (Ayyasamy et al., 2011; Kim et al., 2006). Also, several types of cancer re-express UCP2 (Ayyasamy et al., 2011; Kim et al., 2006). An open mitochondrial permeability transition pore correlates with reduced respiratory coupling in developing cardiomyocytes, and pore closure with further differentiation may increase coupling and mitochondrial ATP production (Hom et al., 2011). A high ratio of inhibitory factor-1 to the β1 subunit of the ETC complex V ATP synthase drives the hydrolysis and consumption of glycolytic ATP at complex V to maintain the mitochondrial membrane potential and block apoptosis in PSCs (Zhang et al., 2011). In cancer, ATP concentrations are also lowered by increased ATP consumption at the endoplasmic reticulum and by a switch in pyruvate kinase isoforms from M1 to M2 with alternative processing of phosphoenolpyruvate to pyruvate without the generation of ATP (Fang et al., 2010; Israelsen and Vander Heiden, 2010; Locasale and Cantley, 2011). All of these mechanisms limit energy production by OXPHOS in PSCs or early differentiating cells in preference to glycolysis and its biosynthetic pathway branches, such as the pentose phosphate pathway. An interesting question is how genes that control metabolism are regulated by core pluripotency transcription factors in PSCs with differentiation or, conversely, during reprogramming.

In Vivo Correlates for PSC Energetics

In mice, one-cell zygotes favor OXPHOS over glycolysis with pyruvate as the preferred carbon source, which is likely a continuation of oocyte metabolism of stored pyruvate before fertilization (Johnson et al., 2003). Morulae (E2.0) increase glucose uptake and anaerobic glycolysis with upregulation of glucose transporter and hexokinase gene expression. Blastocysts (E3.0) further increase anaerobic glycolysis of glucose for continued rapid cell duplication. Following implantation (E6.0), anaerobic glycolysis is maximal and gradually shifts to OXPHOS with germ layer differentiation, similar to in vitro PSC differentiation. Therefore, metabolism in cultured mouse PSCs should reflect derivation from specific embryonic stages. Mouse ESCs (mESCs) originate from the inner cell mass of preimplantation blastocysts, whereas mouse epiblast stem cells (mEpiSCs) derive from the postimplantation epiblast and represent a later stage of pluripotency with distinct epigenetic and genetic features (Nichols and Smith, 2009). The pluripotent state of human ESCs, even though derived from the inner cell mass, resembles mEpiSCs more than mESCs. Consistent with the concept of an embryonic stage-specific metabolism, mEpiSCs (and human ESCs) are also more dependent on glycolysis than OXPHOS for energy compared to mESCs (Zhou et al., 2012). Stabilized expression of HIF1α appears sufficient to advance mESCs to a mEpiSC-like stage, at least by biomarker patterns and colony morphology, although a global epigenetic profile comparison would be beneficial (Zhou et al., 2012). A potential mechanism for this advancement is by HIF1α repression of COX subunits in ETC complex IV, with partial loss of respiratory capacity and concurrent induction of the mEpiSC glycolytic program. It is unknown whether human PSCs ever assume a metabolic program that resembles mESCs. Understandably, this latter issue is complicated by limitations on studies of early human embryogenesis.

Resetting Metabolism by Nuclear Reprogramming

Somatic cells reprogrammed to pluripotency should become dependent on glycolysis. Consistent with this prediction, defined-factor reprogrammed human iPSCs show a high glycolytic carbon flux and increased decoupling of respiration from ATP production in mitochondria (Folmes et al., 2011; Panopoulos et al., 2012; Zhang et al., 2011). Intermediate metabolite levels, epigenetic modifications, and expression levels of metabolic genes resemble human ESCs, with late passage (>p41) iPSCs significantly closer to ESC metabolite levels than early passage (p16) cells (Panopoulos et al., 2012). This progressive resetting of metabolite levels parallels the progressive changes seen in global epigenetic modifications and gene expression with late passage iPSCs over prolonged culture times (Chin et al., 2009). Reprogramming to pluripotency is also more efficient the closer the glycolytic and OXPHOS energy metabolism profile of the starting somatic cells are to the pattern seen in ESCs (Panopoulos et al., 2012). This suggests a type of "metabolic memory" that is similar to the "epigenetic memory" contained within certain differentiated lineages with specific chromatin modifications that may be partially retained through the reprogramming process (Kim et al., 2010). One prediction is that a retained metabolic memory could influence the differentiation potential and function of iPSCs of different cells of origin. Metabolic reprogramming may provide a barrier that must be overcome to enhance reprogramming efficiency.

