Author/s:
Harvey, A;Caretti, G;Moresi, V;Renzini, A;Adamo, S

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Interplay between Metabolites and the Epigenome in Regulating Embryonic and Adult Stem Cell Potency and Maintenance

Alexandra Harvey,[1,4] Giuseppina Caretti,[2,4] Viviana Moresi,[3,4,*] Alessandra Renzini,[3] and Sergio Adamo[3]
[1]School of BioSciences, University of Melbourne, Parkville, VIC 2010, Australia
[2]Department of Biosciences, Università degli Studi di Milano, Milan, Italy
[3]Department of Anatomy, Histology, Forensic Medicine & Orthopedics, Histology & Medical Embryology Section, Sapienza University of Rome and Interuniversity Institute of Myology, Rome, Italy
[4]Co-first author
*Correspondence: viviana.moresi@uniroma1.it
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The environment surrounding stem cells has the ability to elicit profound, heritable epigenetic changes orchestrated by multiple epigenetic mechanisms, which can be modulated by the level of specific metabolites. In this review, we highlight the significance of metabolism in regulating stem cell homeostasis, cell state, and differentiation capacity, using metabolic regulation of embryonic and adult muscle stem cells as examples, and cast light on the interaction between cellular metabolism and epigenetics. These new regulatory networks, based on the dynamic interplay between metabolism and epigenetics in stem cell biology, are important, not only for understanding tissue homeostasis, but to determine *in vitro* culture conditions which accurately support normal cell physiology.

Basic Features of Embryonic and Adult Stem Cells

As a result of their ability to self-renew and to differentiate into different cell types, stem cells play critical roles in development and tissue homeostasis. Both embryonic stem cells (ESCs) and adult stem cells (ASCs) are affected by intrinsic and extrinsic signals, which regulate their self-renewal capacity and the balance between stem cell potency and differentiation (Jaenisch and Young, 2008).

The totipotent cleavage-stage preimplantation embryo gives rise to the blastocyst, within which the inner cell mass (ICM) is representative of a transient, highly proliferative pluripotent cell population from which ESCs are derived. In response to local cues, the pluripotent ICM undergoes lineage specification toward each of the three germ layers, progressively differentiating into more specialized cell types. A number of pluripotent states have been described *in vivo* and *in vitro* (reviewed by Davidson et al., 2015), underpinned by differing growth factor and signaling pathway requirements. Self-renewal and maintenance of pluripotency of mouse ESCs relies on leukemia inhibitory factor (LIF) (Brook and Gardner, 1997), whereas human ESCs, derived from a later "primed" pluripotent cell population likely representative of post-implantation epiblast, rely on activin/Nodal and fibroblast growth factor (FGF) signaling (Vallier et al., 2005). Alternatively, a more naive pluripotent state can be achieved in culture through the inhibition of signaling pathways that regulate differentiation (Ying et al., 2008; Zimmerlin et al., 2017). Thus, pluripotency is a continuum of cell states that give rise to the three germ lineages.

In contrast to the high proliferation rates of ESCs, ASCs generally exist in a quiescent state where transient cell-cycle inhibition prevents exhaustion of the stem cell pool (Ito and Suda, 2014). However, in contrast to pluripotent ESCs, most ASCs are lineage restricted, therefore multipotent, maintaining tissue homeostasis, responding to damage and/or stress. Depending on the tissues, certain ASCs display extensive plasticity and can give rise to different specialized cell types in different organs (Raff, 2003), whereas other ASCs exhibit more restricted plasticity (Wagers and Weissman, 2004). ASCs reside within specialized niches which provide specific cues, including stromal cells, extracellular matrix (ECM), vascularization, and innervation that support their capacity for self-renewal (Jones and Wagers, 2008). ASCs can divide either symmetrically, thereby producing two identical cells that replicate and expand in number, or asymmetrically, thereby producing one identical and one committed stem cell, depending on developmental and environmental signals. Upon recruitment, or in certain pathological conditions, ASCs exit from their quiescent state, re-enter the cell cycle, proliferate, and commit to and differentiate into specific tissue lineages. Most ASCs have the ability to switch between asymmetric and symmetric division, and an imbalance between the two modalities is often associated with disease states.

Muscle stem cells (MuSCs), or satellite cells, are ASCs located between the basal lamina and the sarcolemma of muscle fibers and are crucial for skeletal muscle growth and regeneration (Comai and Tajbakhsh, 2014). Whereas MuSC activation and proliferation rely on Notch activity (Conboy et al., 2003), the commitment and onset of differentiation is due to a transition from Notch to Wnt signaling, the latter being an important regulator of terminal differentiation (Brack et al., 2008). Several growth factors in the satellite cell niche affect MuSCs, in part by influencing the temporal transition from Notch to Wnt signaling. FGF, hepatocyte growth factor, and platelet-derived growth factor promote activation and proliferation

of MuSCs but delay terminal differentiation. Conversely, MuSC differentiation is primarily promoted by the insulin-like growth factor 1 but severely inhibited by transforming growth factor β family members (Kuang et al., 2008).

However, while growth factors, cytokines, and the ECM have traditionally been considered as the signals that regulate cell decisions through pathway activation, it is now becoming increasingly apparent that metabolites can also act as signaling molecules, interacting with their own receptors and regulating a vast array of cellular functions. Increasing evidence supports a role for metabolism in regulating the complexity of early development and lineage specification.

Metabolic Control of Stem Cells

Metabolism underpins cell function, with coordinated nutrient utilization necessary to maintain homeostasis, including cellular energy (ATP) production and biosynthesis to support proliferation (Metallo and Vander Heiden, 2013). Cell function and the surrounding nutrient microenvironment determine cellular metabolic requirements, which are supported by the activity of core metabolic pathways, including glycolysis, the pentose phosphate pathway, the tricarboxylic acid (TCA) cycle and oxidative phosphorylation (OXPHOS), which enable adaptation to nutrient availability. This flexibility promotes cell and organism survival, and supports dynamic, stage-specific energy demands through development. However, long-term adaptation contributes to altered cell health, as demonstrated by numerous diseases characterized by perturbations in metabolism (Cai et al., 2012; Perl, 2017; Wallace, 2012). Consequently, once considered mere by-products of energy production, metabolites are increasingly recognized for their diverse roles in mediating cell signaling, with emerging evidence from stem cells implicating metabolites in the regulation of self-renewal, differentiation, and cell state.

Changing Metabolic Demands during Early Development

The transition of the early preimplantation embryo through differentiation is accompanied by changing energy requirements that require dynamic, coordinated regulation of metabolism to support ongoing development. Although oocytes are unable to utilize glucose during *in vitro* maturation, they preferentially metabolize pyruvate via OXPHOS, supported through the glycolytic activity of the surrounding cumulus cells (Dumesic et al., 2015). Similarly, the early pre-compaction embryo exhibits low levels of oxidative metabolism and oxygen consumption, utilizing pyruvate, lactate, and amino acids (Gardner and Harvey, 2015). With the activation of the embryonic genome, ICM and trophectoderm specification, and the energy requirement to support blastocoel formation, the blastocyst stage embryo increases its requirement for glucose; however, it maintains a high level of oxygen consumption. Indeed, the ICM is predominantly glycolytic, while the trophectoderm is more oxidative (Hewitson and Leese, 1993). Significantly, these metabolic changes coincide with migration of the embryo from the oviduct to the uterus, accompanied by changes in the nutrient composition of reproductive tract fluid (Gardner et al., 1996), including a reduction in oxygen availability from ~7% to 2% (Fischer and Bavister, 1993).

