CellPress

Cell Metabolism
Review

Metabolism in Pluripotent Stem Cells and Early Mammalian Development

Jin Zhang,[1,*] Jing Zhao,[1] Perrine Dahan,[2] Vivian Lu,[3] Cheng Zhang,[4] Hu Li,[4] and Michael A. Teitell[2,5,6,7,8,9,*]
[1]The First Affiliated Hospital and Center for Stem Cell and Regenerative Medicine, Department of Basic Medical Sciences, School of Medicine, Institute of Hematology, Zhejiang University, Hangzhou, Zhejiang 310058, China
[2]Department of Pathology and Laboratory Medicine
[3]Department of Molecular and Medical Pharmacology
David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA 90095, USA
[4]Center for Individualized Medicine, Department of Molecular Pharmacology & Experimental Therapeutics, Mayo Clinic, Rochester, MN 55905, USA
[5]California NanoSystems Institute
[6]Department of Bioengineering
[7]Molecular Biology Institute
[8]Jonsson Comprehensive Cancer Center, David Geffen School of Medicine
[9]Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research
University of California, Los Angeles, Los Angeles, CA 90095, USA
*Correspondence: zhgene@zju.edu.cn (J.Z.), mteitell@mednet.ucla.edu (M.A.T.)
https://doi.org/10.1016/j.cmet.2018.01.008

Emerging and seminal studies have shown that cell metabolism influences gene expression by modifying the epigenome, which can regulate stem cell pluripotency, differentiation, and somatic cell reprogramming. Core pluripotency factors and developmental regulators reciprocally control the expression of key metabolism genes and their encoded pathways. Recent technological advances enabling sensitive detection methods during early mammalian development revealed the state-specific and context-dependent coordination of signal transduction, histone modifications, and gene expression in developing, resting, and malnourished embryos. Here, we discuss metabolism as a potential driver of earliest cell fate through its influence on the epigenome and gene expression in embryos and their *in vitro* surrogate pluripotent stem cells.

Introduction

In its broadest sense, cellular metabolism is a dynamic orchestration of interconnected signaling networks involving the coordination of nutrient utilization to generate energy (ATP) and biomass as well as energy consumption and biomass removal and recycling. The importance of cell metabolism is straightforward in certain contexts, such as building biomass for cell proliferation, compared with less obvious contexts, such as during cell-state transitions driven by epigenetic remodeling and specific gene activation or suppression. There is rapidly accumulating evidence for extensive, interdependent interactions between metabolism and epigenetics in cancerous transformation (Kinnaird et al., 2016), immune system activation and silencing (Raghuraman et al., 2016), and now stem cell maintenance and differentiation (Ryall et al., 2015). A robust example of concurrent changes in epigenome remodeling and cell proliferation occurs during early mammalian embryo development. Blastomeres formed by cleavage divisions do not grow and show minimal gene expression, which suddenly transitions to re-setting of the chromatin structure, massive genome transcription, protein translation, and increased cell proliferation following zygotic genome activation (ZGA) (Jukam et al., 2017). After ZGA, additional changes in epigenetic and gene expression programs occur during pre- and post-implantation embryonic development.

Pluripotent stem cells (PSCs) model cells from pre- and post-implantation embryos and grow indefinitely in culture. They have garnered tremendous attention because of multiple potential applications. Biochemical, molecular, and functional features of PSCs, including metabolism, are being intensively studied. Similarities and distinctions between PSC metabolism and metabolic features from early-stage embryos reported more than 40 years ago are being revisited today with newer, small sample techniques (Leese, 2012). This re-examination has practical consequences as investigators attempt to identify optimal *ex vivo* conditions for generating mature and functional cell types from PSC differentiation protocols.

In contrast to 40 years ago, today there are several types of cultured mammalian PSCs with differing developmental potential that resemble cells at different stages of early embryo development. "Naive"-state PSCs functionally resemble the pre-implantation blastocyst inner cell mass with differentiation potential for all three germ layers and primordial germ cells. "Primed"-state PSCs (Tesar et al., 2007; Brons et al., 2007) resemble post-implantation epiblast cells that have a low capacity to contribute to embryonic chimeras and a low competence for germline differentiation (Weinberger et al., 2016). "Extended" PSCs can form both embryonic and extraembryonic tissues, reminiscent of early blastomeres (Yang et al., 2017). A key challenge for studying early embryos are small sample sizes with limited starting materials. Fortunately, technological advances are beginning to provide versatile omics tools that can analyze the molecular features of small samples directly from early embryos (Lu et al., 2016; Wu et al., 2016; Xue et al., 2013). Profiling

studies are starting to reveal that metabolic programs are regulated by stage-specific transcription factors or other types of regulators and also that, reciprocally, metabolism may promote fate regulator expression in humans and mice. This new insight helps integrate metabolic patterns with upstream regulatory circuits and uncovers potential causal roles for specific aspects of metabolism that confer downstream cell-fate determination. Beyond development, epidemiologic studies on the pathological effects of parental and fetal malnutrition are increasingly being explored using small animal models (Sharma and Rando, 2017). It is possible that understanding metabolism in early development will uncover mechanisms for diseases with embryonic origins and also linked malnutrition causes. In this Review, we evaluate, interpret, and discuss recent metabolic findings for embryos and for *in vitro* surrogate cultured PSCs. We further assess the current state of integration of metabolism into the PSC regulatory circuitry and its potential impact on cell fate with implications for malnutrition in embryo development and causal connections to adult diseases.

Metabolic Features of PSCs

Embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs), collectively referred to as PSCs, are the most accessible cell types to study metabolic events in early mammalian development. Although valuable, PSC studies, nevertheless, may or may not represent the intrinsic metabolic features of their *in vivo* counterparts, as PSCs may acquire unique metabolic features to support infinite self-renewal in several types of culture conditions (Tang et al., 2010). A decade ago, studies began revealing unique patterns of cellular metabolism during PSC differentiation (Chung et al., 2007), during reprogramming to pluripotency (Folmes et al., 2011), and in naive-versus primed-state PSCs (Zhou et al., 2012). Combined, these studies showed a link between metabolic patterns and the loss, gain, or maintenance of PSC pluripotency.