Several studies show that metabolism regulates reprogramming efficiency and that metabolic resetting is an active process during reprogramming. Manipulations that inhibit glycolysis, such as incubation of somatic cells with 2-deoxyglucose to compete for glucose uptake, 3-bromopyruvic acid to block hexokinase 2, or dichloroacetate to impair PDK1, all reduce reprogramming efficiency, whereas augmenting glycolysis with D-fructose-6-phosphate or by PDK1 activation enhances iPSC reprogramming efficiency (Folmes et al., 2011; Panopoulos et al., 2012; Zhu et al., 2010). During the reprogramming process an increase in the expression of specific glycolytic genes precedes the gain of expression in genes that regulate self-renewal, suggesting that metabolic resetting has an early, active role in the return to pluripotency (Folmes et al., 2011). Interestingly, some reprogramming factors directly regulate metabolism. For example, c-Myc promotes glycolysis and represses respiration and Lin28 has been recently shown to stimulate glucose metabolism (Figure 2) (Zhu et al., 2011). The addition of Lin28 to the reprogramming factor cocktail accelerates the reacquisition of pluripotency in a proliferation-dependent manner, potentially by enhancing glycolysis (Hanna et al., 2009). However, the absence of either c-Myc or Lin28 does not block metabolic resetting in successfully reprogrammed somatic cells (Folmes et al., 2011), suggesting that this process is a property of pluripotency that can be achieved independent of these factors.