The glycolytic metabolic state of the pluripotent ICM is replicated *in vitro*, whereby ESCs rely on aerobic glycolysis to support biosynthesis (Harvey et al., 2016a), even in the presence of sufficient oxygen, termed the Warburg effect (Harvey et al., 2013). Furthermore, the transition between naive and primed pluripotency is accompanied by changes in metabolism, whereby naive mouse ESCs exhibit lower rates of glycolysis compared with primed ESCs, accompanied by upregulation of oxidative metabolism (Zhou et al., 2012). In contrast, cultured naive human ESCs exhibit both upregulation of OXPHOS and increased glycolysis relative to their primed counterparts (Gu et al., 2016), suggesting that differing metabolic profiles likely reflect different cell states within pluripotency. However, these divergent metabolic states may also reflect differences in medium composition (and hence relative metabolite levels) and culture conditions (Zhang et al., 2016a). Indeed, the availability of metabolites has been shown to impact the balance between pluripotency and differentiation and is important for the maintenance of cell state.

Glucose and glutamine availability regulate self-renewal in cultured mouse and human ESCs, through the provision of acetyl-CoA and alpha-ketoglutarate (αKG) (Carey et al., 2015; Moussaieff et al., 2015). Unlike primed mouse ESCs, naive mouse ESCs are not dependent on glutamine to maintain proliferation; however, they utilize both glucose and glutamine to maintain self-renewal by maintaining high levels of intracellular αKG (Carey et al., 2015). In support of this, αKG can replace 2i/LIF to sustain naive pluripotency and prevent the transition to a more primed cell state (Tischler et al., 2019). Equally, a requirement for acetyl-CoA is demonstrated by supplementation with dichloroacetate to increase the conversion of pyruvate to acetyl-CoA, which supports the mouse naive cell state (Tischler et al., 2019). Human ESC self-renewal is similarly supported by high rates of glycolysis that support acetyl-CoA production (Moussaieff et al., 2015), such that inhibition of glycolysis with 2-deoxyglucose leads to ESC differentiation *in vitro*, which can be reversed through supplementation of acetate (Carey et al., 2015). Significantly, high glucose has been shown to suppress human ESC-derived cardiomyocyte differentiation and consequently cardiac maturation (Nakano et al., 2017). In contrast, cardiomyocyte differentiation can be enhanced

by supplementation with lactate (Tohyama et al., 2013). These findings are particularly significant, given that the vast majority of media to support both self-renewal and differentiation contain high glucose levels which may interfere with differentiation, purity, and cell function and maturation.

Interestingly, whereas inhibition of glycolysis with 2-deoxyglucose in serum/LIF-cultured mouse ESCs leads to differentiation (Kondoh et al., 2007) similar to that seen in human ESCs, inhibition in naive mouse ESCs has recently been shown to impede the exit from naive pluripotency (Tischler et al., 2019), suggesting temporal differences in metabolic requirements across pluripotent cell states. Significantly, while oxygen availability does not apparently alter ESC pluripotency at the molecular level, physiological conditions (5% oxygen; which replicate the *in vivo* environment) increase glycolytic activity (Harvey et al., 2016b; Lees et al., 2019). Physiological oxygen conditions are also likely to maintain high levels of αKG and acetyl-CoA to underpin cell state. Consequently, the use of atmospheric oxygen conditions will not only modulate nutrient flux, and epigenetic modifications, as has been observed in human ESCs (Lees et al., 2019), but is likely to alter cellular responses to other nutrient changes, plausibly modifying lineage decisions, including kinetics. Therefore, it is important to acknowledge that the majority of studies on metabolic control of stem cells have been performed under 20% oxygen.

Similarly, threonine and methionine, through the generation of S-adenosylmethionine (SAM), are critical for maintaining mouse and human ESC self-renewal, respectively. Threonine dehydrogenase (TDH) is the rate-limiting enzyme for threonine catabolism, converting threonine to acetyl-CoA and glycine, the latter available for SAM generation via the folate cycle. TDH is highly expressed in proliferating mouse ESCs and decreases upon differentiation to embryoid bodies (Wang et al., 2009). Significantly, threonine deprivation reduces mouse ESC proliferation and increases their differentiation *in vitro* (Wang et al., 2009), accompanied by reduced SAM accumulation, leading to altered differentiation potential (Shyh-Chang et al., 2013). Human cells lack the ability to use threonine; however, methionine is likewise important for cultured human ESC survival through the generation of SAM, with short-term methionine deprivation potentiating human ESC differentiation (Shiraki et al., 2014), suggesting a role for metabolites in lineage specification. In support of this, the presence of proline in the growth medium has been shown to induce mouse ESC differentiation (Washington et al., 2010), and may be required for lineage specification within the developing embryo *in vivo* (Tan et al., 2016).

The exit from pluripotency is also accompanied by metabolic change, with embryoid bodies and differentiating ESCs apparently less reliant on glucose uptake, with mitochondrial activity increasing (Harvey et al., 2013; Moussaieff et al., 2015). However, more recently, sequential examination of metabolism during ESC differentiation has identified differing requirements between lineages. Consistent with a requirement for glycolysis to support cell expansion, and with increasing glucose use by *in vivo* derived post-implantation mouse and rat embryos (Clough and Whittingham, 1983; Ellington, 1987), sustained glycolysis is required to support neural differentiation from ESCs (Lees et al., 2018). Combined, these data therefore highlight the ability of metabolites to regulate development, ESC state, and differentiation, and support lineage-specific metabolic requirements (Table 1).

Metabolic State of ASCs

The *in vivo* adult stem cell niche is characterized by low, but physiological (<1%–8%) oxygen levels (Simsek et al., 2010), which supports the high rates of glycolysis that are essential for maintaining ASC quiescence and self-renewal (Simsek et al., 2010). Nutrient availability in the medium, particularly glutamine, has likewise emerged as a key regulator of ASC survival and proliferation, by enhancing mitochondrial function and stimulating mammalian target of rapamycin complex 1 (mTOR complex) (Salabei et al., 2015) as well as recruitment and hematopoietic stem cell (HSC) specification (Oburoglu et al., 2014). Similarly, selective metabolites, such as fructose-1,6-bisphosphate, phosphoenolpyruvic acid, and sodium oxalate, have been shown to induce human mesenchymal stem cell proliferation by increasing glycolytic metabolism (Jeong et al., 2019). Interestingly, caloric restriction in mice (20%–40% reduction in caloric intake) enhances HSC quiescence and increases intestinal and skeletal MuSC functionality, whereas a high-fat diet impairs HSC and neuronal stem cell differentiation both *in vitro* and *in vivo* (reviewed by Mana et al., 2017). Despite this experimental evidence, it cannot be excluded that dietary interventions may result in altered growth factor signaling, rather than merely changes in metabolites.

Fatty acid oxidation is also required for HSC maintenance in the mouse, whereby loss of PPAR-δ or inhibition of mitochondrial fatty oxidation with etomoxir impairs HSC maintenance, while exposure to the PPAR-δ agonist GW-501516 increases HSC maintenance (Ito et al., 2012). Investigation of amino acid regulation of ASC self-renewal is limited; however, valine and cysteine have recently been reported to be indispensable for HSC proliferation *in vitro*, whereas dietary valine is essential to the maintenance of hematopoiesis *in vivo* (Taya et al., 2016).

