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Issue: *Evolving Challenges in Promoting Cardiovascular Health***Energy metabolism plasticity enables stemness programs**

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Engineering pluripotency through nuclear reprogramming and directing stem cells into defined lineages underscores cell fate plasticity. Acquisition of and departure from stemness are governed by genetic and epigenetic controllers, with modulation of energy metabolism and associated signaling increasingly implicated in cell identity determination. Transition from oxidative metabolism, typical of somatic tissues, into glycolysis is a prerequisite to fuel-proficient reprogramming, directing a differentiated cytotype back to the pluripotent state. The glycolytic metabolite supports the anabolic and catabolic requirements of pluripotent cell homeostasis. Conversely, redirection of pluripotency into defined lineages requires mitochondrial biogenesis and maturation of efficient oxidative energy generation and distribution networks to match demands. The vital function of bioenergetics in regulating stemness and lineage specification implicates a broader role for metabolic reprogramming in cell fate decisions and determinations of tissue regenerative potential.

Keywords: bioenergetics; glycolysis; oxidative metabolism; regenerative medicine; induced pluripotent stem cells; embryonic stem cells; lineage specification

Regenerative medicine is poised to transform medical practice by providing the prospect of definitive solutions for patients with degenerative diseases, for which curative therapies are currently lacking.^{1,2} With aging of the global population, chronic non-communicable diseases—led by the surge in cardiovascular disorders—are a recognized emerging pandemic.^{3,4} Expanding the reach of current state-of-the-art therapies, stem cell-based reconstructive strategies aim at repairing disease pathobiology and restoring organ function. Through cell engraftment, growth and lineage specification, and/or recruitment of innate repair mechanisms, regenerative medicine is primed to advance care beyond palliation for a range of diseases, including cardiovascular conditions.^{5,6} To date, clinical experience relies on the use of adult stem cells, which reside in natural body compartments, including the blood, adipose tissue, and bone marrow, but are restricted in their capacity for spontaneous lineage specification.⁷ Beyond use of stem cells in their native state, recent evidence indicates that lineage

prespecification offers enhanced therapeutic benefit.^{8,9} A case in point is cardiopoiesis, whereby guided specification of the stem cell—source has been demonstrated as advantageous in the setting of heart failure therapy.^{10,11} In this context, deconvolution of cellular fate plasticity is a key strategy for advancing the applications of cell-based regenerative medicine.^{12–14}

Cell fate redirection

Remarkably, stem cells are not the sole regenerative source. Indeed, redirection of somatic differentiated cells back to the pluripotent state and transdifferentiation into alternative specialized lineages has recently been reported.^{15–17} Why a specialized cell would maintain the potential to reactivate gene programs typical of another cell type is unknown. Yet, the uncovered cellular plasticity would endow the body with an innate repair capacity, with important implications for regenerative medicine applications. Cell fate redirection is

achieved by perturbing the expression of specific combinations of transcriptional regulators that are naturally dormant in differentiated populations. In this way, nuclear reprogramming by overexpression of primordial transcription factor cocktails is sufficient to reset the gene expression pattern and somatic epigenetic landscape to an embryonic-like state.^{18–20} Such induced pluripotent stem (iPS) cells recapitulate many of the features of natural inner cell mass–derived embryonic stem cells (ESCs), including their ability to give rise to tissues of all lineages, which defines genuine pluripotency.^{21–30} The broad applications of iPS cells range from diagnostic platforms to unravel individual variation in disease susceptibility to personalized biotherapeutics offering next generation tools for functional regeneration.^{31,32}

Metabolism in cell fate decisions

Beyond manipulation of the genetic and epigenetic state, modulation of energy metabolism and metabolic signaling has been implicated in cell fate decisions.^{33–35} Examination of cellular bioenergetics documents that modulation of mitochondrial infrastructure and metabolic pathways is vital for crosstalk with genetic programs ensuring direction of cell fate.^{35,36} With emphasis on dedifferentiation of somatic cells back to the pluripotent ground state and subsequent redifferentiation into specific lineages, this cytotype interconversion implicates mitochondrial dynamics and energy metabolism as a rheostat-controlling cell identity (Fig. 1).