In general, PSCs exhibit a high glycolytic flux to support high energy and biosynthetic demands during rapid proliferation (Folmes et al., 2011; Zhang et al., 2011). These metabolic features are also reacquired during nucleus reprogramming of somatic cells to iPSCs prior to the induction of pluripotency gene expression (Folmes et al., 2011; Kida et al., 2015). Robust metabolism in PSCs supports proliferation and metabolite production for epigenome modifications, as, for example, acetyl-CoA produced by glycolysis maintains histone acetylation and pluripotency (Moussaieff et al., 2015). Naive—or more primitive—and primed—or developmentally more advanced—stages of pluripotency have been defined for mouse PSCs. Naive PSCs and primed epiblast stem cells (EpiSCs) are both metabolically active, but naive PSCs respire more than primed EpiSCs (Zhou et al., 2012), which has been reviewed in detail previously (Teslaa and Teitell, 2015). This metabolic pattern could support the production of the TCA cycle intermediates for amino acid biosynthesis and the mitochondrial folate cycle for nucleotide biosynthesis. Higher oxygen consumption through respiration is consistent with a slightly lower proliferation rate for naive PSCs because nutrient carbons may be lost as CO_2 to lessen the rate of biomass accumulation required for cell replication. Differences in mitochondrial metabolism between naive and primed PSCs likely foster large epigenome differences, such as global hypomethylation in naive PSCs and hypermethylation in primed PSCs, because metabolites serve as co-factors or substrates for epigenome-modifying enzymes, as discussed below. Clearly, there are additional effects from active mitochondria in naive PSCs that need further study and that go beyond support for growth and proliferation. Finally, recent work has also begun to examine metabolic remodeling linked to lineage-specific differentiation, which has refined the concept that differentiation from pluripotency always requires a shift from glycolytic to oxidative metabolism. Sustained high-level glycolysis without an oxidative shift through maintained expression of MYC/MYCN during ectoderm lineage differentiation contrasts with a shift to oxidative metabolism and MYC/MYCN repression for mesoderm and endoderm lineage differentiation (Cliff et al., 2017).

Metabolism in Early Mammalian Embryos

In contrast to our increasing knowledge of cell metabolism in easily expandable PSCs, our understanding of metabolism in early mammalian embryos remains rudimentary and mostly based on whole-embryo nutritional needs (Brown and Whittingham, 1991), metabolic activities (Acton et al., 2004; Houghton et al., 1996), and the abundance of certain metabolites in whole embryos (Barbehenn et al., 1978). Few studies have dissected embryonic metabolism with spatial or temporal resolution or by specific cell types, such as differences and similarities between trophectoderm and the blastocyst inner cell mass (Gopichandran and Leese, 2003; Houghton, 2006; Robinson and Benos, 1991). Cultured PSCs are likely imperfect surrogates for *in vivo* counterparts within a developing embryo because microenvironment differences may cause variation in metabolism gene expression (Tang et al., 2010), which limits our extrapolation of *in vitro* PSC results. New transcriptome profiling studies of individual cells from each stage of early embryo development and each lineage within an embryo are providing expression data for all genes, including metabolism-regulating genes. Concurrently, new epigenome profiling studies requiring just a few cells (Liu et al., 2016; Zhang et al., 2016a) are providing potential mechanistic data for metabolism gene regulation and perhaps how certain environmental insults influence the epigenome at the earliest stages in mammalian development. A missing component for profiling technologies applied to embryogenesis is the metabolome, as current metabolomics and metabolic flux studies remain challenging for small numbers of cells.

Recent transcriptome and epigenome profiling has revealed clues for changes in metabolism during certain stages of early embryo development (Figure 1). Two studies examining single-cell RNA transcriptomes of mouse and human pre-implantation embryos showed the induction of genes controlling OXPHOS during the blastocyst stage of development (Yan et al., 2013; Xue et al., 2013). These results agree with prior studies that showed blastocysts consuming high levels of oxygen, most likely through mitochondrial respiration (Houghton et al., 1996; Leese, 2012). They also align well with naive PSCs that resemble pre-implantation epiblast cells (Boroviak et al., 2015) showing active respiration (Zhou et al., 2012). Open questions include which signals, epigenome modifications, and transcription factors activate expression and translation of OXPHOS genes. Also, what are the carbon fuel sources for the TCA cycle and respiration, and in what way(s) does respiration support the blastocyst and other early stages?

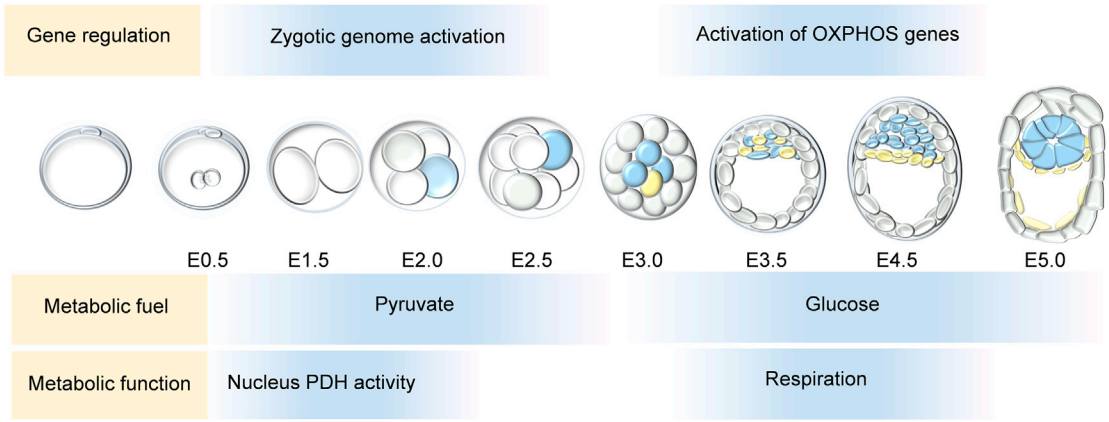

Figure 1. Dynamic Gene and Metabolic Regulation during Early Mouse Embryo Development
The nutrient pyruvate supports the one-cell-to-morula transition in mice prior to glucose becoming an essential nutrient. Enzymatically active PDH and a subset of the TCA cycle enzymes transiently localize into the nucleus at the two-cell stage in mice and between four- and eight-cell stages in human development, coincident with ZGA for each species. Mitochondrial respiration activates during the mouse blastocyst stage and inactivates after implantation, around the same time blastocyst epiblast activation of OXPHOS genes occurs as revealed by transcriptome profiling, and is consistent with high mitochondrial activity in cultured naive-versus primed-state PSCs. PDH, pyruvate dehydrogenase; ZGA, zygotic gene activation.

