Review

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Mitochondria and the dynamic control of stem cell homeostasis

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Abstract

The maintenance of cellular identity requires continuous adaptation to environmental changes. This process is particularly critical for stem cells, which need to preserve their differentiation potential over time. Among the mechanisms responsible for regulating cellular homeostatic responses, mitochondria are emerging as key players. Given their dynamic and multifaceted role in energy metabolism, redox, and calcium balance, as well as cell death, mitochondria appear at the interface between environmental cues and the control of epigenetic identity. In this review, we describe how mitochondria have been implicated in the processes of acquisition and loss of stemness, with a specific focus on pluripotency. Dissecting the biological functions of mitochondria in stem cell homeostasis and differentiation will provide essential knowledge to understand the dynamics of cell fate modulation, and to establish improved stem cell-based medical applications.

Keywords differentiation; metabolism; mitochondria; pluripotency; PSCs
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See the Glossary for abbreviations used in this article.

Introduction

Living cells need to constantly respond to the environment. The cellular responses must be rapid and tightly regulated in order to allow the adaptation to environmental changes and the maintenance of cellular identity. These mechanisms are at the basis of cellular homeostasis and require epigenetic remodeling, that is, chromatin reorganization leading to a different gene expression program without changes in the DNA sequence [1]. Environmental cues can also cause transcriptional responses that challenge the identity of the cell, resulting in survival or cell death depending on whether the cells are plastic enough to adapt.

Adaptation and plasticity are particularly relevant for stem cells, given their ability to generate different progenies. This feature is known as potency and varies among stem cells according to how many distinct "identities" they can give rise to. Pluripotent stem cells (PSCs)—including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs)—can differentiate into virtually any cell of the body (i.e., belonging to all three germ layers). Multipotent adult stem cells—including hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), and neural stem cells (NCSs)—can generate several cell types within a defined lineage. At the same time, all stem cells are capable to continuously proliferate while preserving their identity, a property known as self-renewal. Cellular homeostasis is therefore critical for stem cells, given their need for constant preservation of both potency and self-renewal.

Upon exposure to environmental stimuli, stem cells dynamically regulate the transcriptional machinery and constantly choose between the maintenance of stemness or the exit from stemness, which results in either differentiation or cell death. In the complexity of the cytoplasmic response of stem cells to the environment, mitochondria are poised to play an essential and unique role, given that they are at the center of numerous homeostatic processes [2–4].

In this review, we discuss how mitochondria may contribute to stem cell homeostasis. We focus particularly on pluripotency. In fact, since the discovery of iPSCs in 2006 [5], pluripotent stem cells have acquired a remarkable importance in several biomedical applications, including regenerative medicine, disease modeling, and drug discovery, even in the context of diseases impairing mitochondrial function [6,7]. In order to dissect the role of mitochondria in stem cell homeostasis, we simplify the mitochondrial response to the environment and divide it into three branches: (i) mitochondrial bioenergetics and dynamics, (ii) mitochondrial regulation of redox and calcium balance, and (iii) crosstalk between mitochondrial metabolism and epigenetics (Fig 1).

The picture emerging from studies on PSCs and other stem cell types is that mitochondria can play a contributing role in the instruction of cell fate outcomes, given their dynamic ability to integrate environmental cues to modulate cellular homeostasis. Unraveling the plasticity of the mitochondrial response to the stem cell environment can provide critical insights into how cell fate decisions are established. Moreover, this knowledge may have implications for our understanding of disorders affecting mitochondrial function and could ultimately support the development of improved stem cell-based clinical applications.

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Glossary

ACL	ATP-citrate lyase
aKG	alpha-ketoglutarate
AMPK	AMP-activated protein kinase
BCL-2	B cell lymphoma-2
DNMTs	DNA methyltransferases
DRP1	dynamin-related protein 1
ESCs	embryonic stem cells
GPX2	glutathione peroxidase-2
GSH	glutathione
HDAC	histone deacetylases
HIF1a	hypoxia inducible factor one alpha
HMTs	histone methyltransferases
HSCs	hematopoietic stem cells
iPSCs	induced pluripotent stem cells
JHDMs	Jumonji C domain demethylase
LIF	leukemia inhibitory factor
MFN	mitofusin
MSCs	mesenchymal stem cells
mtDNA	mitochondrial DNA
NAD^+	nicotinamide adenine dinucleotide (oxidized form)
NADH	nicotinamide adenine dinucleotide (reduced form)
NADPH	nicotinamide adenine dinucleotide phosphate (reduced form)
NPCs	neural progenitor cells
NRF2	nuclear respiratory factor 2
NSCs	neural stem cells
OPA1	optic atrophy 1
OXPHOS	oxidative phoshorylation
PDC	pyruvate dehydrogenase complex
PDK	pyruvate dehydrogenase kinases
POLGA	polymerase gamma A
PPP	pentose phosphate pathway
PSCs	pluripotent stem cells
PTP	permeability transition pore
RC	respiratory chain
ROS	reactive oxygen species
TCA	tricarboxylic
TET	ten-eleven translocation
UCP2	uncoupling protein 2

