Published in final edited form as:

Curr Opin Physiol. 2018 June ; 3: 16–24. doi:10.1016/j.cophys.2017.11.006.

More than a powerplant: the influence of mitochondrial transfer on the epigenome

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Abstract

Each cell in the human body, with the exception of red blood cells, contains multiple copies of mitochondria that house their own genetic material, the maternally inherited mitochondrial DNA. Mitochondria are the cell's powerplant due to their massive ATP generation. However, the mitochondrion is also a hub for metabolite production from the TCA cycle, fatty acid beta-oxidation, and ketogenesis. In addition to producing macromolecules for biosynthetic reactions and cell replication, several mitochondrial intermediate metabolites serve as cofactors or substrates for epigenome modifying enzymes that regulate chromatin structure and impact gene expression. Here, we discuss connections between mitochondrial metabolites and enzymatic writers and erasers of chromatin modifications. We do this from the unique perspective of cell-to-cell mitochondrial transfer and its potential impact on mitochondrial replacement therapies.

Keywords

mitochondrial transfer; metabolism; epigenetics; acetylation; methylation

1. Introduction

Considered to originate from an endosymbiotic α -proteobacteria in an ancient cellular host, modern mitochondria occur in dozens to thousands of copies per nucleated mammalian cell

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The authors do not have any conflicts of interest to declare

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and are essential to eukaryotic life. Mitochondria are considered powerplants because they produce up to 90 percent of cellular ATP [1]. However, they are also hubs of metabolic activity and are involved in the production, breakdown, and/or regulation of reactive oxygen species (ROS), fatty acids, one-carbon metabolic intermediates, tricarboxylic acid (TCA) cycle metabolites, and ketone bodies. These metabolites provide cell type-specific and situational control over gene expression by fueling the addition of chemical groups, or epigenetic marks, to DNA or its associated proteins. These marks affect electrostatic and hydrophobic interactions within chromatin, influencing transcription factor or repressor binding and enhancing (euchromatin) or repressing (heterochromatin) gene expression (Figure 1).

In addition to approximately 1,500 different mitochondrial proteins encoded in the nucleus [2], each mitochondrion contains multiple copies of a small, maternally inherited genome, the mitochondrial DNA (mtDNA). In humans, mtDNA is a compact 16,569 bp circular genome coding for 37 genes directly required for oxidative phosphorylation (OXPHOS) and linked to metabolite biosynthesis. Although pathogenic mutations to mtDNA affect 1 in 5,000 people, 1 in 200 are carriers of mtDNA disease-related mutations but do not exhibit pathology because the deleterious effects of mutant mtDNA can be masked by normal mtDNA [3]. The phenomenon of a single cell carrying a heterogeneous population of normal and pathogenic mtDNA genotypes is known as heteroplasmy, and the heteroplasmic ratio of a specific pathogenic mtDNA to other mtDNA genotypes can dictate the severity of a disease. mtDNA mutations are particularly difficult to treat because modifying the mtDNA is currently impossible, however artificial or natural transfer of whole mitochondria containing mtDNA between cells provides mechanisms for altering metabolism and repairing cell damage [3–9], and may be a viable approach for mtDNA diseases (Figure 2). Studies on establishing mitochondrial replacement therapies (MRTs) that allow women carrying mtDNA mutations to have disease-free children have been performed in Mexico [10], and proposed for the United States [11] and the United Kingdom [12]. Investigating the role of mitochondrial function and transfer in nuclear gene regulation and cell fate could provide insight for the long-term feasibility of MRTs and for understanding physiological changes resulting from altered mitochondrial activity.

2. Mitochondrial metabolites and epigenetics

Mitochondria house key metabolic processes including OXPHOS, long-chain fatty acid beta-oxidation, and amino acid metabolism along with the urea, one-carbon, and TCA cycles. A recent study identified 346 distinct metabolites produced within the mitochondria alone [13]. These metabolites regulate the enzymes that modify DNA and histones to modulate gene expression [14–17]. Perturbation of individual electron transport chain (ETC) complexes resulting from hereditary mutations in nuclear DNA and mtDNA can generate distinct mitochondrial metabolite and gene transcription profiles [13]. Intriguingly, cells in vitro and in vivo can donate mitochondria to specific recipient cells, and these donated mitochondria may manipulate cellular metabolism [8], promote tissue repair [18], or alter disease progression [19]. The exact mechanism(s) by which transferred mitochondria and their specific mtDNA sequences fuel these changes likely go beyond altering cellular ATP concentrations and are discussed here.