Transcriptome studies of mammalian diapause embryos, and embryos deficient in mTOR or MYC that resemble diapause (Bulut-Karslioglu et al., 2016; Scognamiglio et al., 2016; Boroviak et al., 2015), are providing new connections between metabolism and this physiologic dormant reproductive state. Naive ESCs deficient in both *c-Myc* and *N-Myc* enter a quiescent state with a strong reduction in protein and nucleic acid biosynthesis, similar to inhibition of MYC in blastocysts, which also induces a dormant state with reduced protein biosynthesis (Scognamiglio et al., 2016). Inhibition of mTOR in ESCs caused global transcription repression, and inhibition of mTOR in blastocysts led to paused embryo development, similar to mammalian diapause (Bulut-Karslioglu et al., 2016). Diapause embryos, with lower expression of c-*Myc* and *N-Myc* (Scognamiglio et al., 2016), maintain naive epiblast gene expression patterns and also show lowered expression of glycolytic genes and serine metabolism genes, such as *Aldoa* and *Phgdh* (Boroviak et al., 2015). Upstream inhibition of the one-carbon serine metabolism pathway might account for reductions in nucleotide biosynthesis, DNA/RNA metabolism, and lower proliferation in diapause (Locasale, 2013; Yang and Vousden, 2016). Whether diapause also induces other one-carbon metabolism changes, such as in S-adenosylmethionine (SAM) production that could alter the methylation state of the epigenome, or changes in the cellular redox state mediated by glutathione is currently unknown. An additional regulator of pluripotency, LIN28, is an intermediate in the MYC to mTOR pathway. LIN28 expression can be induced by c-MYC (Chang et al., 2009) and is co-regulated by ERK/MEK signaling as both c-MYC and LIN28A proteins are phosphorylated and stabilized by ERK (Sears et al., 2000; Tsanov et al., 2017). LIN28 also influences mTOR signaling by targeting IGF/PI3K/mTOR signaling pathway members through *let-7* in mouse embryonic fibroblasts (Zhu et al., 2011). Depletion of LIN28 in mouse ESCs also reduces one-carbon metabolism and nucleotide biosynthesis (Zhang et al., 2016b), similar to MYC-depleted ESCs or mTOR-suppressed ESCs. LIN28 suppression in mouse zygotes arrests development between two-cell and four-cell stages (Vogt et al., 2012), which is reminiscent of MYC or mTOR deficiency-caused embryo developmental dormancy. Whether this arrested transition is due to a metabolic deficiency alone, other gene regulatory network alteration, or both causes is currently unknown.

Remarkably, a subset of TCA cycle enzymes transiently localizes in the nucleus at the two-cell embryo stage in mice and between four- and eight-cell stages in human development, coincident with ZGA for each species, and seems to be critical for ongoing development (Nagaraj et al., 2017). Pyruvate is a nutrient that is required to support the one-cell-to-morula transition in mice prior to glucose becoming an essential nutrient (Brown and Whittingham, 1991). Interestingly, the nucleus localization of several TCA enzymes during the two-cell stage of development in mice is pyruvate dependent. Lack of pyruvate in the media of cultured mouse zygotes led to a two-cell stage arrest and a reduction in citrate and α-ketoglutarate (αKG), along with reduced H3K4Ac, H3K27Ac, and H3K27me3 epigenome marks and decreased global transcription activity. These data provide evidence for a specialized nucleus metabolic program during ZGA that is required for gene activation, likely through changes in the epigenome. The relationship between this metabolic program and waves of transcriptional activity during ZGA is currently unknown (Jukam et al., 2017).

Pluripotency Circuitry and the Metabolic Network

Distinct patterns of metabolic activity associate with specific developmental stages and cell fates. This correlation suggests probable integration between the circuitry that controls pluripotency and early development and the regulation of metabolic gene expression and function (Figure 2). Accumulating evidence from PSC studies supports this suggestion. For example, to maintain or induce pluripotency, developmentally activated *c-Myc* directly *trans*-activates glycolysis genes (Folmes et al., 2013; Cao et al., 2015). Also, development factor LIN28 regulates glycolysis by repressing *let-7*, which targets the glycolysis enzyme *Pdk1* (Ma et al., 2014) and influences OXPHOS gene expression through post-translational mechanisms in mouse