In addition, in response to specific inductive signals initiated by changes in ECM architecture and cytokine levels, recruitment of ASCs away from the stem cell niche has recently been shown to be mediated by nutrient

Table 1. Stage- and Cell State-Specific Metabolic Profiles of the Embryo, and Embryonic and Adult Stem Cells

Cell State	Metabolite/Metabolic Pathway Preference	Reference
Oocyte and Preimplantation Embryo		
Oocyte	pyruvate (OXPHOS)	Dumesic et al., 2015
Early cleavage-stage embryo	↑pyruvate ↑lactate ↓glucose ↑non-essential AA	Gardner and Harvey, 2015
First lineage decision	proline?	Tan et al., 2016
ICM	↓pyruvate ↓lactate ↑glucose ↑non-essential AA ↑essential AA	Gardner and Harvey, 2015 Hewitson and Leese, 1993 Tan et al., 2016
Trophectoderm	↑OXPHOS ↓glucose	Gardner and Harvey, 2015 Hewitson and Leese, 1993
Embryonic Stem Cell Self-Renewal		
Naive mESCs	↓glycolysis ↑OXPHOS glucose- and glutamine-derived αKG αKG can replace 2i/LIF acetyl-CoA	Zhou et al., 2012 Carey et al., 2015 Tischler et al., 2019
Naive hESCs	glycolysis and OXPHOS	Gu et al., 2016
Serum/LIF mESCs	glycolysis threonine-derived SAM	Carey et al., 2015 Wang et al., 2009 Shyh-Chang et al., 2013
Primed ESCs	glycolysis glucose-derived acetyl-CoA methionine-derived SAM	Harvey et al., 2016a Moussaieff et al., 2015 Shiraki et al., 2014
Embryo/ESC Differentiation		
Embryoid bodies	↓glycolysis ↑mitochondrial activity	Harvey et al., 2013 Moussaieff et al., 2015
mESC differentiation	↑proline ↓threonine/SAM αKG	Washington et al., 2010 Arney and Fisher, 2004 TeSlaa et al., 2016
hESCs differentiation	glycolysis/OXPHOS (lineage dependent) ↓methionine/SAM αKG	Lees et al., 2018 Shiraki et al., 2014 TeSlaa et al., 2016
Post-implantation embryo	glycolysis	Clough and Whittingham, 1983 Ellington, 1987
Adult Stem Cell Self-Renewal/Proliferation		
Cardiac progenitors	glutamine (mitochondrial function)	Salabei et al., 2015
MSC	glycolytic intermediates (FBP, PEP, OXA)	Jeong et al., 2019
HSCs	fatty acid oxidation valine, cysteine	Ito et al., 2012 Taya et al., 2016
MuSCs	↓mitochondrial/oxidative activity fatty acid oxidation	Pala et al., 2018 Theret et al., 2017 Machado et al., 2017
Adult Stem Cell Recruitment and Specification		
Cardiac progenitors	glutamine	
HSCs	glutamine mitochondrial complex III activity	Oburoglu et al., 2014 Ansó et al., 2017
MuSCs	↑OXPHOS ↓fatty acid oxidation	Pala et al., 2018 Theret et al., 2017 Gatta et al., 2017

Abbreviations: AA, amino acids; αKG, alpha-ketoglutarate; FBP, fructose 1,6-bisphosphate; PEP, phosphoenolpyruvic acid; OXA, sodium oxalate; OXPHOS, oxidative phosphorylation; SAM, S-adenosylmethionine; 2i/LIF, 2 inhibitors (MEK and ERK)/leukemia inhibitory factor naive embryonic stem cell culture medium.

availability and metabolic activity. Glucose and glutamine use in particular have been shown to modulate HSC fate and commitment both *in vitro* and *in vivo*, with glucose metabolism regulating ASC activation and cell-cycle entry (Oburoglu et al., 2014). Furthermore, loss of the mitochondrial complex III subunit RISP (Rieske iron-sulfur protein) in HSCs impairs differentiation *in vivo*, through a reduction in the multipotent progenitor population and decreased expression of HSC maintenance genes (Ansó et al., 2017). Consequently, cellular metabolism is not a passive bystander in stem cell maintenance or lineage specification. Rather, metabolism regulates cell fate decisions that may bias lineage specification and alter cell function (Table 1).

Metabolic Control of Muscle Stem Cells

Only recently has a well-defined metabolic roadmap for MuSC biology been clarified. Isolated quiescent satellite

cells exhibit low oxygen consumption and mitochondrial activity (Pala et al., 2018), and are characterized by a specific fatty acid oxidation signature (Machado et al., 2017). Their progression to a proliferative state is characterized by a burst of glycolysis, accompanied by higher fatty acid metabolism and OXPHOS, increased mitochondrial biogenesis and complexity, without a significant increase in oxygen consumption (Pala et al., 2018; Ryall et al., 2015), suggesting that other aspects of mitochondrial biology may have regulatory functions. In contrast, differentiation of satellite cells is associated with an increase in OXPHOS (Pala et al., 2018).

This metabolic signature is further demonstrated by decreased MuSC oxidative capacity as a result of 5′ AMP-activated protein kinase (AMPK) deletion, which correlates with an increase in self-renewal and delays their differentiation, compromising skeletal muscle regeneration (Theret et al., 2017); an observation that links innate cell metabolism with self-renewal. Conversely, a reduction in isolated MuSC self-renewal occurs following pharmacological inhibition of lactate dehydrogenase or by activating AMPK with compound 991 (Theret et al., 2017). Inhibition of fatty acid oxidation, which is a signature of the MuSC quiescent state, using the 3-ketoacyl-CoA thiolase inhibitor, trimetazidine, results in MuSC commitment and differentiation (Gatta et al., 2017). Furthermore, treatment with specific pharmacological inhibitors or inhibition of the expression of rate-limiting enzymes for fatty acid oxidation leads to altered differentiation of isolated MuSCs, indicating that satellite cell commitment and differentiation rely on peroxisomal, rather than mitochondrial fatty acid oxidation (Pala et al., 2018). These studies therefore establish a reliance on fatty acid oxidation during satellite cell quiescence, and a switch to OXPHOS and peroxisomal oxidation as MuSCs progress through activation, proliferation, and differentiation.

The transition from quiescence to the activated state is also tightly regulated by nutrient availability directly. Culture of MuSCs in galactose-supplemented medium forces cells to utilize OXPHOS, inhibiting self-renewal and resulting in decreased number of MuSCs (Theret et al., 2017). Moreover, lipid metabolism provides active metabolites for satellite cells. The sphingolipid sphingosine 1-phosphate modulates multiple key biological processes in satellite cells, including cell proliferation, motility, and differentiation, by activating the mitogen-activated protein kinases/extracellular signal-regulated kinases and AKT pathways, thereby promoting muscle regeneration (Calise et al., 2012). Short-term caloric restriction also induces an increase in MuSC number, associated with higher oxidative activity and a concomitant decrease in glycolysis, leading to enhanced muscle regeneration (Cerletti et al., 2012). Improvement in engraftment has been observed following MuSC transplantation into a mouse previously subjected to caloric restriction (Cerletti et al., 2012), suggesting that the nutrient microenvironment may regulate satellite cell biology and engraftment (Table 1). Hence, metabolic reprogramming of MuSCs, or of their niche, can be considered as a potential tool for improving muscle regeneration and/or transplantation efficiency.

Interestingly, distinct satellite cell metabolic features have enabled the identification of satellite cell sub-populations, with different proliferative and regenerative potential. In a rat model, two satellite cell sub-populations have been distinguished within the same muscle, characterized by specific metabolic profiles underpinned by differences in mitochondrial redox state, respiratory CO_2, and ATP production. Consequently, highly proliferative clones express higher levels of the glycolytic enzyme 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 and are stem-like compared with low proliferative clones (Repele et al., 2013). Therefore, different metabolic parameters are likely a basis for intrinsic MuSC diversity, which may be important for skeletal muscle to promptly respond to external stimuli.