Mitochondrial metabolism and dynamics in stem cell homeostasis

The first and best known function of mitochondria is the production of energy in the form of ATP via oxidative phosphorylation (OXPHOS). This process takes place in the mitochondrial cristae through the action of respiratory chain (RC) complexes [8]. A structural change to the morphology of the cristae or to the overall mitochondrial shape can have an impact on the cellular bioenergetic output. In fact, mitochondria do not operate as individual organelles but rather as an interconnected intracellular network [3]. This mitochondrial network is constantly modulated by continuous cycles of mitochondrial fusion and fission, a process collectively known as mitochondrial dynamics [9]. Given the lack of *de novo* mitochondrial biogenesis, the fusion and fission balance is essential for mitochondria to acquire the morphological structure needed to fulfill the specific cellular requirements. Hence, mitochondrial dynamics allow the cells to rapidly respond to environmental cues and adapt the bioenergetic needs. A fused interconnected mitochondrial architecture is generally present in cells that are metabolically active and rely on OXPHOS for energy production. Non-fused spherical mitochondria are instead common in cells that are quiescent or that are using glycolytic metabolism [10]. The state of the mitochondrial network is also changing in response to the nutrient availability, as nutrient-rich environments associate with mitochondrial fragmentation and nutrient-poor environments with mitochondrial elongation [11].

The first studies investigating the mitochondrial changes occurring during the induction of pluripotency observed that mitochondria in iPSCs acquire a non-fused morphology with underdeveloped cristae [12,13]. At the same time, the metabolic profile of the reprogrammed cells shifts from OXPHOS to glycolysis [12,14–16] (Fig 2). The activation of DRP1 (dynamin-related protein 1), the protein regulating mitochondrial fission, is indeed critical for reprogramming to iPSCs [17,18]. During the differentiation of PSCs, oxidative metabolism is activated [12,19]. Consequently, the proteins that drive mitochondrial fusion, MFN (mitofusin) 1 and 2 and OPA1 (optic atrophy 1) are required for the differentiation of stem cells into cells that depend on OXPHOS metabolism, like cardiomyocytes and neurons [20,21]. Interestingly, reprogramming to iPSCs is significantly improved under high-glucose conditions [22], which are supportive of non-fused mitochondrial network [11]. These findings underscore the importance of nutrient availability in the conversion to pluripotency and in the achievement of its correct mitochondrial and metabolic state [4,23].

The metabolic switch from OXPHOS metabolism to glycolysis occurring during iPSC generation is reminiscent of the effect noticed by Otto Warburg in the context of cancer cells, which he described as being able to maintain high glycolytic rates even in the presence of oxygen, a phenomenon known as "aerobic glycolysis" or "Warburg effect" [24]. The glycolytic state of both tumor cells and PSCs has been suggested to be related to their high proliferative rates that require biomass precursors derived from the higher branches of glycolysis and the pentose phosphate pathway (PPP) [25]. In fact, non-replicative cells, such as neurons and cardiomyocytes, typically rely on OXPHOS [26]. However, adult stem cells, including HSCs and NSCs, also depend on glycolysis despite being lowly proliferative or even quiescent [27–29]. This suggests that the preference of glycolysis over mitochondrial function may represent a feature of stemness irrespective of their proliferative features. One likely reason for the glycolytic state of stem cells may be that the reduction in mitochondrial metabolism allows the maintenance of low levels of harmful free radicals (see below).

Despite the importance of glycolysis, mitochondrial metabolism can also play a role in stemness. Even in the context of cancer, it is now evident that mitochondria are not simply defective, as initially postulated by Warburg, but are instead essential for tumor growth and progression and may even represent a therapeutic target [30]. Accordingly, PSCs express high level of the mitochondrial protein uncoupling protein 2 (UCP2) [31], which is involved in the transport of metabolites out of the mitochondria, thereby regulating glucose oxidation [32]. Although a glycolytic switch is required for the acquisition of pluripotency, the early phases of iPSC generation are characterized by an initial burst of OXPHOS activity and by the up-regulation of RC complexes [33–35]. Mitochondrial metabolism may also be important in the self-renewal of human PSCs, as its activation is increased when the lipid presence