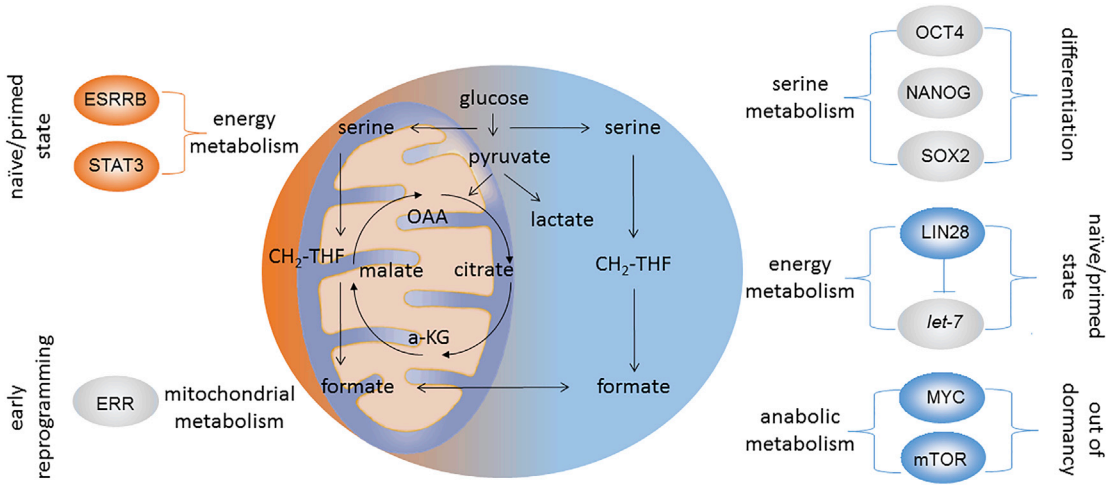

Figure 2. Glucose and One-Carbon Metabolic Pathways Regulated by Pluripotency and Early Development Factors

Naive PSC factor ESRRB activates mitochondrial OXPHOS, and STAT3 binds to the mitochondrial genome to enhance oxidative metabolism. Primed PSC factor LIN28 coordinates with *let-7* to enhance glycolysis and repress OXPHOS. Pluripotency core factors OCT4, NANOG, and SOX2 bind the serine metabolism *Psat1* gene promoter to activate transcription and influence mouse ESC differentiation. Nuclear factor ESRRB activates OXPHOS and c-MYC activates glycolysis at early stages of somatic cell reprogramming to iPSCs to pre-set the metabolism program before induction of pluripotency gene expression. MYC and mTOR are required for anabolic metabolism in mouse ESCs and embryos, with loss of mTOR and MYC generating a dormant state that resembles reproductive diapause and maintains features of naive pluripotency. In the diagram, orange-colored objects are regulators associated with naive-state metabolism. Blue-colored objects depict regulators that, upon depletion, may confer mouse ESCs' naive-like state features. Gray-colored objects indicate regulators related to metabolism in maintenance of general pluripotency, differentiation, and reprogramming.

ESCs (Zhang et al., 2016b). Pluripotency-associated *mir-290* cluster microRNAs in mouse ESCs activate glycolysis by repressing a reader for methylated CpG dinucleotides, MBD2, which represses *c-Myc* directly and glycolysis genes *Pkm2* and *Ldha* (Cao et al., 2015). The transcription factor ERR, which is a co-factor of PGC-1α that is transiently induced by somatic cell reprogramming with OSKM factors, generates a burst of OXPHOS activity at day 3 or 5 of mouse and human reprogramming, respectively (Kida et al., 2015). Finally, a serine metabolism gene, *Psat1*, is *trans*-activated by core pluripotency factors *Oct4/Sox2/Nanog* and mediates the production and effects of αKG on mouse ESC differentiation (Hwang et al., 2016). Combined, the evidence is strong and mounting that pluripotency and development factors coordinately regulate metabolic gene expression and activity in early mammalian development.

A transition between mouse PSC naive and primed states engages development factors *Stat3*, *Esrrb*, *Zic3*, and *Lin28a/b* and provides new details for how the metabolic network of these two pluripotent stages is regulated by this circuitry (Figure |Fig. 2) (Sone et al., 2017; Zhang et al., 2016b; Carbognin et al., 2016). For naive-state PSCs, *Stat3* sustains the nucleus naive gene expression profile and also interacts with the mitochondrial genome to activate genes promoting respiration (Carbognin et al., 2016). *Esrrb* induces OXPHOS through an unknown mechanism during the primed-to-naive-state transition, and it has a similar function during somatic cell reprogramming to pluripotency (Sone et al., 2017). *Esrrb* and *Zic3* synergistically enhance glycolysis during iPSC generation, and they combine to maintain pluripotency through direct binding of glycolysis promoting genes. Conversely, *Lin28a/b* represses OXPHOS to support state conversion in the opposite direction from the naive to primed state (Zhang et al., 2016b), and *Zic3* alone can repress OXPHOS and activate glycolysis to help establish the primed-state metabolic profile (Sone et al., 2017). Collectively, these reports show that key metabolic parameters are controlled by state-specific pluripotency and development factors, strongly suggesting that metabolism has an active, required role in shaping different pluripotent states.

To what Extent Can Metabolism Influence PSC and Early Embryo Fates?

Changes in metabolism could passively participate in PSC and early embryo fate determination driven by other regulatory factors, or they could help shape and even control fate outcomes (Zhang et al., 2012). Accumulating data strongly suggest an active role for metabolism in early cell-fate determination. Fate-influencing mechanisms are centered on several key metabolism-regulating signal transduction pathways, such as AMPK/mTOR (Bulut-Karslioglu et al., 2016), and intermediate metabolites, such as αKG, SAM, and acetyl-CoA (Ryall et al., 2015) (Figure 3). Changes in bioenergetics or nutrient availability resulting in a high ADP/ATP ratio inactivates the AMPK/mTOR pathway, as do mTOR inhibitors, and consequently stalls development similar to the physiologic diapause in rodent embryos (Bulut-Karslioglu et al., 2016). Additionally, AMPK/mTOR pathway activity is also required for post-implantation proliferation of embryonic and extraembryonic cells *in vivo* (Murakami et al., 2004). The TCA cycle metabolite αKG is a required co-factor for dioxygenase enzymes that include the Jumonji C (JmjC)-domain-containing histone demethylases (JHDMs) and Ten-eleven translocation (TET) DNA methylcytosine hydroxylases. αKG levels impact histone H3K9m3 and H3K27m3 marks to facilitate cell-fate outcomes between LIF/2i naive-state PSCs and a LIF/serum metastable PSC state (Carey et al., 2015) or FGF2/Activin-induced primed PSC state (Zhang et al., 2016b). αKG levels can also promote PSC differentiation through altering histone