With age, MuSCs become dysfunctional, decreasing the ability of skeletal muscle to successfully regenerate after damage (Blau et al., 2015). The interplay between metabolism and proteostasis maintains the reversible quiescence of satellite cells, which is lost in senescence. Pharmacological inhibition of mTOR with rapamycin improves MuSC function *in vivo*, at least in part by increasing autophagy and thereby promoting proteostasis (García-Prat et al., 2016). Interestingly, aged satellite cells are characterized by a reduction in most metabolic pathways, including the TCA cycle, respiratory electron transport chain, and mitochondrial fatty acid oxidation (Pala et al., 2018; Zhang et al., 2016b). Contrastingly, glycolysis is an exception, whereby aged satellite cells rely on glycolysis, rather than OXPHOS, for ATP production (Pala et al., 2018). These metabolic changes are consistent with mitochondrial dysfunction and a decrease in NAD+ levels associated with aging (Zhang et al., 2016b). Importantly, dietary supplement with NAD+ increases senescent MuSC number and the ability to regenerate muscles, by improving mitochondrial functions (Zhang et al., 2016b). In conclusion, nutrients and metabolism are not merely energy sources for the cell but finely define MuSC stage progression and senescence, thereby affecting regenerative potential and transplantation efficiency.

Epigenetics and Stem Cell Identity

Epigenetics is a key contributor to stemness, cell fate determination, and the maintenance of cell identity. Epigenetic modifications regulate gene expression, without changing the DNA sequence. These modifications represent an

Table 2. Stage-Specific Epigenetic Changes, Regulators, and Metabolites, Affecting Stem Cell Differentiation

Cell State	Epigenetic Marks	Biological Function	Writers/Erasers	Metabolites	Cell Fate Transition	Reference
Pluripotent	low levels DNA methylation	permissive chromatin compaction	DNMT/TET1, 2, 3	SAM, αKG, GlcNAc	favors undifferentiated state	Clara Lopes Novo et al., 2018 Shyh-Chang et al., 2013
	H3K4me3	transcriptional activation	Wdr5 Ash2 (TrxG)/JARID1 KDM2B	SAM, GlcNAC	favors self-renewal	Liu et al., 2016
	high levels histone acetylation	transcriptional activation	HATs (PCAF, p300, CBP, MOZ)/HDACs SIRTs	acetyl-CoA NAD^+	favors pluripotency	Battle et al., 2019
Differentiating	H3K9me3	gene repression	HMTs/JHDM	αKG	lineage commitment	Meshorer et al., 2006
	reduced histone acetylation	gene repression	HATs (PCAF, p300, CBP, MOZ)/HDACs SIRTs	acetyl-CoA NAD^+	lineage commitment	Ryall et al., 2015
	increased DNA methylation		DNMTs/TET1, 2, 3	SAM, αKG, GlcNAc	prevents alternative fates and allow differentiation	Chamberlain et al., 2008
	H3K27me3	transcriptional repression	PcG/UTX JMJD3	SAM, GlcNAc	prevents alternative fates	O'Carroll et al., 2001

Abbreviations: Ash2, ASH2-like histone lysine methyltransferase complex subunit; αKG, α-ketoglutarate; CBP, CREB-binding protein; DNMTs, DNA methyltransferases; GlcNAc, N-acetylglucosamine; H3K4me3, histone H3 lysine 4 trimethylation; H3K9me3, histone H3 lysine 9 trimethylation; H3K27me3, histone H3 lysine 27 trimethylation; HATs, histone acetyltransferases; HDACs, histone deacetylases; HMTs, histone methyltransferases; JARID1, histone demethylase; JMJD3, KDM1 lysine (K)-specific demethylase 6B; KDM2B, lysine demethylase 2B; MOZ, monocytic leukemia zinc finger protein; NAD^+, oxidized nicotinamide adenine dinucleotide; PCAF, P300/CBP-associated factor; SAM, S-adenosylmethionine; SIRTs, sirtuins; TET, ten-eleven translocation methylcytosine dioxygenase; TrxG, Trithorax Group; UTX, lysine (K)-specific demethylase 6A; Wdr5, WD repeat domain 5.

additional layer of genome interpretation and are mediated by DNA methylation, histone modifications, histone variants, chromatin remodeling, and gene regulation by non-coding RNAs (Atlasi and Stunnenberg, 2017). Intrinsic and extrinsic cues influence the acquisition of daughter cell identity during cell division, such that an epigenetic signature is gained as they progress toward lineage commitment and differentiation. The exclusive signature of these modifications allows sister cells sharing an identical genome to harbor a cell-type-specific landscape which defines their specific cell identity (Atlasi and Stunnenberg, 2017).

Epigenetic Control of ESCs

Progressive loss of pluripotency with differentiation of the ICM results in the establishment of the three germ layers, ultimately giving rise to more than 200 specialized cell types of the mammalian body. In this specialization process, the progressive decrease in cell differentiation potential is accompanied by a restriction in epigenetic plasticity, which delineates a cell-specific epigenetic pattern in resultant somatic cells. Thus, during lineage commitment and differentiation, a stage-specific epigenetic landscape grants the transcriptional machinery access to certain genes and defines a specific transcriptional profile (Table 2). The gradual transcriptional and epigenetic transition through different developmental stages in the embryo can be largely recapitulated *in vitro* with ESC differentiation.

The epigenetic landscape of undifferentiated ESCs features a permissive chromatin configuration, with limited DNA methylation and higher levels of histone acetylation than lineage-restricted cells (Arney and Fisher, 2004; Mousaieff et al., 2015). In addition, mouse and human ESC chromatin has been shown to be very dynamic, displaying a higher exchange rate of architectural histone proteins H1, H2B, H3, and HP1α, when compared with differentiated cells (Battle et al., 2019). Chromatin immunoprecipitation assays in ESCs and in the developing embryo have shown that regulatory regions of genes encoding key differentiation-associated transcription factors are characterized by the co-occurrence of the repressive histone modification, H3K27me3, with smaller regions of the permissive modification, H3K4me3, on the same allele (Bernstein et al., 2006). These regions have been termed "bivalent" domains and have been proposed to silence developmental regulators while keeping them poised for future activation. H3K4me3 is deposited by the chromatin factors Trithorax Group proteins, favoring gene activation, and counteracting the repressive action of Polycomb Group (PcG) silencing. Significantly, in murine ESCs, knockdown of the H3K4me3 regulators, Wdr5 and Ash2l, negatively

impacts self-renewal and impairs differentiation (Ang et al., 2011; Wan et al., 2013). These features in chromatin architecture and histone modifications suggest that ESCs are characterized by euchromatin, prone to transcriptional activation.

H3K27me3 is found in promoters of developmental genes in undifferentiated murine ESCs (Bernstein et al., 2006) and is detectable from the 2- to the 16-cell-stage of mouse embryo development, robustly increasing during the early blastocyst stage (Liu et al., 2016). The H3K27me3 mark is established by a protein complex belonging to the PcG proteins, which play a pivotal role in transcriptional repression during development. In undifferentiated ESCs, developmental genes bound by the pluripotency factors OCT4, NANOG, and SOX2 are targeted by PcG repressive complexes (Lee et al., 2006). Methylation of H3K27 is catalyzed by the PcG complex PRC2, which is composed of three fundamental proteins: embryonic ectoderm development (EED), suppressor of zeste 12, and the histone methyltransferase (HMT) enhancer of zeste homolog 2 (EZH2). PRC2 complex proteins are not required for ESC pluripotency (Chamberlain et al., 2008), but are indispensable for early mammalian embryo development and the correct formation of the three germ layers (O'Carroll et al., 2001). Indeed, knockdown of the EED component of PRC2 results in increased expression of certain lineage-related genes and aberrant differentiation (Chamberlain et al., 2008). Overall, these data highlight the key role played by the repressive mark H3K27me3 in ESC biology.