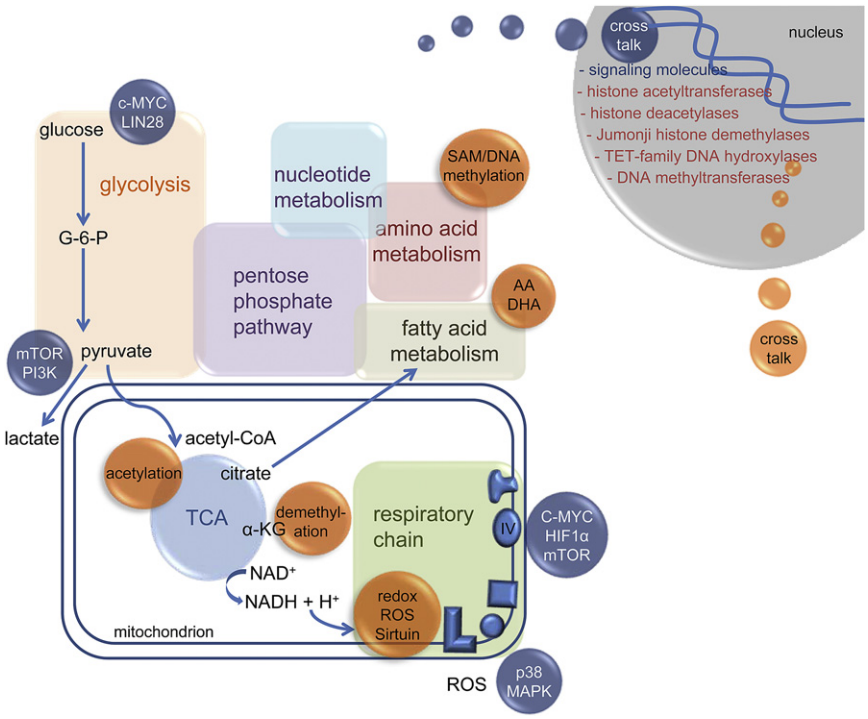

Figure 2. Metabolic, Signaling, Genetic, and Epigenetic Network Crosstalk

Glucose enters glycolysis (orange panel) and is metabolized to lactate. The expression and activity of glycolytic regulating enzymes are controlled by mTOR and PI3K signaling pathways (crosstalk). Reprogramming factors c-Myc and Lin28 promote glucose metabolism and c-Myc also suppresses respiration (crosstalk). Alternatively, pyruvate can be converted into two molecules of acetyl-CoA and enter the TCA cycle. Acetyl-CoA can traffic to the nucleus as a substrate for acetyltransferase enzymes (crosstalk). TCA cycle intermediates can be siphoned into amino acid or lipid biosynthesis pathways, or can participate in the control of gene expression. For example, α-ketoglutarate (α-KG) is a cofactor for Jumonji-domain containing histone demethylases (crosstalk). Methionine adenosyltransferase, an enzyme in amino acid biosynthesis, also converts methionine and ATP into S-adenosylmethionine (SAM) for DNA methyltransferases and other transmethylation reactions (crosstalk). The NAD^+/NADH ratio reflects cellular redox state and regulates deacetylase enzymes such as the Sirtuins (crosstalk). Reactive oxygen species (ROS) are required for lineage-specific differentiation and are regulated by the p38 MAPK stress signaling pathway (crosstalk). Lipid biosynthesis supports membrane formation but also provides signaling molecules, including arachidonic acid (AA) and docosahexanoic acid (DHA) (crosstalk). All together, metabolites, the redox environment, and metabolism-regulated signaling cascades communicate with the nucleus (gray panel) to directly impact chromatin structure and gene expression programs that may control PSC self-renewal, differentiation, and reprogramming. Blue buttons indicate nucleus and signaling control of metabolic pathways; orange buttons indicate metabolic pathways that influence gene expression changes within the nucleus.

Redox Homeostasis

Cellular redox states are regulated by metabolic activities and impact many biological processes. In some settings, such as dividing glial precursor cells, the redox state modulates the balance between self-renewal versus differentiation (Smith et al., 2000). ROS is minimized in PSCs by a reduction in substrate oxidation and respiratory coupling, and by the elevated expression of antioxidant stress genes *UCP2*, *SOD2*, and *GPX2*, which are repressed with the induction of differentiation (Saretzki et al., 2008; Zhang et al., 2011). Increased ROS promotes lineage-specific differentiation, which for cardiomyocyte precursors is through the induced activity of the p38 MAPK stress signaling pathway (Figure 2) (Crespo et al., 2010). By contrast, increased ROS promotes cell proliferation and not the differentiation of cancer cells (Weinberg et al., 2010). The underlying mechanisms for this differential response to ROS in distinct cellular contexts are unknown. During mESC differentiation, the ratio of reduced to oxidized glutathione decreases as does the level of NADH, and the unsaturated metabolome with carbon-carbon double bonds is replaced by an oxidative metabolome, effectively increasing the overall cellular oxidation state (Wang et al., 2009; Yanes et al., 2010). Blocking the oxidative eicosanoid signaling cascade in ESCs can also delay differentiation (Yanes et al., 2010). Tight regulation of specific redox species, such as the NAD^+/NADH ratio, is critical for signaling events that require NAD^+-dependent deacetylase activities (Han et al., 2008). An important unexplored question is how changes in PSC metabolism influence the cellular redox state to regulate signal transduction and cell fate. Since the NAD^+/NADH ratio is regulated by glycolytic and mitochondrial activities that change dramatically during differentiation or reprogramming (Locasale and Cantley, 2011), the NAD^+/NADH redox state may have a role in driving PSC fate.

Amino Acid and Lipid Metabolism

Proliferating cells use glucose and anapleurotic, or replenishing, fuels such as glutamine to supply the TCA cycle and generate energy and intermediate metabolites for biosynthetic reactions (Vander Heiden et al., 2009). The recently discovered mitochondrial pyruvate transporter may provide a pathway to the TCA cycle for PSCs and early differentiating cells (Divakaruni and Murphy, 2012). As expected, amino acids and fatty acids are also required for PSC self-renewal and differentiation. An amino acid "drop-out" study showed that mESCs and embryos, but not fibroblasts, require the catabolism of threonine for survival (Wang et al., 2009). Whole metabolome studies showed that threonine provides glycine through one-carbon metabolism for purine biosynthesis to support rapid DNA replication in mESCs, which is not required for slower proliferating fibroblasts. Threonine dehydrogenase (TDH), which catalyzes the initial rate-limiting step in threonine catabolism, is highly expressed in mESCs and disappears with lineage nonspecific differentiation to embryoid bodies. Metabolomic data also indicate that purine nucleotides are differentially present between human ESCs and somatic cells (Panopoulos et al., 2012), although a gene encoding a functional human TDH enzyme is lacking, being replaced by a pseudogene with three nonfunctional mutations. It will be interesting to determine how human