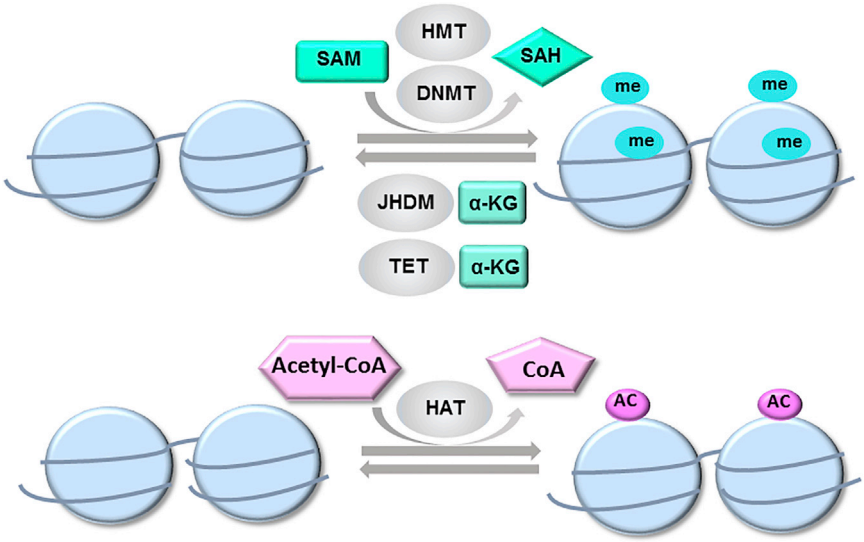

Figure 3. Reported Intermediate Metabolites that Regulate Histone and DNA Epigenetic Modifications in PSCs

S-adenosylmethionine (SAM) is a substrate for histone methyltransferases (HMTs) and DNA methyltransferases (DNMTs) in PSCs. Conversely, erasure of histone and DNA methylation marks by Jumonji C (JmjC)-domain-containing histone demethylases (JHDMs) and Ten-eleven translocation (TET) proteins requires α-ketoglutarate (αKG) as an enzyme co-factor. Acetyl-CoA is a substrate for histone acetyltransferases (HATs) in PSCs.

and DNA methylation levels in spontaneous mouse ESC differentiation (Hwang et al., 2016) and in directed human neuroectoderm differentiation (TeSlaa et al., 2016) even though the mechanisms of αKG production may vary in different cell contexts. Levels of the one-carbon metabolism pathway metabolite SAM influence H3K4me3 levels in mouse ESCs (Shyh-Chang et al., 2013) and in human ESCs (Shiraki et al., 2014). Acetyl-CoA produced during glycolysis affects histone acetylation and gene expression in both human and mouse ESCs, and modulation of acetyl-CoA levels either by inhibiting glycolysis enzymes or by supplementing acetate influences PSC pluripotency versus differentiation fates (Moussaieff et al., 2015). Key metabolites, including those that are produced intracellularly or those that are imported from the microenvironment or culture media, can substantially influence histone and/or DNA epigenetic marks. Nevertheless, our current understanding of how metabolic pathways influence cell fate is complicated by two unresolved challenges. One challenge is to provide evidence that the physiologic fluctuation of metabolite levels can cause significant changes of global or local chromatin modifications. Another challenge is to provide evidence that metabolism-mediated epigenetic changes can translate to specific changes in gene expression that are causal for cell-fate outcomes, which is briefly discussed below.

Interpreting the Influence of Metabolites on Epigenetics and Cell Fate

Studies to date provide relatively consistent and reinforcing data for interconnections between metabolite levels, epigenetic alterations, and fate outcomes for PSCs without resolving questions of specificity and causality. Which chromatin modifications during embryo development arise from differences in which metabolites? Which genes affected by metabolism-mediated changes in histone and/or DNA modifications are involved in cell-fate determination? Reporting correlations between metabolic rewiring, epigenetic modifications, gene expression, and cell fate is the status quo, and definitive studies showing that metabolism influences PSC fate through epigenetic alterations are still lacking. Direct evidence for causal connections and molecular mechanisms requires further studies with new tools and technologies, such as site-specific epigenetic modifiers that are under development to confer causality.

A second open area is to determine how much each nutrient source contributes to a metabolite pool of interest. This is a difficult question to address because it is technically infeasible to knock out all the genes controlling a specific metabolite, leaving extracellular metabolite addition or omission as the leading way to perturb some metabolites of interest. Also, metabolite pools arise from multiple sources, making assignments of nutrient origins potentially complex. For example, in PSCs, αKG originates from the TCA cycle via isocitrate dehydrogenase, from glutaminolysis through glutaminase (TeSlaa et al., 2016), or from serine metabolism through phosphoserine aminotransferase (Hwang et al., 2016). Also, SAM can be produced from exogenous or glucose-derived serine, from threonine metabolism in mouse ESCs (Shyh-Chang et al., 2013), or from methionine metabolism in human ESCs (Shiraki et al., 2014).

Another open question is evidence *in vivo* showing that metabolite levels control biochemical reactions to modify chromatin. For example, it has not been shown within a PSC nucleus that fluctuating αKG levels impact dioxygenase or demethylase enzyme activities. This would be difficult to do currently because, unlike *in vitro* biochemical reactions, there is no way to add or reduce only αKG without perturbing other systems within a cell. Similarly, a one-carbon subunit, such as a methyl group, from any nutrient source has not yet been shown to generate a methylation mark on a histone or deoxynucleotide. Therefore, development of new tools to monitor, quantify, and even manipulate specific metabolite levels within living cells will potentially provide causative links between changes in cell metabolism, the epigenome, and cell fate.