Metabolic control of dedifferentiation

Metamorphosis of metabolic infrastructure

Dedifferentiation of somatic cells back to the pluripotent ground state requires dramatic remodeling of the metabolic infrastructure to support the anabolic and catabolic requirements of pluripotent cells. Nuclear reprogramming induces a reduction in mitochondrial DNA (mtDNA), which results in diminished mitochondrial density compared to the parental somatic source, and similar to that observed in ESCs, the quintessential stemness archetype.^{37–46} Mitochondrial localization also transitions during nuclear reprogramming from extensive cytoplasmic networks to a predominately embryonic perinuclear localization.^{37,39,41,43,47–51} The perinuclear mitochondrial localization has been proposed to be a marker of stemness, as it is also observed in human hematopoietic and mesenchymal stem cells.^{52,53} Remaining mitochondria undergo structural regression from mature mitochondria of somatic cells, characterized by branched and elongated structures with extensive intracellular membranes (cristae), to the predominantly spherical and cristae poor structures of iPS cells.^{37,39,41–43}

Transcriptional profiling has revealed a significant remodeling of genes contributing to mitochondrial function and energy metabolism, with the upregulation of mitochondrial biogenesis genes during reprogramming, while expression of nuclear encoded mitochondrial genes remains constant.^{37,54} A significant reconfiguration of glucose metabolism also occurs, with the upregulation of the initial and final steps of glycolysis and the nonoxidative branch

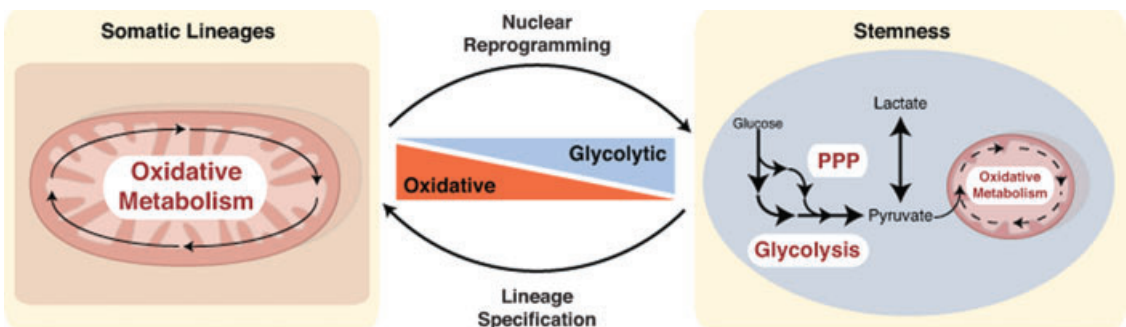


Figure 1. Energy metabolism plasticity regulates the balance between stem cell pluripotency and lineage specification. Somatic lineages efficiently generate ATP through complete oxidation of substrates in the mitochondria. In contrast, stem cells use glycolysis and the pentose phosphate pathway (PPP) to meet the anabolic and catabolic demands of stemness. The balance between glycolysis and oxidative metabolism contributes to the determination of cell fate, with nuclear reprogramming–induced mitochondrial regression and a greater reliance on glycolysis, while lineage specification results in mitochondrial biogenesis and the maturation of oxidative metabolism.