ESCs support purine biosynthesis independent of a functional TDH enzyme.

Lipids are essential components of plasma and organelle membranes, and fatty acids arising by de novo synthesis or liberated from stored fat can function as secondary messengers for specific signal transduction pathways. Lipids that participate in signaling cascades, such as arachidonic acid, diacylglycerol, and prostaglandins, are among the most highly expressed metabolites in PSCs (Yanes et al., 2010). How these messenger lipids affect PSC fate decisions and how their levels are regulated are interesting questions, especially when considering their identified signaling roles in cancer. For example, monoacylglycerol lipase regulates a fatty acid network enriched in oncogenic signaling lipids, such as prostaglandins, that promotes tumor aggressiveness, such as cell motion and metastasis (Nomura et al., 2010). It would be interesting to determine whether lipid metabolites influence PSC survival, movement, or differentiation potential and, conversely, reprogramming efficiency. Also, free fatty acids can provide energy when glucose availability is restricted, and fatty acid oxidation supports mouse blastocyst inner cell mass growth and the maintenance of ESC lipid homeostasis (Dunning et al., 2010; Zaugg et al., 2011). Disruption of carnitine palmitoyltransferase, the rate-limiting enzyme for fatty acid oxidation, leads to ATP depletion and to decreased resistance to nutrient deprivation, indicating a key role for fatty acids in mESC energy generation (Zaugg et al., 2011).

Adult-Type Stem Cells and the Environment

Similar to PSCs, hematopoietic stem cells (HSCs) consume fatty acids but probably rely mainly on glycolysis and increase OXPHOS upon differentiation (Figure 1) (Ito et al., 2012; Suda et al., 2011). However, unlike PSCs or their more differentiated multipotent progenitor cells, HSCs are quiescent and cycle slowly, indicating that a high cell replication rate is not required to favor glycolytic metabolism. This fundamental difference might reflect an HSC adaptation to a bone marrow niche with limited oxygen perfusion (Suda et al., 2011). One potential advantage of this difference is reduced ROS production. HSCs are sensitive to ROS, and they either die or differentiate with excessive ROS. With physiologic demand HSCs differentiate, which increases OXPHOS and ROS production as progenitors are recruited away from the hypoxic stem cell niche for functions in the animal periphery. Therefore, the higher level of glycolysis in HSCs relative to their multipotent progenitors may reflect an adaptive strategy to compensate for reduced mitochondrial energy production in the hypoxic niche that supports HSC self-renewal.

Additional types of adult stem cells reside in hypoxic or normoxic niches. Some of these adult stem cells cycle slowly, such as skin stem cells in the hair follicle, whereas some cycle rapidly, such as intestinal crypt stem cells. It will be interesting to characterize the metabolic state of these stem cells to determine whether the relative dependence on glycolysis versus OXPHOS is an environmental adaptation strategy or is determined by intrinsic metabolic demands to maintain stem cell identity. In addition, ESCs originate from a hypoxic preimplantation blastocyst microenvironment, although they are routinely grown in ambient oxygen following derivation, and reprogrammed iPSCs may never experience hypoxia. Low oxygen content also helps to maintain ESC self-renewal and increases iPSC reprogramming efficiency (Wion et al., 2009). Yet, both ESCs and iPSCs share similar metabolic features. So, for PSCs, the metabolic program is integrated with stemness independent of oxygen availability, whereas certain adult stem cell types may use different molecular mechanisms for distinct purposes to arrive at a relative dependence on glycolysis.