Embryo Malnutrition and Disease

Improved understanding of metabolism during embryonic development can lay a foundation for comparative studies of abnormal development due to environmental insults such as malnutrition. Malnutrition can predispose individuals to diseases that manifest later in life but whose origins begin during early development (Barker, 1997). Studies using small animal models have revealed that poor nutrition may impact germ cells, and this information can be transmitted between generations and show up as changes in epigenetic modifications and/or the expression of

small noncoding RNAs (Chen et al., 2016; Huypens et al., 2016; Sharma et al., 2016), as recently reviewed (Sharma and Rando, 2017). It is technically more difficult to tease out maternal effects of malnutrition for studies on early embryo development because placental insufficiency or hormone deregulation can also be caused by malnutrition in a pregnant female. In addition to malnutrition models, genetics studies are also useful. There is evidence that the consequences of malnutrition in the unborn depend upon genetic polymorphisms in *IGF2BP2* and *PPAR-γ2* from the Dutch Famine Birth Cohort (van Hoek et al., 2009; de Rooij et al., 2006). Therefore, genetic manipulations of key metabolism-related genes may help to model fetal malnutrition in animals, and such models can be potentially disentangled from maternal effects. For example, conditional knockout of *Lin28a* and *Lin28b* in embryos using tamoxifen-induced excision can cause aberrations in growth and glucose metabolism in adults (Shinoda et al., 2013). Since most metabolism genes are ubiquitously expressed and required for normal adult tissue development and physiology, transient reduction of these genes during embryo development may be preferable for modeling effects of transient malnutrition in utero. For instance, with an inducible dCAS9 fused to an activating or repressive domain, it is possible to transiently change a metabolism gene in the embryo, then determine acute metabolic alterations, and later determine long-term adult phenotype abnormalities and epigenome changes after the animal grows up. Once an animal model is established, critical questions can be asked, such as how a malnutrition signal is translated to long-lasting epigenetic memories that can be manifested in adult life, or even in the next generation.

Concluding Remarks

Great advances in our understanding of metabolism in PSCs have been made, with next steps to understand upstream regulators and downstream impact that are directly hard wired into PSC fates. Additionally, there are tremendous opportunities to understand metabolism in the physiological context of early embryo development, and in the pathological context of malnutrition and the early origins of diseases.

ACKNOWLEDGMENTS

Support for studies in the Zhang lab is by The Thousand Talents Plan Startup. Support for H.L. is by a grant from the Paul F. Glenn Foundation. Support for studies in the Teitell lab are by an Air Force Office of Scientific Research grant FA9550-15-1-0406; by National Institute of Health grants GM114188, GM073981, and CA185189; and by a California Institute for Regenerative Medicine grant RT3-07678.

REFERENCES

Acton, B.M., Jurisicova, A., Jurisica, I., and Casper, R.F. (2004). Alterations in mitochondrial membrane potential during preimplantation stages of mouse and human embryo development. Mol. Hum. Reprod. *10*, 23–32.

Barbehenn, E.K., Wales, R.G., and Lowry, O.H. (1978). Measurement of metabolites in single preimplantation embryos; a new means to study metabolic control in early embryos. J. Embryol. Exp. Morphol. *43*, 29–46.

Barker, D.J.P. (1997). Maternal nutrition, fetal nutrition, and disease in later life. Nutrition *13*, 807–813.

Boroviak, T., Loos, R., Lombard, P., Okahara, J., Behr, R., Sasaki, E., Nichols, J., Smith, A., and Bertone, P. (2015). Lineage-specific profiling delineates the emergence and progression of naive pluripotency in mammalian embryogenesis. Dev. Cell *35*, 366–382.

Brons, I.G.M., Smithers, L.E., Trotter, M.W.B., Rugg-Gunn, P., Sun, B., Chuva de Sousa Lopes, S.M., Howlett, S.K., Clarkson, A., Ahrlund-Richter, L., Pedersen, R.A., and Vallier, L. (2007). Derivation of pluripotent epiblast stem cells from mammalian embryos. Nature *448*, 191–195.

Brown, J.J., and Whittingham, D.G. (1991). The roles of pyruvate, lactate and glucose during preimplantation development of embryos from F1 hybrid mice in vitro. Development *112*, 99–105.

Bulut-Karslioglu, A., Biechele, S., Jin, H., Macrae, T.A., Hejna, M., Gertsenstein, M., Song, J.S., and Ramalho-Santos, M. (2016). Inhibition of mTOR induces a paused pluripotent state. Nature *540*, 119–123.

Cao, Y., Guo, W.-T., Tian, S., He, X., Wang, X.-W., Liu, X., Gu, K.-L., Ma, X., Huang, D., Hu, L., et al. (2015). miR-290/371-Mbd2-Myc circuit regulates glycolytic metabolism to promote pluripotency. EMBO J. *34*, 609–623.

Carbognin, E., Betto, R.M., Soriano, M.E., Smith, A.G., and Martello, G. (2016). Stat3 promotes mitochondrial transcription and oxidative respiration during maintenance and induction of naive pluripotency. EMBO J. *35*, 618–634.

Carey, B.W., Finley, L.W.S., Cross, J.R., Allis, C.D., and Thompson, C.B. (2015). Intracellular α-ketoglutarate maintains the pluripotency of embryonic stem cells. Nature *518*, 413–416.

Chang, T.-C., Zeitels, L.R., Hwang, H.-W., Chivukula, R.R., Wentzel, E.A., Dews, M., Jung, J., Gao, P., Dang, C.V., Beer, M.A., et al. (2009). Lin-28B transactivation is necessary for Myc-mediated let-7 repression and proliferation. Proc. Natl. Acad. Sci. USA *106*, 3384–3389.

Chen, Q., Yan, M., Cao, Z., Li, X., Zhang, Y., Shi, J., Feng, G.H., Peng, H., Zhang, X., Zhang, Y., et al. (2016). Sperm tsRNAs contribute to intergenerational inheritance of an acquired metabolic disorder. Science *351*, 397–400.

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.

Cliff, T.S., Wu, T., Boward, B.R., Yin, A., Yin, H., Glushka, J.N., Prestegaard, J.H., and Dalton, S. (2017). MYC controls human pluripotent stem cell fate decisions through regulation of metabolic flux. Cell Stem Cell *21*, 502–516.e9.

de Rooij, S.R., Painter, R.C., Phillips, D.I.W., Osmond, C., Tanck, M.W.T., Defesche, J.C., Bossuyt, P.M.M., Michels, R.P.J., Bleker, O.P., and Roseboom, T.J. (2006). The effects of the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor-γ2 gene on glucose/insulin metabolism interact with prenatal exposure to famine. Diabetes Care *29*, 1052–1057.