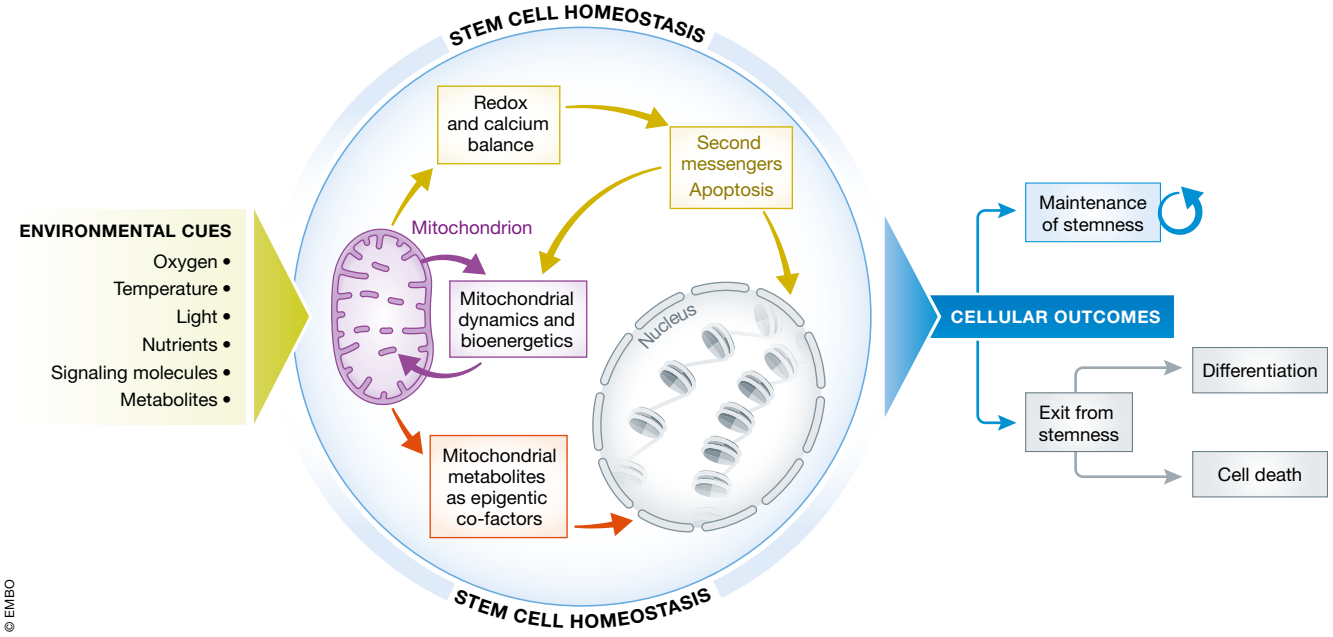

Figure 1. Mitochondrial regulation of stem cell homeostasis.
Summary of the three mechanisms that we describe in the text as being associated with the regulation of stem cell homeostasis. Upon exposure to environmental cues (left), mitochondria respond by modulating (i) their network morphology and bioenergetics (purple arrows), (ii) the redox and calcium balance (light green arrows), and (iii) the epigenetic landscape of the cells (orange arrows). Following these mitochondria-based cellular responses, stem cells can either maintain their identity or exit stemness. This latter route can either lead to physiological differentiation or cell death (right).

in the media is reduced [36], further highlighting how nutrients in the environment can shape the metabolic and functional state of stem cells.

The relevance of mitochondrial metabolism for pluripotency is illustrated by recent studies aiming at dissecting the functional and molecular differences between two PSC states, namely naïve and

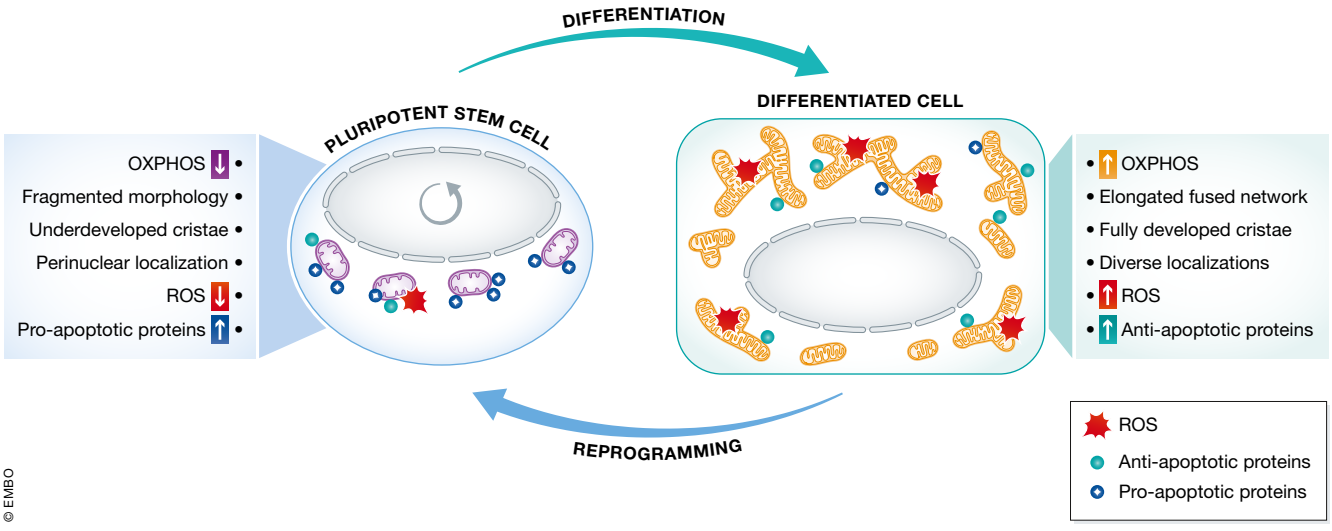

Figure 2. Mitochondrial plasticity during reprogramming and differentiation.
Mitochondria undergo several changes during the reprogramming of somatic cells into pluripotent stem cells (PSCs) and upon the differentiation of PSCs. These modifications impact the OXPHOS activity, the morphology and localization of the mitochondrial network, the appearance of the mitochondrial cristae, the production of reactive oxygen species (ROS), and the balance between pro-apoptotic and anti-apoptotic BCL-2-like proteins.

primed. Naïve PSCs are believed to correspond to the pre-implantation stage of embryo development, while primed PSCs should reflect the post-implantation stage [37]. Despite being potentially less developmentally mature, naïve PSCs exhibit higher OXPHOS activity than primed PSCs [38–41]. In accordance, the conversion from primed to naïve PSCs is facilitated by STAT3-mediated activation of mitochondrial respiration [42] and by the induction of OXPHOS genes following the down-regulation of LIN28 [43], whose expression is low in naïve PSCs. However, the OXPHOS increase in naïve PSCs does not necessarily translate into reduced glycolysis. Naïve PSCs display a bivalent metabolism dependent on both glycolysis and OXPHOS [39] and also exhibit increased glycolytic metabolism [44–46]. Therefore, the role of mitochondrial activity in pluripotency may be independent from glycolytic regulation.