Outlook: Stem Cell Metabolism Links to Epigenetics and Gene Expression

A key unresolved question is how metabolism integrates with epigenetic and genetic programs to coordinately regulate PSC function and fate. Metabolite fluxes can be controlled by enzymes that are regulated by transcription factors including c-Myc and HIF1α, and by signaling network molecules including PI3K, AKT, and mTOR (Figure 2) (DeBerardinis et al., 2008). Downstream studies in mammalian cells have shown that intermediate metabolites can function as substrates or cofactors for enzymes that regulate chromatin structure and gene expression. For example, acetyl-CoA generated by catabolism of glucose, fatty acids, or certain amino acids in mitochondria can be transported into the cytosol and enter the nucleus to increase histone acetylation (Cai et al., 2011; Wellen et al., 2009). Similarly, α-ketoglutarate can exit the mitochondria to function as a cofactor for dioxygenase enzymes including Jumonji-family histone demethylases, TET-family DNA hydroxylases, and possibly prolyl hydroxylases that control HIF1α/2α transcription factor stability (Figure 2) (Xu et al., 2011). Defects in TCA cycle enzymes, such as succinate dehydrogenase, fumarate hydratase, and isocitrate dehydrogenase, cause inherited benign or malignant tumors by altering DNA and histone modifications to cause widespread transcriptional dysregulation (Nunnari and Suomalainen, 2012). Deacetylase enzymes, such as Sirtuin family proteins, are sensitive to redox state and can impact histone modifications and posttranscriptional changes through nonhistone protein deacetylation (Haigis and Guarente, 2006). The production of S-adenosylmethionine is tightly linked to amino acid metabolism and is a donor for DNA methylation with high levels present in iPSCs (Panopoulos et al., 2012). These and likely additional metabolites could exert tremendous influence and broad regulatory control over the PSC genome. Because cofactors and modifying enzymes are always present at some level, specificity must be imparted or all genes would be regulated synchronously, which is not observed with metabolite dysregulation in cancer. Therefore, a hierarchy of target chromatin regions and associated genes, miRNAs, and noncoding elements likely exists, which could be regulated by enzyme recruitment with DNA-binding factors, local depletion or excess cofactors, or the modification of spatial and temporal sublocalization of enzymes within the nucleus (Katada et al., 2012). Links being forged between metabolism and epigenetics in the dysregulated setting of cancer provide added impetus for determining how metabolism and metabolites exert influence over epigenetic and genetic circuits that establish and maintain PSC self-renewal and differentiation, or that reestablish pluripotency with somatic cell reprogramming.

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REFERENCES

Ayyasamy, V., Owens, K.M., Desouki, M.M., Liang, P., Bakin, A., Thangaraj, K., Buchsbaum, D.J., LoBuglio, A.F., and Singh, K.K. (2011). Cellular model of Warburg effect identifies tumor promoting function of UCP2 in breast cancer and its suppression by genipin. PLoS ONE *6*, e24792.

Birket, M.J., Orr, A.L., Gerencser, A.A., Madden, D.T., Vitelli, C., Swistowski, A., Brand, M.D., and Zeng, X. (2011). A reduction in ATP demand and mitochondrial activity with neural differentiation of human embryonic stem cells. J. Cell Sci. *124*, 348–358.

Boyer, L.A., Lee, T.I., Cole, M.F., Johnstone, S.E., Levine, S.S., Zucker, J.P., Guenther, M.G., Kumar, R.M., Murray, H.L., Jenner, R.G., et al. (2005). Core transcriptional regulatory circuitry in human embryonic stem cells. Cell *122*, 947–956.

Cai, L., Sutter, B.M., Li, B., and Tu, B.P. (2011). Acetyl-CoA induces cell growth and proliferation by promoting the acetylation of histones at growth genes. Mol. Cell *42*, 426–437.

Chin, M.H., Mason, M.J., Xie, W., Volinia, S., Singer, M., Peterson, C., Ambartsumyan, G., Aimiuwu, O., Richter, L., Zhang, J., et al. (2009). Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures. Cell Stem Cell *5*, 111–123.