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., Martinez-Fernandez, A., Faustino, R.S., Yamada, S., Perez-Terzic, C., Nelson, T.J., and Terzic, A. (2013). Nuclear reprogramming with c-Myc potentiates glycolytic capacity of derived induced pluripotent stem cells. J. Cardiovasc. Transl. Res. *6*, 10–21.

Gopichandran, N., and Leese, H.J. (2003). Metabolic characterization of the bovine blastocyst, inner cell mass, trophectoderm and blastocoel fluid. Reproduction *126*, 299–308.

Houghton, F.D. (2006). Energy metabolism of the inner cell mass and trophectoderm of the mouse blastocyst. Differentiation *74*, 11–18.

Houghton, F.D., Thompson, J.G., Kennedy, C.J., and Leese, H.J. (1996). Oxygen consumption and energy metabolism of the early mouse embryo. Mol. Reprod. Dev. *44*, 476–485.

Huypens, P., Sass, S., Wu, M., Dyckhoff, D., Tschöp, M., Theis, F., Marschall, S., Hrabě de Angelis, M., and Beckers, J. (2016). Epigenetic germline inheritance of diet-induced obesity and insulin resistance. Nat. Genet. *48*, 497–499.

Hwang, I.Y., Kwak, S., Lee, S., Kim, H., Lee, S.E., Kim, J.H., Kim, Y.A., Jeon, Y.K., Chung, D.H., Jin, X., et al. (2016). Psat1-dependent fluctuations in α-ketoglutarate affect the timing of ESC differentiation. Cell Metab. *24*, 494–501.

Jukam, D., Shariati, S.A.M., and Skotheim, J.M. (2017). Zygotic genome activation in vertebrates. Dev. Cell *42*, 316–332.

Kida, Y.S., Kawamura, T., Wei, Z., Sogo, T., Jacinto, S., Shigeno, A., Kushige, H., Yoshihara, E., Liddle, C., Ecker, J.R., et al. (2015). ERRs mediate a

metabolic switch required for somatic cell reprogramming to pluripotency. Cell Stem Cell *16*, 547–555.

Kinnaird, A., Zhao, S., Wellen, K.E., and Michelakis, E.D. (2016). Metabolic control of epigenetics in cancer. Nat. Rev. Cancer *16*, 694–707.

Leese, H.J. (2012). Metabolism of the preimplantation embryo: 40 years on. Reproduction *143*, 417–427.

Liu, X., Wang, C., Liu, W., Li, J., Li, C., Kou, X., Chen, J., Zhao, Y., Gao, H., Wang, H., et al. (2016). Distinct features of H3K4me3 and H3K27me3 chromatin domains in pre-implantation embryos. Nature *537*, 558–562.

Locasale, J.W. (2013). Serine, glycine and one-carbon units: cancer metabolism in full circle. Nat. Rev. Cancer *13*, 572–583.

Lu, F., Liu, Y., Inoue, A., Suzuki, T., Zhao, K., and Zhang, Y. (2016). Establishing chromatin regulatory landscape during mouse preimplantation development. Cell *165*, 1375–1388.

Ma, X., Li, C., Sun, L., Huang, D., Li, T., He, X., Wu, G., Yang, Z., Zhong, X., Song, L., et al. (2014). Lin28/let-7 axis regulates aerobic glycolysis and cancer progression via PDK1. Nat. Commun. *5*, 5212.

Moussaieff, A., Rouleau, M., Kitsberg, D., Cohen, M., Levy, G., Barasch, D., Nemirovski, A., Shen-Orr, S., Laevsky, I., Amit, M., et al. (2015). Glycolysis-mediated changes in acetyl-CoA and histone acetylation control the early differentiation of embryonic stem cells. Cell Metab. *21*, 392–402.

Murakami, M., Ichisaka, T., Maeda, M., Oshiro, N., Hara, K., Edenhofer, F., Kiyama, H., Yonezawa, K., and Yamanaka, S. (2004). mTOR is essential for growth and proliferation in early mouse embryos and embryonic stem cells. Mol. Cell. Biol. *24*, 6710–6718.

Nagaraj, R., Sharpley, M.S., Chi, F., Braas, D., Zhou, Y., Kim, R., Clark, A.T., and Banerjee, U. (2017). Nuclear localization of mitochondrial TCA cycle enzymes as a critical step in mammalian zygotic genome activation. Cell *168*, 210–223.e11.

Raghuraman, S., Donkin, I., Versteyhe, S., Barrès, R., and Simar, D. (2016). The emerging role of epigenetics in inflammation and immunometabolism. Trends Endocrinol. Metab. *27*, 782–795.

Robinson, D.H., and Benos, D.J. (1991). Glucose metabolism in the trophectoderm and inner cell mass of the rabbit embryo. J. Reprod. Fertil. *91*, 493–499.

Ryall, J.G., Cliff, T., Dalton, S., and Sartorelli, V. (2015). Metabolic reprogramming of stem cell epigenetics. Cell Stem Cell *17*, 651–662.

Scognamiglio, R., Cabezas-Wallscheid, N., Thier, M.C., Altamura, S., Reyes, A., Prendergast, A.M., Baumgärtner, D., Carnevalli, L.S., Atzberger, A., Haas, S., et al. (2016). Myc depletion induces a pluripotent dormant state mimicking diapause. Cell *164*, 668–680.

Sears, R., Nuckolls, F., Haura, E., Taya, Y., Tamai, K., and Nevins, J.R. (2000). Multiple Ras-dependent phosphorylation pathways regulate Myc protein stability. Genes Dev. *14*, 2501–2514.

Sharma, U., and Rando, O.J. (2017). Metabolic inputs into the epigenome. Cell Metab. *25*, 544–558.

Sharma, U., Conine, C.C., Shea, J.M., Boskovic, A., Derr, A.G., Bing, X.Y., Belleannee, C., Kucukural, A., Serra, R.W., Sun, F., et al. (2016). Biogenesis and function of tRNA fragments during sperm maturation and fertilization in mammals. Science *351*, 391–396.