The epigenetic plasticity of pluripotent stem cells is progressively restricted during lineage commitment, with differentiation accompanied by the loss of chromatin accessibility, increased DNA methylation, and re-distribution of histone marks (Atlasi and Stunnenberg, 2017). As differentiation proceeds, bivalent loci are resolved, losing one of the two marks and becoming fully activated or stably silenced (Bernstein et al., 2006). Furthermore, heterochromatic markers display a dispersed localization in murine ESCs, but are detected as concentrated distinct foci in differentiated cells, with increased overall levels of H3K9me3, a histone mark linked to gene repression, and reduced levels of acetylated histones H3 and H4 (Meshorer et al., 2006). In contrast, DNA methylation and H3K27me3 are mutually exclusive at CpG islands in ESCs, where DNA methylation antagonizes H3K27me3 deposition (Atlasi and Stunnenberg, 2017). DNA methylation is catalyzed by DNA methyltransferases (DNMTs), which add a methyl group to the cytosine residue of CpG dinucleotides. DNMT1 and DNMT3A/B depletion in ESCs blocks their differentiation (Jackson et al., 2004), suggesting that regulation of DNA methylation is essential for differentiation. During ESC differentiation, DNMT3A is recruited to deacetylated regulatory regions of pluripotency genes and contributes to their transcriptional repression. In addition, DNMT3A also methylates promoters at a subset of genes that will be induced at later stages of differentiation. DNA methylation at these sites will be removed during the course of differentiation by the ten-eleven translocation (TET) family of enzymes, which uses O_2 to decarboxylate α-ketoglutaric acid for the conversion of 5mC to 5hmC, which is required for the formation of bivalent domains at CpG islands of these developmental genes (Kong et al., 2016). Therefore, the interplay between DNA methylation and histone modifications plays a key role in the regulation of ESC self-renewal and differentiation.

As differentiation progresses during implantation *in vivo*, chromatin accessibility becomes more selective, and repressive epigenetic marks, such as H3K27me3, and DNA methylation are deposited to prevent transcription of regulators of alternative fates, or to avoid expression of genes that would precociously lead to activation of other differentiation pathways (Spivakov and Fisher, 2007). Consequently, methylated DNA is thought to impair recruitment of transcription factors to DNA and to promote repressive chromatin through the association of methyl-binding proteins (e.g., MeCP2 or MDB1) and histone deacetylases (HDACs) (Klose and Bird, 2006), thus affecting chromatin accessibility and enhancer activity. During differentiation and lineage commitment, transcription factors establish the specific gene expression program that defines cell identity. Genome-wide studies have shown that transcription factor binding at distal enhancers activates epigenetic events that result in increased transcription. Enhancers are characterized by DNaseI hypersensitivity and enrichment in histone acetylases (HATs) p300/CBP, H3K4me1 and H3K27ac marks, and RNA polymerase II recruitment. More recently, a set of larger open chromatin domains has been described in mouse and human ESCs, denoted "super-enhancers." These regulatory regions control transcriptional activation of genes crucial for stem cell behavior and cell identity, and are markedly rewired in the epiblast-derived stem cell state relative to mouse and human ESCs (Clara Lopes Novo et al., 2018). Global histone acetylation increases at genes that are transcribed from day 0 to 5 during embryoid bodies formation (Markowetz et al., 2010). Therefore, dynamic and controlled regulation of the epigenetic landscape and gene activation permits the acquisition of cell-type-specific signatures to establish cell identity.

Overall, tight regulation of epigenetic events by chromatin modifiers shapes ESC plasticity and their changing transcriptional program toward commitment and differentiation. Identification of novel epigenetic marks and their regulation, along with the role of less-characterized modifications, such as GlcNAcylation, crotonylation,

propionylation, or succinylation, will further enhance our understanding of the control of ESC self-renewal and differentiation.

Epigenetic Control of ASCs

Within adult tissues, ASCs similarly maintain stemness and retain the potential to give rise to multiple differentiated cell types, preserving tissue homeostasis, and allowing tissue regeneration, through epigenetic modulation. H3K27me3 profiles in ASCs regulate and maintain lineage fidelity by preventing precocious induction of non-lineage-specific differentiation genes, repressing muscle differentiation. PRC2 loss of function affects stem cell proliferation capacity and tissue regeneration in different ASC types, such as neural and MuSCs (Pereira et al., 2010). The inability to self-renew is attributed to the upregulation of the Ink4a/Arf/Ink4b locus following PRC2 depletion (Caretti et al., 2004; Juan et al., 2011), and consequent cell-cycle arrest. EZH2 is expressed in MuSCs and its levels decrease during differentiation. During differentiation of MuSCs, when EZH2 levels decrease, myogenesis-related genes are de-repressed (Caretti et al., 2004). In addition, in response to tumor necrosis factor alpha, EZH2 interacts with p38 and represses PAX7 expression during regeneration, thus controlling the balance between proliferation and differentiation of satellite cells (Caretti et al., 2011; Palacios et al., 2010). These insights support a pivotal role for PRC2 and H3K27me3 in tissue regeneration and regulation of ASC self-renewal.

Although bivalent marks, indicative of a poised configuration, are also found in ASCs as in ESCs, they co-occupy fewer genes. In HSCs, many bivalent domains are resolved during differentiation, but a subset of promoters maintain bivalent marks after terminal differentiation (Abraham et al., 2013). Nevertheless, bivalency is not exclusive to stem or progenitor cells, because cells of restricted potency, including T cells, fibroblasts, and adult tissues, can also acquire *de novo* bivalency (Jadhav et al., 2016). Thus, bivalency may not simply represent a transient, plastic chromatin state during differentiation, but more likely a condition present in most cell types, which may provide the cell with a flexible chromatin configuration to respond to a changing environment.

Recent findings also hint to a dynamic role for DNA methylation in ASCs. At regulatory regions of developmental genes, the interplay between DNMTs and TET enzymes defines dynamic alterations in DNA methylation, which influence transcription factor association. For instance, in blood and skin stem cells, regulatory regions of differentiation-associated genes are methylated in progenitor cells and become progressively demethylated during cell differentiation (Bock et al., 2012). Similarly, DNMT1 is highly expressed in the central nervous system during embryogenesis and prevents neural stem cell differentiation (Namihira et al., 2009). During neurogenesis, DNMT3A methylates CpG islands of glial differentiation genes, preventing their activation (Wu et al., 2010).

Conversely, histone acetylation also influences chromatin accessibility to transcriptional machinery in ASCs, by modulating packed chromatin to a more relaxed state and providing a permissive chromatin state for recruitment of additional regulatory complexes. Loss of the acetyltransferase monocytic leukemia zinc finger protein severely reduces HSC lineage-committed progenitors and B lineage cells, revealing a critical function in hematopoiesis (Katsumoto et al., 2006). Histone acetylation is counterbalanced by the activity of HDACs, such as HDAC and sirtuins (SIRT). Combined HDAC1/2 ablation leads to severe hematopoietic failure (Wilting et al., 2010). HDAC1/2 deletion in epidermal cells impairs transcriptional repression by p63 on genes important in maintaining the proliferative potential of stem cells in the stratified epithelia, leading to the failure of mature hair follicle structures and epidermal proliferation and stratification (LeBoeuf et al., 2010).