Chung, S., Dzeja, P.P., Faustino, R.S., Perez-Terzic, C., Behfar, A., and Terzic, A. (2007). Mitochondrial oxidative metabolism is required for the cardiac differentiation of stem cells. Nat. Clin. Pract. Cardiovasc. Med. *4*(*Suppl 1*), S60–S67.

Crespo, F.L., Sobrado, V.R., Gomez, L., Cervera, A.M., and McCreath, K.J. (2010). Mitochondrial reactive oxygen species mediate cardiomyocyte formation from embryonic stem cells in high glucose. Stem Cells *28*, 1132–1142.

Dang, C.V. (2012). Links between metabolism and cancer. Genes Dev. *26*, 877–890.

DeBerardinis, R.J., Lum, J.J., Hatzivassiliou, G., and Thompson, C.B. (2008). The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. Cell Metab. *7*, 11–20.

Divakaruni, A.S., and Murphy, A.N. (2012). Cell biology. A mitochondrial mystery, solved. Science *337*, 41–43.

Dunning, K.R., Cashman, K., Russell, D.L., Thompson, J.G., Norman, R.J., and Robker, R.L. (2010). Beta-oxidation is essential for mouse oocyte developmental competence and early embryo development. Biol. Reprod. *83*, 909–918.

Fang, M., Shen, Z., Huang, S., Zhao, L., Chen, S., Mak, T.W., and Wang, X. (2010). The ER UDPase ENTPD5 promotes protein N-glycosylation, the Warburg effect, and proliferation in the PTEN pathway. Cell *143*, 711–724.

Filosa, S., Fico, A., Paglialunga, F., Balestrieri, M., Crooke, A., Verde, P., Abrescia, P., Bautista, J.M., and Martini, G. (2003). Failure to increase glucose consumption through the pentose-phosphate pathway results in the death of glucose-6-phosphate dehydrogenase gene-deleted mouse embryonic stem cells subjected to oxidative stress. Biochem. J. *370*, 935–943.

Folmes, C.D., Nelson, T.J., Martinez-Fernandez, A., Arrell, D.K., Lindor, J.Z., Dzeja, P.P., Ikeda, Y., Perez-Terzic, C., and Terzic, A. (2011). Somatic oxidative bioenergetics transitions into pluripotency-dependent glycolysis to facilitate nuclear reprogramming. Cell Metab. *14*, 264–271.

Folmes, C.D.L., Dzeja, P.P., Nelson, T.J., and Terzic, A. (2012). Metabolic Plasticity in Stem Cell Homeostasis and Differentiation. Cell Stem Cell *11*, this issue, 596–606.

Haigis, M.C., and Guarente, L.P. (2006). Mammalian sirtuins—emerging roles in physiology, aging, and calorie restriction. Genes Dev. *20*, 2913–2921.

Han, M.K., Song, E.K., Guo, Y., Ou, X., Mantel, C., and Broxmeyer, H.E. (2008). SIRT1 regulates apoptosis and Nanog expression in mouse embryonic stem cells by controlling p53 subcellular localization. Cell Stem Cell *2*, 241–251.

Hanna, J., Saha, K., Pando, B., van Zon, J., Lengner, C.J., Creyghton, M.P., van Oudenaarden, A., and Jaenisch, R. (2009). Direct cell reprogramming is a stochastic process amenable to acceleration. Nature *462*, 595–601.

Hom, J.R., Quintanilla, R.A., Hoffman, D.L., de Mesy Bentley, K.L., Molkentin, J.D., Sheu, S.S., and Porter, G.A., Jr. (2011). The permeability transition pore controls cardiac mitochondrial maturation and myocyte differentiation. Dev. Cell *21*, 469–478.

Israelsen, W.J., and Vander Heiden, M.G. (2010). ATP consumption promotes cancer metabolism. Cell *143*, 669–671.

Ito, K., Carracedo, A., Weiss, D., Arai, F., Ala, U., Avigan, D.E., Schafer, Z.T., Evans, R.M., Suda, T., Lee, C.H., and Pandolfi, P.P. (2012). A PML-PPAR-δ pathway for fatty acid oxidation regulates hematopoietic stem cell maintenance. Nat. Med. Published online August 19, 2012. http://dx.doi.org/10.1038/nm.2882.