Self-renewing proliferative progenitors also show active mitochondrial metabolism. This is the case for both mouse embryonic NPCs and adult NPCs, which activate the OXPHOS program already during the initial stages of neurogenesis [47]. NPCs derived from human PSCs also display a tubular mitochondrial network and OXPHOS-dependent metabolism when they are generated and cultured using leukemia inhibitory factor (LIF) [48], itself capable of activating mitochondrial respiration [42].

The relationship between the architecture of the mitochondrial network and the cellular metabolism of stem cells is however more complex than previously thought [3]. In fact, even if naïve PSCs show higher OXPHOS activity than primed PSCs, their mitochondrial morphology is less tubular and non-fused compared to that of primed PSCs [4,39–41]. Moreover, glycolytic embryonic mouse NSCs have been found to contain a relatively connected mitochondrial network [49]. The findings have been corroborated in adult mouse NSCs [47]. Mesenchymal stem cells, although mainly glycolytic [50], also exhibit tubular mitochondria that can further elongate upon differentiation [51]. Even HSCs, which are known to rely on glycolysis [27], show elongated mitochondria and require the expression of the fusion protein MFN2 [52]. Therefore, the general assumption that glycolytic cells, like stem cells, have fragmented non-fused mitochondrial network is likely to be imprecise. In fact, by looking at different stem cell types and at cancer cells, it becomes clear that the association between proliferation, mitochondrial metabolism, and mitochondrial dynamics is rather complex and probably highly plastic and environment-dependent (Table 1).

Mitochondrial control of redox and calcium balance in stem cell homeostasis

Mitochondria play a critical role in the balance between cell survival and cell death. These mechanisms are at the bases of cellular homeostasis and regulate the potential outcomes in response to environmental cues. Mitochondria influence cell death pathways associated with necrosis, apoptosis, and autophagy [53] mainly through the modulation of redox and calcium balance [54].

The constant and tight regulation of cell death is essential for stem cells in order to preserve genome integrity, which ensures the faithful derivation of functional progeny [55]. The main insult causing loss of genome integrity is evoked by oxidative stress that occurs when the amount of free radicals is not properly counterbalanced by the cellular antioxidant defenses. Reactive oxygen species (ROS), generated upon OXPHOS metabolism during the transport of electrons in the RC [56], can have detrimental consequences on DNA, proteins, or lipids. Oxidative damage of DNA is particularly harmful for stem cells as it can cause unwanted mutations [57]. ROS may also damage mitochondrial DNA (mtDNA), which is particularly susceptible to oxidative stress, given the proximity to free radicals and the lack of histones [58,59]. Importantly, PSCs carrying high mtDNA mutation load have been shown to generate defective differentiated cells [60–63]. Furthermore, mtDNA mutagenesis can impair the derivation of iPSCs [64]. Increased ROS levels and mtDNA mutagenesis can also lead to defective NSCs and HSCs [65].

In order to limit the occurrence of DNA lesions, stem cells must be equipped with a specific strategy. To this aim, the glycolytic metabolism of stem cells may represent a protective mechanism, as it avoids excessive ROS production by lowering OXPHOS activity. In accordance, PSCs have been found to exhibit reduced ROS and reduced ROS-mediated damage to lipids, proteins, and DNA [12,66]. Pluripotent stem cells are also highly proficient in DNA repair capacity and antioxidant defenses, including glutathione (GSH) and glutathione peroxidase-2 (GPX2), which undergo down-regulation

Table 1. Mitochondrial properties in stem cells and cancer.

Stem cell type	Proliferation	Metabolism	Mitochondrial network	ROS	Sensitivity to cell death
Primed PSCs	High	Glycolysis	Non-fused with underdeveloped cristae, but some tubular	Low	High
Naïve PSCs	High	Glycolysis but also OXPHOS	Non-fused with underdeveloped cristae	Low	High
NSCs	Low	Glycolysis	Tubular	Low	Intermediate
NPCs	High	Glycolysis; OXPHOS (when grown with LIF)	Non-fused; tubular (when grown with LIF)	Low	Intermediate
HSCs	Low	Glycolysis	Tubular	Low	Intermediate
MSCs	High	Glycolysis	Tubular	Low	Intermediate
Cancer cells	High	Glycolysis	Tubular with disorganized cristae	High	Low

In Table 1, we report some of the mitochondrial features in distinct stem cell types and cancer cells. It is interesting to point out that OXPHOS metabolism can be associated also with stem/progenitor cells and that tubular mitochondrial network may also be present in stem/progenitor cells regardless of their metabolic state. The proliferative rate of the cells also does not seem to be uniquely correlated with a specific metabolism. By comparing the mitochondrial properties of stem/progenitor cells with that of cancer cells, it becomes clear that they exhibit key differences in the response to cell death.

upon differentiation [67–69]. Furthermore, the high energy flux of glycolysis and PPP support stem cell antioxidant defenses by providing NADPH that maintains GSH in its reduced form [70]. HSCs also display enhanced self-renewal potential under conditions of low ROS content [71]. NPCs exhibit decreased amount of free radical production in comparison with neuronal and astrocytic counterparts [72]. MSCs show low levels of ROS and high level of GSH [73].