In MuSCs, SIRT1 mediates the H4K16 deacetylation of MyoD regulatory regions, and SIRT1 ablation leads to premature differentiation *in vitro* and reduced regeneration ability *in vivo* (Ryall et al., 2015). HDAC4 deletion, instead, induces a derepression of the cyclin inhibitor p21 and Sharp1 gene expression, thereby modulating MuSC proliferation and differentiation *in vitro*, and muscle regeneration *in vivo* (Marroncelli et al., 2018). Moreover, HDAC4 deletion in skeletal muscle inhibits MuSC proliferation and differentiation via soluble factors (Renzini et al., 2018). Thus, the interplay between HDACs and HATs orchestrates transcriptional regulation both in ESCs and in adult tissue homeostasis.

Super-enhancers have been also described in ASCs, including hair follicle stem cells, where they undergo profound remodeling during lineage progression, resulting in selective decommission or activation of a subset of these regulatory regions (Adam et al., 2015). Overall, annotation of super-enhancers through the characterization of histone marks and transcription factor occupancy at these regulatory regions will be a future development in the epigenetic field to understand gene regulation in ESCs and ASCs and the regulation of cell identity.

Our knowledge of the epigenetic landscape of ASCs has improved significantly in the last decades, thanks to advances in genome technology and the development of improved isolation methods for ASCs. In the future, we expect to witness further progress toward characterization of epigenetic changes that occur during differentiation and ASC activation, particularly at the single-cell level, to understand specific spatial and temporal epigenetic states of ASCs in their niche.

Metabolic Regulation of Epigenetics in Stem Cells

As well as providing cellular energy, dynamic and temporal changes in metabolism directly affect nutrient availability and metabolite production that lead to changes in gene expression through alterations in epigenetic regulation. Metabolic control of the enzymes that regulate chromatin accessibility—e.g., HDACs, DNMTs, HMTs, or HATs—involves the use of metabolites as cofactors, the availability of which is strictly related to the energetic status of the cell (Berger and Sassone-Corsi, 2016). This implies a direct link between changes in metabolism and global consequences on the stem cell and differentiating epigenome, thereby influencing cell state, cell fate and identity, and consequently cell function.

The metabolic requirements of ESCs not only support the provision of energy, but also provide intermediates that underpin the pluripotent epigenetic landscape (Figure 1). Most proliferating cells rely on the catabolism of glucose to support biosynthetic requirements. Inhibition of glycolysis, with 2-deoxyglucose, leads to ESC differentiation (Carey et al., 2015) accompanied by decreased H3K9/H3K27 acetylation (Moussaieff et al., 2015). High rates of glycolysis drive the production of acetyl-CoA to modulate acetylation (Moussaieff et al., 2015), supporting a role for glycolytic activity in maintaining the pluripotent epigenetic landscape. Indeed, acetyl-CoA production through glycolysis rapidly decreases during differentiation of pluripotent stem cells, and supplementation of differentiating ESCs with acetate, a precursor of acetyl-CoA, is sufficient to prevent differentiation-induced loss of H3K9 and H3K27 acetylation, while fatty acid oxidation is relatively low in human ESCs (Moussaieff et al., 2015).

Glucose-dependent glutamate production by naive mouse ESCs similarly maintains low levels of histone methylation through the generation of αKG, an essential cofactor for both histone demethylases members of the Jumonji family (JHDM) and TET enzymes, involved in DNA demethylation (Figure 1). Glutamine deprivation leads to increased H3K9, H3K27, H3K26, and H4K20 trimethylation in naive ESCs, which can be reversed through supplementation with cell permeable αKG (Carey et al., 2015). As cells transition from naive to primed pluripotency, glucose-dependent glutamate production and intracellular αKG levels decrease, accompanied by a concomitant increase in histone methylation and a dependence on glutamine (Carey et al., 2015). In contrast, human ESC neural and endodermal differentiation is enhanced by the inclusion of αKG during induction, as is mouse differentiation induced by LIF withdrawal (TeSlaa et al., 2016), indicates cell state-dependent nutrient requirements. Importantly, dysregulation in αKG levels leads to deregulation of both DNA 5′-hydroxymethylcytosine and histone H3K9 tri-methylation levels in the regulatory regions of pivotal transcription factors, hampering ESC differentiation (Hwang et al., 2016), confirming that αKG can be modulated to manipulate stem cell fate.

Threonine metabolism also contributes significantly to the acetyl-CoA pool in mouse ESCs, but also to SAM synthesis, which acts as a cofactor for both DNMTs and HMTs (Shyh-Chang et al., 2013) (Figure 1). The reduction in mouse ESC proliferation and increase in differentiation observed following removal of threonine is accompanied by an increase in the SAM/S-adenosylhomocysteine ratio and a concomitant decrease in H3K4me3, which can be rescued by supplementation with threonine, glycine, or pyruvate (Shyh-Chang et al., 2013). Similarly, human ESCs display a requirement for SAM to support self-renewal, underpinned by regulation of methylation, via the metabolome or the catabolism of methionine (Shiraki et al., 2014; Sperber et al., 2015). Methionine deprivation leads to a significant decrease in H3K4 trimethylation and a reduction in global DNA methylation; however, the loss of methylation following short-term (5 h) deprivation could be abrogated by supplementation with SAM (Shiraki et al., 2014). These data therefore suggest that amino acids, including threonine and methionine, are critical for maintaining mouse and human ESC self-renewal via modulation of the pluripotent epigenetic landscape (Shiraki et al., 2014; Wang et al., 2009). Likewise, differentiation of ESCs induced by the amino acid proline are likewise associated with alterations in histone methylation (Comes et al., 2013), further linking amino acid metabolism to the regulation of the pluripotent and differentiating ESC epigenome.

In addition to glycolytic activity, glucose flux contributes to the activity of the hexosamine biosynthetic pathway. The hexosamine biosynthetic pathway is sensitive to amino acid, fatty acid, and nucleotide metabolism, and produces uridine diphosphate N-acetylglucosamine, a substrate necessary for the addition of O-linked-N-acetylglucosamine (O-GlcNAc) to serine or threonine residues of intracellular proteins, catalyzed by O-GlcNAc transferase (OGT) (Hanover et al., 2012). Indeed, reduced glucose availability or inhibition of O-GlcNAcylation leads to an impairment in ESC self-renewal and increased expression of several markers of differentiation, whereas increased global O-GlcNAc levels inhibits ESC differentiation (Jang et al., 2012). Such nutrient-sensitive post-translational histone modification further links metabolic state to stem cell fate via epigenetic regulation of gene transcription. Although OGT can associate with the TET protein family, the PRC2 complex is required for the correct cellular distribution of O-GlcNAcylation in mouse ESCs and to maintain normal OGT levels (Myers et al., 2011). Thus, O-GlcNAcylation likely modulates chromatin compaction and gene transcription either positively or