Johnson, M.T., Mahmood, S., and Patel, M.S. (2003). Intermediary metabolism and energetics during murine early embryogenesis. J. Biol. Chem. *278*, 31457–31460.

Katada, S., Imhof, A., and Sassone-Corsi, P. (2012). Connecting threads: epigenetics and metabolism. Cell *148*, 24–28.

Kim, J.W., Tchernyshyov, I., Semenza, G.L., and Dang, C.V. (2006). HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. Cell Metab. *3*, 177–185.

Kim, K., Doi, A., Wen, B., Ng, K., Zhao, R., Cahan, P., Kim, J., Aryee, M.J., Ji, H., Ehrlich, L.I., et al. (2010). Epigenetic memory in induced pluripotent stem cells. Nature *467*, 285–290.

Locasale, J.W., and Cantley, L.C. (2011). Metabolic flux and the regulation of mammalian cell growth. Cell Metab. *14*, 443–451.

Manganelli, G., Fico, A., Masullo, U., Pizzolongo, F., Cimmino, A., and Filosa, S. (2012). Modulation of the pentose phosphate pathway induces endodermal differentiation in embryonic stem cells. PLoS ONE *7*, e29321.

Nichols, J., and Smith, A. (2009). Naive and primed pluripotent states. Cell Stem Cell *4*, 487–492.

Nomura, D.K., Long, J.Z., Niessen, S., Hoover, H.S., Ng, S.W., and Cravatt, B.F. (2010). Monoacylglycerol lipase regulates a fatty acid network that promotes cancer pathogenesis. Cell *140*, 49–61.

Nunnari, J., and Suomalainen, A. (2012). Mitochondria: in sickness and in health. Cell *148*, 1145–1159.

Orkin, S.H., and Hochedlinger, K. (2011). Chromatin connections to pluripotency and cellular reprogramming. Cell *145*, 835–850.

Panopoulos, A.D., Yanes, O., Ruiz, S., Kida, Y.S., Diep, D., Tautenhahn, R., Herrerías, A., Batchelder, E.M., Plongthongkum, N., Lutz, M., et al. (2012). The metabolome of induced pluripotent stem cells reveals metabolic changes occurring in somatic cell reprogramming. Cell Res. *22*, 168–177.

Prigione, A., Fauler, B., Lurz, R., Lehrach, H., and Adjaye, J. (2010). The senescence-related mitochondrial/oxidative stress pathway is repressed in human induced pluripotent stem cells. Stem Cells *28*, 721–733.

Samudio, I., Fiegl, M., and Andreeff, M. (2009). Mitochondrial uncoupling and the Warburg effect: molecular basis for the reprogramming of cancer cell metabolism. Cancer Res. *69*, 2163–2166.

Saretzki, G., Walter, T., Atkinson, S., Passos, J.F., Bareth, B., Keith, W.N., Stewart, R., Hoare, S., Stojkovic, M., Armstrong, L., et al. (2008). Downregulation of multiple stress defense mechanisms during differentiation of human embryonic stem cells. Stem Cells *26*, 455–464.

Shyh-Chang, N., Zheng, Y., Locasale, J.W., and Cantley, L.C. (2011). Human pluripotent stem cells decouple respiration from energy production. EMBO J. *30*, 4851–4852.

Singh, A.M., and Dalton, S. (2009). The cell cycle and Myc intersect with mechanisms that regulate pluripotency and reprogramming. Cell Stem Cell *5*, 141–149.

Smith, J., Ladi, E., Mayer-Proschel, M., and Noble, M. (2000). Redox state is a central modulator of the balance between self-renewal and differentiation in a dividing glial precursor cell. Proc. Natl. Acad. Sci. USA *97*, 10032–10037.

Suda, T., Takubo, K., and Semenza, G.L. (2011). Metabolic regulation of hematopoietic stem cells in the hypoxic niche. Cell Stem Cell *9*, 298–310.

Vander Heiden, M.G., Cantley, L.C., and Thompson, C.B. (2009). Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science *324*, 1029–1033.