As mentioned above, aerobic glycolysis can be favored by stem cells and proliferative cells even in the presence of oxygen. The simple absence of oxygen also causes increased glycolysis, a phenomenon known as "anaerobic glycolysis". The underlying mechanisms might be similar and may involve the induction of a gene expression program activated by hypoxia inducible factors such as the hypoxia inducible factor one alpha (HIF1a). Accordingly, low oxygen conditions increase the glycolytic flux of ESCs [74,75] and improve iPSC derivation [76]. At the same time, glycolysis and HIF1a are activated during reprogramming to pluripotency even under normoxic culture [77,78]. Low oxygen in *in vitro* cultures is more reminiscent of the actual *in vivo* situation in the inner cell mass, where the physiological oxygen concentration is lower than 5% and thus far less of the 20% of atmospheric oxygen [2]. Physiological oxygen levels are also present in the *in vivo* niches of adult stem cells, including HSCs and MSCs [79,80], and NSCs [81,82]. Given the direct effect of environmental oxygen on transcriptional regulation, it may be possible that differences in the oxygen exposure could cause slightly altered gene expression programs in distinct cells within the *in vivo* niche. This effect may contribute to the functional heterogeneity of stem cells *in vivo* [83].

Despite the decreased OXPHOS-mediated ROS generation, oxidative damage may still occur in stem cells. Therefore, there should be mechanisms in place to ensure the efficient elimination of damaged cells. Consequently, PSCs are highly sensitive to agents causing DNA lesions, including pro-apoptotic chemicals and gamma irradiation [84,85]. This hyper-sensitivity of PSCs has been dubbed "mitochondrial priming", as the cell death pathways of PSCs appear specific for mitochondria-triggered apoptosis and not receptor-mediated apoptosis [68,86,87]. Pluripotent stem cells exhibit a specific state of BCL-2 (B cell lymphoma-2) family proteins, with high amount of pro-apoptotic BCL-2-like proteins (like BAX, BAK, and BOK/MTD) and low levels of anti-apoptotic BCL-2-like proteins (like BCL-2, BCL-XL, BCL-W, and MCL-1) [84,88]. This mitochondrial priming allows PSCs to rapidly undergo cell death when DNA damage has occurred. The mitochondrial apoptotic pathway is also crucial during the early exit from pluripotency, where it can cause the elimination of those cells that are not efficiently undergoing differentiation. Interestingly, cellular apoptosis typically involves mitochondrial fission. Accordingly, BCL-2 family members can contribute to the regulation of mitochondrial dynamics [89]. This suggests that the processes underlying the changes mentioned above in mitochondrial morphology and metabolism occurring during the acquisition and loss of pluripotency may also be important for the configuration of a PSC-like apoptotic sensitivity (Fig 2).

Adult stem cells are considered to be more resistant to cell death than PSCs [90]. Nonetheless, adult stem cells may still retain the ability to undergo cell death upon DNA damage [91–93]. On the other hand, the sensitivity to cell death is radically different in cancer cells, which are capable of surviving in the presence of high levels of ROS and DNA damage (Table 1).

In addition to their role in redox biology, free radicals can act as second messengers to modulate cellular processes. The biological role of mitochondrial ROS may be particularly relevant for stem cell homeostasis [94]. An increase in ROS, independently from OXPHOS, can promote the differentiation of PSCs [95] and adult stem cells, including MSCs [73,96] HSCs [97] and NSCs [98]. Modulation of ROS may also be important in the regulation of stem cell self-renewal [99]. Physiological free radicals can trigger a nuclear response that includes the expression of nuclear respiratory factor (NRF) 2, which in turn leads to stem cell differentiation [49]. ROS-mediated NRF2 induction occurs also during the initiation of iPSC reprogramming, where it causes an initial burst of OXPHOS followed by the activation of glycolytic metabolism [35].

Reactive oxygen species are also important for the stem cell response to low oxygen [100]. In order for the physiological ROS production to successfully induce a HIF1a response, the mitochondrial network acquires a perinuclear clustering. The typical perinuclear localization of mitochondria that has been detected in PSCs [12,101,102] has been therefore suggested to play a role in the oxygen-dependent regulation of cell fate in PSCs [2].

In accordance with the physiological importance of ROS in stem cell homeostasis, the exogenous administration of antioxidants may not always be beneficial. Although vitamin C was initially found to improve the derivation of iPSCs [103], the use of various antioxidants failed to increase the efficiency of iPSC reprogramming and did not ameliorate the growth defects of iPSCs carrying mtDNA mutations [64]. Even in the context of tumor cells, antioxidants may promote cancer proliferation by down-regulating the endogenous protective mechanisms [104].

Intracellular calcium is another mechanism that is regulated by mitochondria and is implicated in both cell death activation and physiological signaling pathways [105,106]. Mitochondria regulate calcium balance in the cells by acting as high capacity buffers [53]. Intra-mitochondrial calcium positively affects energy metabolism through the stimulation of ATP production by OXPHOS. At the same time, excessive accumulation of calcium into the mitochondria can lead to apoptosis through the opening of the permeability transition pore (PTP) and the consequent release of cytochrome c in the cytoplasm [107].

The regulation of intracellular calcium might be crucial for the physiological differentiation of PSCs [21] and adult stem cells, including MSCs [108] and NSCs [109]. Nonetheless, the specific importance of mitochondrial calcium regulation for stem cell homeostasis remains overall under-investigated [110,111].