2.1. Methylation of histones and DNA

DNA methylation at transcriptional start sites usually represses gene expression by altering chromatin structure, inhibiting transcription factor binding, and/or recruiting transcription repressors to methylated nucleotides [20]. However, DNA methylation may also promote transcriptional activation when methylation occurs in a gene body [21]. The most prevalent mammalian DNA modification is 5-methylcytosine in regions of CpG islands that is catalyzed by de novo DNA methyltransferases DNMT3A and DNMT3B and sustained through DNA replication by the DNA maintenance methyltransferase, DNMT1 [22]. Histone-tail amino acids are also subject to methylation, which can repress or activate gene expression depending on the residue, number, and epigenetic context of added or removed methyl groups. Histone-tail methylation occurs on any basic residue, but epigenetic modifications to arginine and lysine are most common [23–26].

Methyltransferase activity depends upon mitochondrial function since these enzymes require *S*-adenosylmethionine (AdoMet), which is synthesized by methionine adenosyltransferase from ATP and methionine. One-carbon cycle intermediates are required for AdoMet synthesis, and cells with impaired one-carbon cycling show decreased AdoMet levels and reduced DNA and histone methylation [27,28]. The one-carbon cycle occurs in both the cytosol and mitochondria, however mitochondrial serine catabolism is the predominant source of cellular one carbon metabolites [29]. Serine catabolism requires coupled respiration and its ATP and NAD⁺ byproducts, closely tying methylation to respiration and mitochondrial activity [30,31].

DNA and histone methylation marks are removed by Ten-Eleven Translocation (TET) or Jumonji catalytic (JmjC) domain-containing demethylases, respectively [14]. To remove 5-methylcytosine, TET dioxygenases convert 5-methylcytosine to 5-hydroxymethylcytosine, which is further oxidized to 5-formylcytosine and 5-carboxylcytosine before final removal by base excision repair [22,32,33]. Methyl groups on lysine residues in histones are removed by demethylases, 27 of which have been identified [34]. Lysine demethylases may also exhibit some arginine demethylase activity [35,36].

2.2. Mitochondrial metabolites and demethylation activities

Several TCA cycle-associated metabolites competitively inhibit demethylase activity and have profound effects on gene expression. Fumarate and succinate accumulate in cells with mutations in fumarate hydratase and succinate dehydrogenase [37], which inhibits TET demethylases and 5-hydroxymethylcytosine formation, reduces histone demethylation, activates the hypoxia inducible pathway [38,39], and may lead to tumorigenesis [37,40,41]. 2-hydroxyglutarate (2HG) is a TCA cycle-associated metabolite that can inhibit demethylases, and elevated 2HG levels can promote tumorigenesis [42,43], enhance mTOR signaling, and alter T cell differentiation (Figure 3) [44,45]. 2HG occurs as two enantiomers, D-R-2-hydroxyglutarate or L-S-2-hydroxyglutarate, depending on the biosynthetic pathway. D-R-2-hydroxyglutarate generation occurs by neomorphic mutations in isocitrate dehydrogenase 1 and 2 (IDH1/2) [46] or by transamination via glutamate-oxaloacetate transaminase 1 (GOT1) [44]. Conversely, both hypoxia [47,48] and promiscuous activity of lactate and malate dehydrogenase enzymes at acidic pH [49,50] generate L-R-2-

hydroxyglutarate. It is unclear whether these enantiomers have different cellular activity as both occur naturally, but a shift to favor L-S-2-hydroxyglutarate appears to result in T cell activation due to histone and DNA demethylation (Figure 3) [45].

The cellular concentrations of TCA cycle metabolites alpha-ketoglutarate (α KG) and succinate are dependent upon mitochondrial activity and are important regulators of DNA and histone methylation patterning and cell fate. Both TET and JmjC-containing demethylases require O_2 , α KG, and complexed iron (II) for activity, and produce succinate and CO_2 as reaction byproducts [14,51]. Adjusting α KG to succinate ratios can cause phenotype switching in cells. In macrophages, a high α KG to succinate ratio causes demethylation of H3K27me3 marks and drives M2 macrophage differentiation for anti-inflammatory and tissue repair responses, whereas a low ratio promotes lipopolysaccharide-sensing M1 macrophages and a pro-inflammatory response (Figure 3) [52]. Elevated superoxide radicals from superoxide dismutase 2 depletion in stromal precursor cells may impede differentiation by promoting the accumulation of α KG, impairing glycolysis, and enhancing oxidative metabolism [53]. The mechanism(s) by which α KG causes epigenetic changes in this case are unknown, although α KG involvement may suggest a demethylase related activity that requires exploration. Alternatively, the extracellular levels of these metabolites may influence gene expression and cell function by serving as ligands of G-Protein Coupled Receptors (GPCRs). For example, succinate and α KG can bind to GPCR91 and GPCR99, respectively, to regulate cellular functions and tissue physiology [54,55]. Further investigation is needed to determine the extent to which mitochondrial metabolites influence cellular phenotypes by epigenetic modification or through non-canonical signaling pathways.