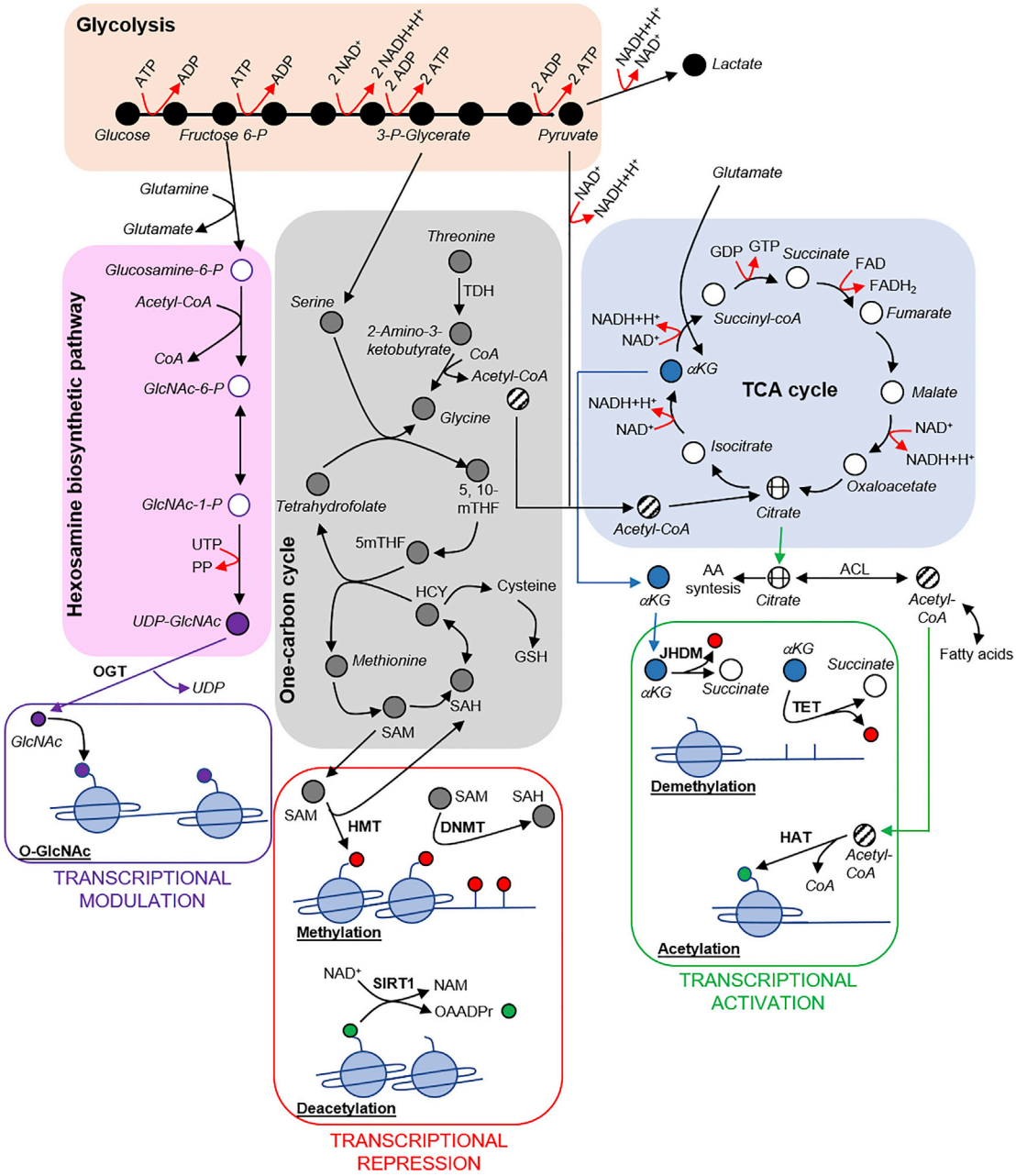

Figure 1. Metabolism Finely Regulates Chromatin Structure and Gene Transcription

Glycolysis provides intermediates such as fructose-6-phosphate (Fructose 6-P), 3-phosphoglycerate (3-P-Glycerate), and pyruvate, which trigger the hexosamine biosynthetic pathway, the one-carbon cycle, and the TCA cycle, respectively. The hexosamine biosynthetic pathway generates uridine diphosphate N-acetylglucosamine (UDP-GlcNAc), an N-acetylglucosamine (GlcNAc) donor for addition of O-linked N-acetylglucosamine (O-GlcNAc) to histone proteins. The one-carbon cycle produces S-adenosylmethionine (SAM), a donor for methylation of both DNA and histone proteins. The TCA cycle generates α-ketoglutarate (αKG), the main cofactor for both the histone demethylases members of the Jumonji family (JHDM) and TET enzymes, involved in DNA methylation. In addition, the TCA cycle generates citrate, which is converted by ATP-citrate lyase (ACL) to acetyl-CoA, which acts as an acyl donor for histone acetylation. Acetyl-CoA can derive from either glycolysis or fatty acid oxidation. NAD+ is a main cofactor for SIRT1-mediated histone deacetylation. Intracellular NAD+ levels are determined by glycolysis and oxidative phosphorylation. SIRT1 catalyzes the NAD+-dependent deacetylation of target proteins, which are regulated by this reversible lysine modification. During deacetylation, NAD+ is converted to nicotinamide (NAM) and the ribose accepts the acetyl group from substrate to produce O-acetyl-ADP-ribose (OAADPr). Red dots, methyl groups; green dots, acetyl groups.

(legend continued on next page)

negatively, depending on the partners with which it is associated (Figure 1).

Metabolites have also been shown to affect epigenetics and ASC behavior. As in ESCs, acetyl-CoA is an important acetyl donor for histone acetylation in ASCs. By regulating the availability of acetyl-CoA, the enzyme ATP-citrate lyase alters histone H3 acetylation status in the MyoD locus, promoting MuSC differentiation (Das et al., 2017). Similarly, NAD+, a co-substrate for SIRTs, regulates acetylation (Figure 1). During the exit from the quiescence state, a decrease in cellular NAD+ levels leads to an increase in the acetylation of H4K16 and contributes to the induction of the myogenic cell fate (Ryall et al., 2015). In response to high glucose, for instance, SIRT1 represses the Hes-1 promoter, thereby impairing neural stem cell proliferation. Conversely, upon caloric restriction, the transcription factor cAMP response element-binding protein replaces SIRT1 on Hes-1 promoter and activates neural stem cell proliferation (Fusco et al., 2016). Considering the aging-dependent decline in NAD+ cellular levels (Imai and Guarente, 2014), the epigenome may be directly and globally affected by this metabolic change. While the links between metabolism and the epigenome are still being elucidated, because aging induces both epigenetic and metabolic changes in stem cells, it will be important to delineate their intimate connections to better understand the molecular mechanisms underlying aging.

Flavin adenine dinucleotide (FAD), a redox coenzyme derived from the vitamin riboflavin (B2), is involved in several important enzymatic reactions in metabolism. FAD can also influence epigenetic changes via lysine-specific demethylase 1 (LSD1), a FAD-dependent histone 3 demethylase that suppresses gene expression by converting dimethylated H3K4 to mono- and unmethylated H3K4 (Shi et al., 2004). Increasing intracellular FAD has been shown to promote neural stem cell differentiation by increasing LSD1 nuclear localization and enzymatic activity (Hirano and Namihira, 2017).

In addition to the significant evidence that metabolites directly influence epigenetics, an inverse relationship has also been demonstrated, such that epigenetic factors regulate stem cell metabolism and fate. For instance, inactivation of SIRT1 in neural stem cells induces oligodendrocyte progenitor expansion and altered expression of metabolic genes (Rafalski et al., 2013). In HSCs, SIRT7 is important for maintaining their regenerative capacity, by repressing the nuclear respiratory factor 1-dependent metabolic genes and the mitochondrial unfolded protein response (Mohrin et al., 2015).

Further investigation focusing on how metabolism and nutrient availability affects more recently identified histone post-translational modifications (such as crotonylation, propionylation, or succinylation), as well as other acetylation/methylation residues, and linking these epigenetic modifications to changes in stem cell is needed.

Intergenerational Epigenetic Modifications Associated with Changes in Metabolism

Changes in nutrient intake have clearly been associated with changes in the epigenome in different species (Zhang and Kutateladze, 2018), with some alterations inherited by subsequent generations (Skvortsova et al., 2018). Indeed, metabolic challenges might affect either the epigenome of parental gametes or the development of primordial germ cells, thereby influencing next generations.