Mitochondrial metabolism–epigenetics crosstalk in stem cell homeostasis

A growing body of evidence suggests that the metabolic profile of the cells can influence the cytoplasmic signaling, connecting environmental inputs with transcriptional programming [1,112]. Metabolism-driven chromatin regulation is crucial for cellular plasticity for dictating the changes required to modulate cellular identity, a key process during cancer transformation and reprogramming to iPSCs [4].

Mechanistically, the importance of mitochondrial metabolism for stem cell fate regulation may be due to the action of metabolites

generated in the tricarboxylic (TCA) cycle that can function as co-factors or substrates for chromatin modifying enzymes. The mechanisms through which mitochondrial metabolites influence stem cell epigenetics is actively investigated in the context of PSCs. However, the metabolism–epigenetic crosstalk could be important for stemness in general [113]. A schematic representation of the interplay

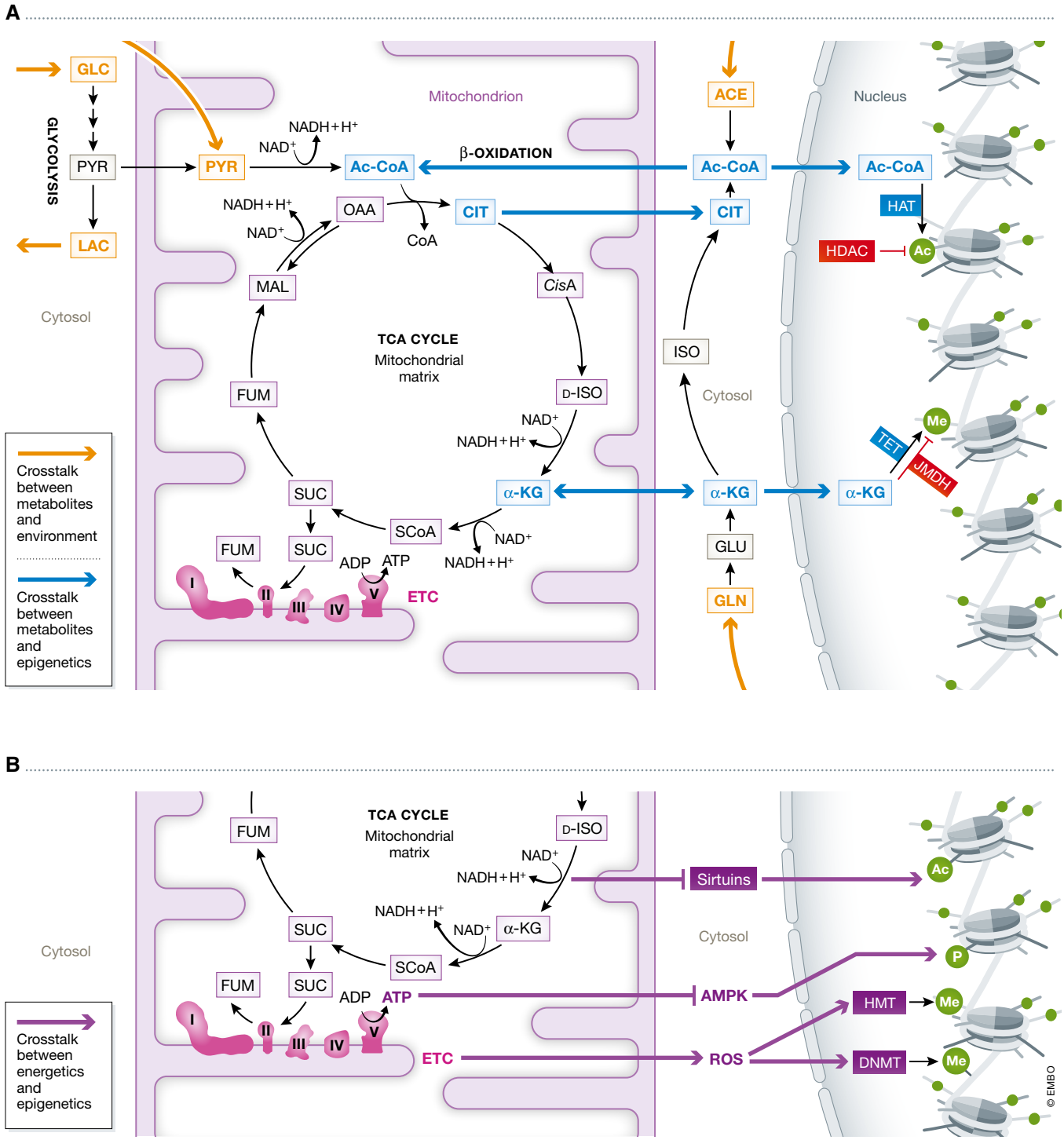

Figure 3. Mitochondria metabolism and epigenetic modulation.
Schematic representation of the interaction between mitochondrial metabolism and chromatin regulation mechanisms. (A) Interplay between mitochondrial metabolites and epigenetics. Orange arrows indicate the connection with the extracellular environment. Blue arrows refer to the mitochondrial metabolites (like citrate, acetyl-CoA, and aKG). (B) Interplay between mitochondrial bioenergetics and epigenetics. Bordeaux arrows indicate pathways related to mitochondrial bioenergetics (like AMPK, sirtuins, and ROS).

between mitochondrial metabolism and epigenetics in stem cells is shown in Fig 3A and B.