Varum, S., Rodrigues, A.S., Moura, M.B., Momcilovic, O., Easley, C.A., 4th, Ramalho-Santos, J., Van Houten, B., and Schatten, G. (2011). Energy metabolism in human pluripotent stem cells and their differentiated counterparts. PLoS ONE *6*, e20914.

Wang, Y., Baskerville, S., Shenoy, A., Babiarz, J.E., Baehner, L., and Blelloch, R. (2008). Embryonic stem cell-specific microRNAs regulate the G1-S transition and promote rapid proliferation. Nat. Genet. *40*, 1478–1483.

Wang, J., Alexander, P., Wu, L., Hammer, R., Cleaver, O., and McKnight, S.L. (2009). Dependence of mouse embryonic stem cells on threonine catabolism. Science *325*, 435–439.

Warburg, O. (1956). On the origin of cancer cells. Science *123*, 309–314.

Ward, P.S., and Thompson, C.B. (2012). Metabolic reprogramming: a cancer hallmark even warburg did not anticipate. Cancer Cell *21*, 297–308.

Weinberg, F., Hamanaka, R., Wheaton, W.W., Weinberg, S., Joseph, J., Lopez, M., Kalyanaraman, B., Mutlu, G.M., Budinger, G.R., and Chandel, N.S. (2010). Mitochondrial metabolism and ROS generation are essential for Kras-mediated tumorigenicity. Proc. Natl. Acad. Sci. USA *107*, 8788–8793.

Wellen, K.E., Hatzivassiliou, G., Sachdeva, U.M., Bui, T.V., Cross, J.R., and Thompson, C.B. (2009). ATP-citrate lyase links cellular metabolism to histone acetylation. Science *324*, 1076–1080.

Wion, D., Christen, T., Barbier, E.L., and Coles, J.A. (2009). PO(2) matters in stem cell culture. Cell Stem Cell *5*, 242–243.

Xu, W., Yang, H., Liu, Y., Yang, Y., Wang, P., Kim, S.H., Ito, S., Yang, C., Wang, P., Xiao, M.T., et al. (2011). Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of α-ketoglutarate-dependent dioxygenases. Cancer Cell *19*, 17–30.

Yanes, O., Clark, J., Wong, D.M., Patti, G.J., Sánchez-Ruiz, A., Benton, H.P., Trauger, S.A., Desponts, C., Ding, S., and Siuzdak, G. (2010). Metabolic oxidation regulates embryonic stem cell differentiation. Nat. Chem. Biol. *6*, 411–417.

Zaugg, K., Yao, Y., Reilly, P.T., Kannan, K., Kiarash, R., Mason, J., Huang, P., Sawyer, S.K., Fuerth, B., Faubert, B., et al. (2011). Carnitine palmitoyltransferase 1C promotes cell survival and tumor growth under conditions of metabolic stress. Genes Dev. *25*, 1041–1051.

Zhang, J., Khvorostov, I., Hong, J.S., Oktay, Y., Vergnes, L., Nuebel, E., Wahjudi, P.N., Setoguchi, K., Wang, G., Do, A., et al. (2011). UCP2 regulates energy metabolism and differentiation potential of human pluripotent stem cells. EMBO J. *30*, 4860–4873.

Zhou, W., Choi, C., Margineantu, D., Margaretha, L., Hesson, J., Cavanaugh, C., Blau, C.A., Horwitz, M.S., Hockenbery, D., Ware, C., and Ruohola-Baker, H. (2012). HIF1α induced switch from bivalent to exclusively glycolytic metabolism during ESC-EpiSC transition. EMBO J *31*, 2103–2116.

Zhu, S., Li, W., Zhou, H., Wei, W., Ambasudhan, R., Lin, T., Kim, J., Zhang, K., and Ding, S. (2010). Reprogramming of human primary somatic cells by OCT4 and chemical compounds. Cell Stem Cell *7*, 651–655.

Zhu, H., Shyh-Chang, N., Segrè, A.V., Shinoda, G., Shah, S.P., Einhorn, W.S., Takeuchi, A., Engreitz, J.M., Hagan, J.P., Kharas, M.G., et al; DIAGRAM Consortium; MAGIC Investigators. (2011). The Lin28/let-7 axis regulates glucose metabolism. Cell *147*, 81–94.