Shinoda, G., Shyh-Chang, N., Soysa, T.Y., Zhu, H., Seligson, M.T., Shah, S.P., Abo-Sido, N., Yabuuchi, A., Hagan, J.P., Gregory, R.I., et al. (2013). Fetal deficiency of lin28 programs life-long aberrations in growth and glucose metabolism. Stem Cells *31*, 1563–1573.

Shiraki, N., Shiraki, Y., Tsuyama, T., Obata, F., Miura, M., Nagae, G., Aburatani, H., Kume, K., Endo, F., and Kume, S. (2014). Methionine metabolism regulates maintenance and differentiation of human pluripotent stem cells. Cell Metab. *19*, 780–794.

Shyh-Chang, N., Locasale, J.W., Lyssiotis, C.A., Zheng, Y., Teo, R.Y., Ratanasirintrawoot, S., Zhang, J., Onder, T., Unternaehrer, J.J., Zhu, H., et al. (2013). Influence of threonine metabolism on S-adenosylmethionine and histone methylation. Science *339*, 222–226.

Sone, M., Morone, N., Nakamura, T., Tanaka, A., Okita, K., Woltjen, K., Nakagawa, M., Heuser, J.E., Yamada, Y., Yamanaka, S., and Yamamoto, T. (2017). Hybrid cellular metabolism coordinated by Zic3 and Esrrb synergistically enhances induction of naive pluripotency. Cell Metab. *25*, 1103–1117.e6.

Tang, F., Barbacioru, C., Bao, S., Lee, C., Nordman, E., Wang, X., Lao, K., and Surani, M.A. (2010). Tracing the derivation of embryonic stem cells from the inner cell mass by single-cell RNA-seq analysis. Cell Stem Cell *6*, 468–478.

Tesar, P.J., Chenoweth, J.G., Brook, F.A., Davies, T.J., Evans, E.P., Mack, D.L., Gardner, R.L., and McKay, R.D. (2007). New cell lines from mouse epiblast share defining features with human embryonic stem cells. Nature *448*, 196–199.

Teslaa, T., and Teitell, M.A. (2015). Pluripotent stem cell energy metabolism: an update. EMBO J. *34*, 138–153.

TeSlaa, T., Chaikovsky, A.C., Lipchina, I., Escobar, S.L., Hochedlinger, K., Huang, J., Graeber, T.G., Braas, D., and Teitell, M.A. (2016). α-Ketoglutarate accelerates the initial differentiation of primed human pluripotent stem cells. Cell Metab. *24*, 485–493.

Tsanov, K.M., Pearson, D.S., Wu, Z., Han, A., Triboulet, R., Seligson, M.T., Powers, J.T., Osborne, J.K., Kane, S., Gygi, S.P., et al. (2017). LIN28 phosphorylation by MAPK/ERK couples signalling to the post-transcriptional control of pluripotency. Nat. Cell Biol. *19*, 60–67.

van Hoek, M., Langendonk, J.G., de Rooij, S.R., Sijbrands, E.J.G., and Roseboom, T.J. (2009). Genetic variant in the IGF2BP2 gene may interact with fetal malnutrition to affect glucose metabolism. Diabetes *58*, 1440–1444.

Vogt, E.J., Meglicki, M., Hartung, K.I., Borsuk, E., and Behr, R. (2012). Importance of the pluripotency factor LIN28 in the mammalian nucleolus during early embryonic development. Development *139*, 4514–4523.

Weinberger, L., Ayyash, M., Novershtern, N., and Hanna, J.H. (2016). Dynamic stem cell states: naive to primed pluripotency in rodents and humans. Nat. Rev. Mol. Cell Biol. *17*, 155–169.

Wu, J., Huang, B., Chen, H., Yin, Q., Liu, Y., Xiang, Y., Zhang, B., Liu, B., Wang, Q., Xia, W., et al. (2016). The landscape of accessible chromatin in mammalian preimplantation embryos. Nature *534*, 652–657.

Xue, Z., Huang, K., Cai, C., Cai, L., Jiang, C.Y., Feng, Y., Liu, Z., Zeng, Q., Cheng, L., Sun, Y.E., et al. (2013). Genetic programs in human and mouse early embryos revealed by single-cell RNA sequencing. Nature *500*, 593–597.

Yan, L., Yang, M., Guo, H., Yang, L., Wu, J., Li, R., Liu, P., Lian, Y., Zheng, X., Yan, J., et al. (2013). Single-cell RNA-seq profiling of human preimplantation embryos and embryonic stem cells. Nat. Struct. Mol. Biol. *20*, 1131–1139.

Yang, M., and Vousden, K.H. (2016). Serine and one-carbon metabolism in cancer. Nat. Rev. Cancer *16*, 650–662.

Yang, Y., Liu, B., Xu, J., Wang, J., Wu, J., Shi, C., Xu, Y., Dong, J., Wang, C., Lai, W., et al. (2017). Derivation of pluripotent stem cells with in vivo embryonic and extraembryonic potency. Cell *169*, 243–257.e25.

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.

Zhang, J., Nuebel, E., Daley, G.Q., Koehler, C.M., and Teitell, M.A. (2012). Metabolic regulation in pluripotent stem cells during reprogramming and self-renewal. Cell Stem Cell *11*, 589–595.

Zhang, B., Zheng, H., Huang, B., Li, W., Xiang, Y., Peng, X., Ming, J., Wu, X., Zhang, Y., Xu, Q., et al. (2016a). Allelic reprogramming of the histone modification H3K4me3 in early mammalian development. Nature *537*, 553–557.

Zhang, J., Ratanasirintrawoot, S., Chandrasekaran, S., Wu, Z., Ficarro, S.B., Yu, C., Ross, C.A., Cacchiarelli, D., Xia, Q., Seligson, M., et al. (2016b). LIN28 regulates stem cell metabolism and conversion to primed pluripotency. Cell Stem Cell *19*, 66–80.

Zhou, W., Choi, M., 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-to-EpiSC/hESC transition. EMBO J. *31*, 2103–2116.

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.