An important TCA metabolite associated with epigenetic modulation is citrate. Citrate can be exported outside the mitochondria, where it is converted into acetyl-CoA by the enzyme ATP-citrate lyase (ACL) [114]. Cytosolic acetyl-CoA is the primary donor for the acetylation of histones, an epigenetic modification associated with transcriptional activation. High levels of acetyl-CoA induce the expression of proliferation-related genes in yeast [115], a response that is highly relevant for cancer transformation [116]. Acetyl-CoA-dependent histone acetylation plays a role in the maintenance of primed PSCs [117].

Mitochondrial citrate is primarily obtained from mitochondrial acetyl-CoA. Mitochondrial acetyl-CoA can be imported from the cytoplasm under conditions promoting lipid oxidation. Alternatively, mitochondrial acetyl-CoA can be generated from glycolysis-derived pyruvate through the action of pyruvate dehydrogenase complex (PDC). This latter route is the preferred one of primed PSCs [117]. At the same time, pyruvate dehydrogenase kinases (PDK) 1 and 3, which inhibit the PDC and redirect pyruvate away from the mitochondria into lactate, have been reported to be over-expressed in cancer cells and primed PSCs [16,78,118,119]. This apparent controversy may perhaps be explained by the fact that PDC has been suggested to translocate into the nuclear compartment in response to mitochondrial inhibition [120]. Therefore, we can speculate that pyruvate (glucose-derived and/or exogenously provided) might have two fates in PSCs. On the one hand, pyruvate could be shunt away from mitochondria to generate lactate following PDC inhibition within the mitochondria. On the other hand, pyruvate might be converted into acetyl-CoA by PDC localized in the nucleus in order to enable rapid histone acetylation. Further studies are needed to fully address these mechanisms (Fig 4).

Another important TCA metabolite is alpha-ketoglutarate (aKG). aKG is transported into the cytosol via the aKG-malate shuttle that also facilitates the NAD^+/NADH mitochondria–cytosol transfer [121]. alpha-ketoglutarate can enter the nucleus where it is used as a substrate of ten-eleven translocation (TET) proteins for DNA methylation (associated with transcriptional repression) and of Jumonji C domain demethylase (JHDMs) for histone demethylation (linked to context-dependent gene silencing or activation) [116]. Global DNA hypomethylation is a known feature of PSCs that must be obtained during cellular reprogramming for the faithful establishment of pluripotency [122]. The level of genomic methylation can