of the pentose phosphate pathway and downregulation of the intermediate reactions of glycolysis (*GPI*, *PFK*, and *ALDO*).^{43,55} Bisulfite sequencing identified a large number of nuclear reprogramming-induced epigenetic modification of genes involved in glycolysis and oxidative metabolism.⁵⁶ In somatic cells undergoing reprogramming, upregulation of glycolytic genes precedes expression of pluripotent markers, suggesting a requirement for a metabolic switch in fueling reprogramming processes.³⁹ Transcriptional remodeling translates into a predominant downregulation of the subunits of the electron transport chain and upregulation of glycolytic enzymes in iPS cells, similar to that observed in ESCs.³⁹ Specifically, the isoform switch from hexokinase I to II and the upregulation of pyruvate dehydrogenase kinase—critical components at the mitochondrial–glycolysis interface—contribute significantly to induction of pluripotency, as inhibition of either of these targets reduces reprogramming efficiency.^{39,43} Remodeling of the metabolic infrastructure is thus an essential and consistent attribute of nuclear reprogramming and supports the bioenergetic transition during pluripotent induction.

Metabolic switch defines pluripotency

Somatic cells completely oxidize metabolic substrates in the mitochondria to meet the energetic demands of cellular homeostasis. Nuclear reprogramming-induced regression of mitochondrial morphology would therefore suggest a significant impact on mitochondrial function. Pluripotent cells have elevated mitochondrial membrane potential compared to their parental somatic source, maintaining these cells in an energetically nascent, yet responsive state poised to meet the demands imposed by redifferentiation.^{38,39,57} Direct assessment of mitochondrial oxidative competence indicates that pluripotent cells have reduced basal oxygen consumption and limited reserve capacity.^{39,43} Although iPS cells have reduced energy turnover and total cellular adenosine triphosphate (ATP) levels compared to their parental sources,^{37,39,42,43} the reduced oxidative capacity would require the use of alternative ATP-generating pathways to meet bioenergetic demands of self-renewal and proliferation. In this regard, recent metabolomics studies have indicated a bioenergetic switch from somatic oxidative metabolism to glycolysis during nuclear reprogramming (Fig. 1).^{39,56} iPS cells have a

metabolome resembling that of ESCs but significantly different than parental cells, consistent with the upregulation of glycolysis, including elevated use of glucose and accumulation of lactate, and the downregulation of metabolites involved in tricarboxylic acid cycle and cellular respiration.^{37,39,43,55,56} This observation is consistent across species and cell lines, indicating that the metabolic transition is a required marker of nuclear reprogramming success.^{37,39,40,43,55}

Although the metabolome of iPS and ESCs are convergent, they are not necessarily identical, with iPS cells demonstrating lower abundance of unsaturated fatty acids and higher abundance of metabolites in the S-adenosyl methionine cycle.⁵⁶ High levels of unsaturated metabolites commonly found in ESCs are important for suppression of oxidative metabolism and maintenance of the pluripotent state, while S-adenosyl methionine is a key substrate for transmethylation reactions.^{56,58} Indeed, supplementation with metabolites from either of these pathways significantly reduces nuclear reprogramming efficiency.⁵⁶ It remains unknown if other metabolic pathways that help to maintain pluripotency in ESCs, such as threonine metabolism and purine biosynthesis,⁵⁹ would significantly alter the efficiency of nuclear reprogramming.

Targeting energy metabolism for stemness induction

Energy metabolism is a novel target that can be manipulated to regulate the efficiency of nuclear reprogramming. Hypoxic stimulation of glycolytic flux improves the maintenance of stem cell pluripotency^{60–63} and augments reprogramming efficiency.⁶⁴ Alternatively, inhibition of the p53 pathway, which in part stimulates glycolysis, also potentiates reprogramming.^{65–70} Direct pharmacological modulation of energy metabolism or supplementation with glycolytic intermediates, accelerates glycolysis to augment reprogramming efficiency.^{39,71} As proof of principle, agents that inhibit glycolysis and/or stimulate oxidative metabolism significantly suppress reprogramming efficiency.^{39,71} The significance of optimizing energy metabolism during nuclear reprogramming is evidenced by the ability to reprogram cells with only a single stemness factor, OCT4, when glycolysis is stimulated in the presence of histone deacetylase, TGF β , and MAPK/ERK inhibitors.⁷¹ Therefore,

a thorough understanding of the bioenergetic requirements of nuclear reprogramming, will allow for optimization of energy metabolism to promote pluripotency induction.