DNA methylation has been extensively studied as a transgenerational epigenetic mark, owing to its relative stability. In mice, starvation of pregnant F0 females results in metabolic phenotypes with altered DNA methylation at discrete loci in adult F1 sperm, which leads to significantly altered transcriptional profiles in F2 genes involved in metabolic functions (Radford et al., 2014). Intrauterine growth restriction has also been associated with F2 changes in blastocyst gene expression and nutrient utilization (Master et al., 2015), which manifest as metabolic disease in subsequent offspring (Master et al., 2014). In humans, deficiency in nutrients during early gestation impacts DNA methylation of the offspring (Heijmans et al., 2008). Moreover, small RNAs have been recently implicated in the intergenerational transmission of metabolic phenotypes in mammals. Indeed, small tRNA fragments inherited from sperm of fathers fed either a low-protein or high-fat diet, or those from obese, prediabetic fathers, mediate the repression of metabolic genes in mouse embryos (Cropley et al., 2016; St. John et al., 2019). Significantly, autologous mitochondrial supplementation has recently been shown to alter weight gain and gene expression in F1-F3 generations, accompanied by lesions of, and thickening to, the connective tissue of the atria-ventricular valves in several F1 and F2 mice (Portela and Esteller, 2010) suggesting that altered metabolism may be in part responsible for these effects.

ACL, ATP-citrate lyase; ADP, adenosine diphosphate; αKG, α-ketoglutarate; ATP, adenosine triphosphate; DNMT, DNA methyltransferase; FAD, flavin adenine dinucleotide; GlcNAc, N-acetylglucosamine; HAT, histone acetyltransferase; HCY, homocysteine; HMT, histone methyltransferase; JHDM, Jumonji domain-containing histone demethylase; NAD+, oxidized nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; NAM, nicotinamide; OAADPr, O-acetyl-ADP-ribose; O-GlcNAc, O-linked N-acetylglucosamine; OGT, O-linked β-N-acetylglucosamine transferase; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; SIRT1, sirtuin 1; TET, ten-eleven translocation methylcytosine dioxygenase; TDH, threonine dehydrogenase; THF, tetrahydrofolate.

Although these studies implicate broad changes in metabolism in transgenerational epigenetic inheritance, no studies have examined specific changes in individual co-substrates as regulators of chromatin-modifying proteins beyond the F1 generation. In addition, it is difficult to demonstrate a direct link between changes in the germ line epigenome and regulation of gene expression of the offspring (Lappalainen and Greally, 2017), or to exclude that the differences in DNA sequence among individuals may contribute to the inheritance of epigenetic states (Eckersley-Maslin et al., 2018). However, because the epigenome inherited from the gametes is heavily programmed during embryo development (Eckersley-Maslin et al., 2018), how the environment affects disease risk across generations may nonetheless be linked with metabolic regulation of the epigenome and highlights the need for further studies to assess the transgenerational impact(s) of individual metabolic perturbations at a systems biology level.

Implications for Stem Cell Application and Disease Models

The continued ability of stem cells to generate representative cell types is viewed as indicative of cell health. Indeed, stem cells have been noted as having remarkable plasticity with regard to their surrounding microenvironment *in vitro*, supporting their differentiation into many specified cell types. However, subtle changes in nutrient availability and medium composition can impact on pathway flux that result in an altered metabolic state and consequently epigenetic state (Zhang et al., 2016a). Although cells must balance cell stability and flexibility to respond to external stimuli, metabolic plasticity, however, comes at a price. Numerous studies have demonstrated a link between non-physiological nutrient availability and post-implantation embryo survival and development, as well as transgenerational impacts of diet. Consequently, perturbations persist, and often manifest post-implantation and, in the adult, mediated through permanent changes to the epigenome. Indeed, exposure of pre-implantation embryos to altered nutrient availability for even 6 h is sufficient to induce a loss in implantation potential (Lane and Gardner, 1998), highlighting the sensitivity of early development to nutrient availability. In an extension of this, perturbations in nutrient availability within the *in vitro* and *in vivo* stem cell niche will likely have consequences not only for self-renewal, recruitment, and differentiation, but may program cell function and viability through modulation of the epigenetic landscape, leading to dysfunction and disease (Moresi et al., 2015).

Our evolving understanding of the link between metabolic state and the epigenome therefore raises significant questions as to whether *in vitro* manipulation of nutrient availability induces perturbations in downstream cell function and health, a concern that is particularly relevant for the efficacy and safety of cell transplantation and disease models. Similarly, whether and/or how the *in vivo* metabolic environment hinders cell integration following transplantation, beyond our understanding of ischemia, is likely to lead to metabolic supplements as adjuncts to cell therapy. Plausibly, sub-optimal nutrient formulations may underpin poor stem cell transplantation retention and integration to date. Furthermore, metabolic and epigenetic factors may have developmental, stage- and tissue-specific functions, and the range of consequences of their disruption may vary depending on cellular activity. Whether modulation of the culture environment is therefore capable of expanding the number of cell types that can be differentiated remains to be explored. Such insights will assist in the design of more physiological media formulations and culture protocols to support long-term cell viability. One valuable and easily implemented starting point that must be considered is the incubator atmosphere. Although often viewed as innocuous and insignificant, the metabolite oxygen has been shown to alter the epigenetic landscape (Lamadema et al., 2019; Lees et al., 2019). However, given oxygen significantly alters nutrient use, particularly glycolysis (Harvey et al., 2016b; Lees et al., 2019) impacting on the availability of metabolites such as αKG and acetyl-CoA, the effects of modulating the availability of additional metabolites, including those described above that regulate stem cell state and epigenetics, in combination with physiological oxygen conditions is likely to further alter differentiation kinetics and cell fate decisions. The reluctance to change to a physiological atmosphere, while concerning, is, however, impeded by the decades of scientific work and understanding that have relied on cells surviving atmospheric oxygen culture (undoubtedly a selection), and apparent "plasticity" of cells to grow and differentiate under such sub-optimal conditions. However, as the aspirations for the clinical use of stem cells become a reality, the question becomes "at what cost" do we ignore the elephant in the room and risk destroying the future of stem cell translation should cells start to fail?

Conclusions

Cell metabolism intrinsically regulates the molecular signature of stem cells, affecting stemness and differentiation. Energetic state directly affects the epigenetic regulators that modulate gene transcription by altering the availability of cellular metabolites, pivotal cofactors of many chromatin remodeling factors, such as histone methyltransferases or acetylases. Since the local energetic reservoir regulates stem cell identity, metabolic reprogramming of stem cells should be considered as a potent tool for improving tissue regeneration.

There is clear need to understand the impact of *in vitro* culture on the interplay between nutrient availability and

epigenetic regulation. A wide knowledge gap exists relating to the impact of nutrient availability on both long-term stem cell maintenance and expansion, and during differentiation, particularly given often limited examination of cell physiology of endpoint cell types, as well as the permanence or reversibility of the epigenetic responses. Moreover, from a therapeutic perspective, additional studies are necessary to better understand the metabolic and epigenetic alterations that cause, rather than correlate with, diseases, and those which support tissue regeneration. The consideration of nutrient regulation of cell fate and function is of particular importance given emerging clinical trials exploring the use of *in vitro* cultured and expanded cells differentiated from pluripotent and ASCs, and their engraftment potential into a damaged niche. Plausibly, strategies to enhance regenerative therapies will need to consider nutrient modifications in parallel to ensure not only cell survival and engraftment, but also support the establishing epigenetic landscape.

AUTHOR CONTRIBUTIONS

A.H., G.C., and V.M. conceived, wrote, and edited the review, contributing equally to this work. A.R. and S.A. critically revised the manuscript and generated the figure and tables. All authors read and approved the final manuscript.

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