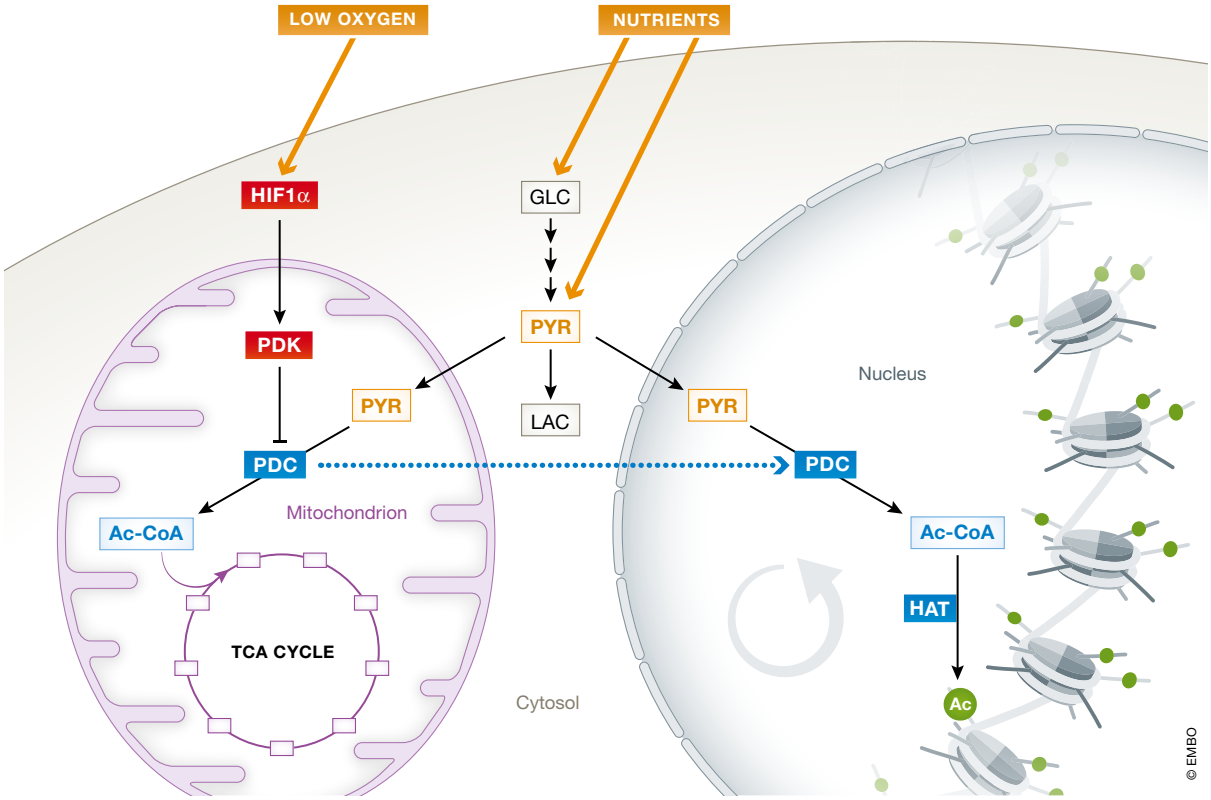

Figure 4. Energy flux and pyruvate fate in PSCs.
Intracellular pyruvate can be obtained from glucose or directly taken up from the environment. The fate of pyruvate depends on the activity of pyruvate dehydrogenase complex (PDC), which converts pyruvate into acetyl-CoA that is in turn used in the TCA cycle in the mitochondria. In PSCs, hypoxia inducible factors (HIF) are activated and lead to increased expression of pyruvate dehydrogenase kinase (PDK)1-3 that inhibit mitochondrial PDC. The inhibition of mitochondrial PDC re-routes pyruvate outside the mitochondria to be used for lactate generation. However, this mechanism is in apparent contrast with the importance of pyruvate-derived acetyl-CoA for histone acetylation. Given that it has been reported that PDC can translocate outside the mitochondria and into the nucleus under conditions of mitochondrial inhibition, we speculate that a PDC translocation may also occur in PSCs. This might help explaining the double importance of pyruvate in PSCs for glycolytic metabolism and epigenetic regulation.

Box 1: In need of answers

(i) Does mitochondrial impairment affect stem cell function *in vivo*? Can stem cells be a therapeutic target for diseases causing mitochondrial dysfunction?
(ii) Is it possible to modulate cell fate identity only through metabolic-related changes? If so, are these mechanisms also relevant *in vivo*, suggesting for example that our environment can shape the function of our somatic stem cells?
(iii) Can we untangle the relationship between energy metabolism, cellular proliferation, and mitochondrial dynamics?
(iv) What is the importance of calcium balance for cell fate plasticity?
(v) How can we effectively target redox homeostasis to enhance stem cell function and improve efficient differentiation?

also have an impact on the mtDNA copy number. PSCs and cancer cells may in fact maintain low level of mtDNA replication by reducing the activity of the nuclear-encoded mitochondrial polymerase gamma A (POLGA) through the hypermethylation at its exon 2 [123]. Finally, aKG-dependent DNA and histone methylation may be important for promoting naïve pluripotency [124,125]. In primed PSCs, aKG has been found to both accelerate differentiation [126] and support the undifferentiated state [127]. Therefore, aKG might exert opposite effects in cell fate regulation depending on the specific cellular context.

Tricarboxylic intermediates can be derived not only from glucose but also from glutamine, which is converted into glutamate and afterward into aKG in the cytoplasm. aKG can then function in the cytoplasm or be transferred into the mitochondria. The glutamine dependence of the TCA cycle is known as reductive carboxylation, and it is believed to be important for cancer metabolism [128]. Glutamine appears also indispensable for the survival of primed PSCs [129]. This supports the notion that mitochondrial metabolism and active TCA cycle are maintained during pluripotency and can be fueled by different sources.

In addition to TCA metabolites, the bioenergetic state of the cells can influence the epigenetic landscape. The energy sensor AMP-activated protein kinase (AMPK), which is activated when intracellular ATP levels lower, can phosphorylate histones and histone acetylases. By doing so, AMPK induces the expression of genes involved in cellular metabolic stress and inhibits cellular growth [130]. AMPK activation may function as a barrier for reprogramming to pluripotency [131]. Another example of bioenergetics-related regulation of epigenetics is represented by sirtuins. Sirtuins are activated following increased NAD^+/NADH ratio that occurs for example upon energy starvation due to glucose depletion. Sirtuins can act as histone deacetylases (HDAC) to achieve transcriptional silencing [1,116,132]. The chromatin regulation of sirtuins may contribute to cell fate transition. Indeed, sirtuins have been implicated in enhancing the generation of iPSCs [133,134].

Finally, the epigenetic landscape might also be affected by the redox state of the cells. In addition to their described importance in signaling, free radicals can alter chromatin remodeling through the oxidation of DNA methyltransferases (DNMTs), histone methyltransferases (HMTs), or through the direct oxidation of nucleotide bases [135,136]. The role of vitamin C in the reprogramming to pluripotency may in fact be linked to both its antioxidant features and its action as cofactor for epigenetic enzymes [103].

Concluding remarks

The ability of mitochondria to integrate environmental cues to influence cellular homeostatic responses is emerging as a key aspect of stem cell biology. A growing body of work suggests that mitochondria play an active role in shaping the cellular fate through the modulation of bioenergetics, redox and calcium balance, and epigenetics. Moreover, mitochondria regulate cell death pathways, which are crucial to allow stem cells to preserve their genome integrity and the functionality of the differentiated progeny.

Dissecting the importance of mitochondria for stemness (see also Box 1) will help designing improved stem cell-based medical applications. Furthermore, it may lead us to broaden our understanding of the pathogenetic mechanisms of diseases causing mitochondrial dysfunction. If mitochondrial impairment can impact stem cell function, then mitochondrial disorders may also affect the stem cell compartment and not only fully differentiated cells. This might be the case of aging and neurological diseases, where defects in neural stem cells and neurogenesis are starting to be identified [47,137,138]. Finally, uncovering the mitochondrial control of stem cell homeostasis will shed light on the cellular strategies underlying the establishment of cell fate identity and how to modulate its plasticity.

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Conflict of interest

The authors declare that they have no conflict of interest.

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