The glycolytic state in pluripotent cells may be required to fuel both catabolic and anabolic requirements.^{33,72} Under the abundant supply of metabolic substrates in normal cell culture conditions, pluripotent cells may benefit from a faster rate of ATP generation from glycolysis, without being limited by the pathway's inefficient ATP generation.^{33,35,73} Glycolysis in conjunction with the pentose phosphate pathway also provides a source of biosynthetic substrates and reducing cofactors that could match the anabolic requirements of cell proliferation. As the environment and complete oxidation of metabolic substrates cannot meet the demand for these cellular constituents, the glycolytic network can provide a capacity for generation and distribution of ATP and anabolic precursors to support cell proliferation and cellular homeostasis.^{33,35,47,72} Anaerobizing of somatic oxidative metabolism into pluripotent glycolysis thus fuels nuclear reprogramming and allows pluripotent cells to meet both anabolic and catabolic requirements.

Metabolic control of redifferentiation

Oxidative metabolism maturation fuels lineage specification

Remodeling of the mitochondrial and metabolic infrastructure matches the evolving bioenergetic requirements, as cells with a high energetic demand, such as the cardiomyocyte, drive the requirement for efficient oxidative ATP generation.^{35,47,57} Spontaneous differentiation of stem cells is initiated by downregulation of pluripotent genes and the stimulation of mtDNA replication, which ultimately results in elevated mtDNA copy number to support mitochondrial biogenesis.^{37,42,46,50,51,57,74} Concomitant to the increasing density of mitochondria is a maturation of their ultrastructure and localization to form networks of elongated and cristae-rich structures to allow energy transfer between specific cellular compartments.^{37,42,47,50,51,57,75–77} Differentiation into lineages, especially those with high energetic demands, involves upregulation of tricarboxylic acid enzymes and electron transport chain subunits to support acceleration of cellular respiration and oxygen consumption to increase ATP produc-

tion.^{47,50,57,74} Maturation of metabolic signaling and phosphotransfer infrastructure supports the glycolytic to oxidative metabolism transition in lineage specification (Fig. 1).^{75,78} Neuronal and cardiac progeny derived from ESCs have a more saturated metabolome compared to their parental counterparts, consistent with a significant change in redox status during differentiation.⁵⁸ Treatment of ESCs with saturated substrates, including eicosanoids, saturated fatty acids, and acyl-carnitines promotes neuronal and cardiac differentiation.⁵⁸ Although these changes in bioenergetics coincide with differentiation of pluripotent cells, it remains unknown if a specific mitochondrial function and/or metabolic capacity must be obtained to overcome bioenergetic barriers and define specific lineages.

Metabolic markers of differentiation

The recent literature has demonstrated a growing role for mitochondrial dynamics and energy metabolism in driving pluripotent cell fate specification.^{34,35,39} Inherent cell bioenergetic markers have been utilized to define the differentiation capacity of pluripotent cells. Despite these cells having similar expression of pluripotency markers and morphology, subsets of cells with less perinuclear localized mitochondria⁷⁹ and low-resting mitochondrial membrane potential have greater spontaneous differentiation.⁸⁰ Inhibition of maturation of the mitochondria and extended metabolic network inhibits differentiation,^{57,78,81,82} although inhibition of the mammalian target of rapamycin, which significantly reduces mitochondrial oxygen consumption, increases mesodermal differentiation.⁸⁰ Mitochondrial content and function also defines the differentiation capacity of mesoangioblasts, a pre-committed cardiac progenitor, with slow dividing cells, that contain abundant mitochondria with high membrane potential, efficiently differentiating into cardiomyocytes, while fast-dividing cells with few mitochondria do not respond to differentiation stimuli.⁸³ However, mitochondrial function may only be required during differentiation not for the maintenance of the progenitor state, as oxygen consumption is only elevated in the fast-dividing cells following induction of differentiation.⁸³ The differentiation block in fast-dividing mesoangioblasts can be reversed by increasing mitochondrial content, while loss of mitochondria in the slow-dividing

cells impairs differentiation.⁸³ Mitochondrial dynamics also facilitate *in vivo* cardiac development as cardiomyocytes from early embryonic development contain few fragmented and immature mitochondria that transit into extensive networks of mature mitochondria in close proximity to the developing contractile filaments to ensure energetically competent development.⁸⁴

Regulators of bioenergetic maturation

Some of the molecular components contributing to mitochondrial and bioenergetic maturation leading to pluripotent cell lineage specification have recently been elucidated. The mitochondrial permeability transition pore (mPTP), a non-selective conduit residing in the inner mitochondrial membrane has been identified as a gating mechanism underlying *in vivo* mitochondrial maturation and cardiomyocyte differentiation.^{84,85} Early cardiomyocytes have a higher mPTP open probability, resulting in mitochondria with lower mitochondrial membrane potential and greater reactive oxygen species (ROS) generation, which ultimately impairs development.⁸⁴ Closure of mPTP facilitates maturation of mitochondria resulting in subsequent cardiomyocyte differentiation.⁸⁴ Additional regulators of permeability transition, including mitofusins-2, have been also implicated in pluripotent cell differentiation.^{57,86} Permeability transition can affect cardiac differentiation via a number of vital downstream processes, including ROS production and energy metabolism. ROS flashes appear to modulate cardiomyocyte differentiation in both a time- and concentration-dependent fashion. Early commitment of cardiac progenitors may require ROS, with subsequent cardiomyocyte differentiation and maturation occurring under reduced ROS load, as addition of stable oxidants to immature cardiomyocytes impairs differentiation, while addition of antioxidants promotes cardiomyogenesis.^{84,87,88} Low levels of ROS, potentially from high levels of glucose provided in cell culture, accelerate cardiac differentiation of stem cells by stimulation of cardiac genes and transcription factors,^{89–92} while high levels of ROS appear to delay the process.^{88,93} Transient mPTP opening also uncouples oxidative metabolism from ATP synthesis, which would maintain a high glycolytic capacity to support the immature state, while mPTP closure would promote oxidative metabolism to facilitate cardiac

differentiation.^{34,35,85} This metabolic shift may also be regulated at the level of mitochondrial substrate supply, which is under the control of uncoupling protein 2 (UCP2).⁹⁴ Unlike UCP1, UCP2 has yet to display physiological uncoupling activity; however, it has been shown to suppress pyruvate oxidation and increase fatty acid and glutamine oxidation.^{95–97} Owing to UCP2 expression in, predominately, glycolytic tissues and cancer cells and its ability to block mitochondrial pyruvate entry, UCP2 may promote glycolytic glucose use.^{94,98–100} Indeed, gene silencing of UCP2 in pluripotent cells significantly reduces extracellular acidification rate (a surrogate marker of glycolysis) and ATP levels.⁹⁴ In contrast, UCP2 expression is reduced during differentiation of pluripotent cells, with ectopic expression of UCP2 both impairing oxygen consumption and pluripotent cell differentiation by blocking the metabolic shift from glycolysis to oxidative metabolism.⁹⁴ Taken together, maturation of mitochondrial function and energy metabolism is an essential component of pluripotent cell differentiation; however, further examination is required to define the intimate metabolic reprogramming conditions that drive the specification of diverse cell lineages.

Conclusion

Recent evidence has revealed a previously unrecognized role for energy metabolism in controlling the balance between maintenance of stemness and differentiation into specific lineages. The reliance on glycolysis has been documented to fuel the anabolic and catabolic requirements to maintain stem cells in the pluripotent state, while mitochondrial biogenesis and maturation of mitochondrial oxidative metabolism appears integral to match the energetic demands of differentiation. An enabling role for metabolic reprogramming in cell fate decisions offers a novel perspective on molecular events managing cell identity.

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Conflicts of interest

The authors declare no conflicts of interest.

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