Finally, naïve mouse embryonic stem cells maintain DNA/histone demethylase function and pluripotency by sustaining an elevated α KG to succinate ratio [56]. Artificially reducing this ratio impairs TET demethylase activity, increases trimethylation and decreases monomethylation of H3K9, H3K27, H3K36, and H4K20, and promotes cell differentiation [56]. In less naïve, primed-state human pluripotent stem cells and mouse epiblast stem cells, elevated α KG levels promote stimulated differentiation [57]. Furthermore, elevated α KG causes a decrease in H3K4me3 and H3K27me3, while an increased succinate concentration enhances these marks [57]. These results show that in vitro, specific mitochondrial metabolites differentially regulate epigenome modifying enzymes and cellular differentiation depending upon cell state and microenvironment cues (Figure 3). Because these studies often involve the extracellular addition of membrane-permeable derivatives to cells under laboratory conditions, how robust this regulation is in vivo needs further study. Additionally, while it is known that the transfer of mitochondria occurs between cells in vitro and in vivo, the biological consequences of transfer are unclear and it is unknown whether this process occurs in glycolytic hypoxic stem cells niches, which could alter mitochondrial metabolites and specific patterns of differentiation.

2.3. Mitochondrial metabolites and histone acetylation

In addition to methylation, acetylation occurs on histone tail residues, promoting euchromatin conformation and gene activation [58,59]. The twenty-two known HATs use

the cofactor acetyl-coenzyme A (acetyl-CoA) to bind acetyl groups to lysine residues [58]. Histone acetylation is dependent upon the availability of acetyl-CoA, which accumulates within the nucleus, cytoplasm, and mitochondria [60]. Mitochondrial acetyl-CoA is made from fatty acid beta-oxidation, from branched-chain amino acids, and from pyruvate, citrate, and acetate via pyruvate decarboxylase, ATP citrate lyase (ACL), and acetyl-CoA synthetase 1 enzymes, respectively [61,62]. Furthermore, not only is TCA cycle generated citrate exported to the cytoplasm or nucleus and converted by ACL into acetyl-CoA, but ACL is also essential for histone acetylation and epigenome remodeling during nutrient sensing and adipocyte differentiation [63–65]. 2HG can enhance histone H3 acetylation by stimulating ACL and histone acetyltransferases (HATs) [42]. Finally, macrophages exposed to IL-4 show enhanced Akt-mTORC1 pathway activity, which increases the production of acetyl-CoA by ACL and results in H3/H4 histone acetylation that, similar to elevated demethylase activity, favors M2 macrophage activation [66].

Acetyl marks are removed by the eighteen histone deacetylases (HDACs), which are divided into four classes. Class I, II, and IV (HDACs 1–11) are Zn²⁺-dependent, whereas class III HDACs, also known as the sirtuins (SIRT1–7), require NAD⁺ for activity [58,67,68]. Each class of HDAC is inhibited by different mitochondrial metabolites, which can result in histone H3/H4 tail hyperacetylation [69]. Class I and IIa HDACs are inhibited by the ketone body D-beta-hydroxybutyrate, a metabolic intermediate produced in liver mitochondria by fatty acid beta-oxidation during starvation conditions and when the TCA cycle is unable to produce sufficient amounts of acetyl-CoA [70,71]. This inhibition results in histone hyperacetylation, transcription changes, and activation of oxidative stress transcription factors FOXO3A and MT2, similar to the depletion of HDAC1 and HDAC2 [71,72]. Understanding how mitochondrial function and transfer may influence this regulation requires further study but also seems critical in animal models. Altering histone acetylation and gene expression can improve spatial memory impairment caused by hypoxia [73] and extend lifespan in the roundworm *Caenorhabditis elegans* [74], although it can also induce tumorigenicity in certain cases [75].

Depletion of the class III HDAC SIRT1 results in increased H3K9 acetylation in hepatocellular carcinoma cells [76]. NAD⁺ depletion results in hyperacetylation and reduced trimethylation at H3K9 as well as reduced cell proliferation in human cancer models [77]. The simultaneous change in both acetylation and methylation highlights the multilayered regulation of the epigenome by a single metabolite. However, the specific role of mitochondrial NAD⁺ is currently unknown due to the technical challenges of studying how specific subcellular NAD⁺ pools influence enzymatic activities [78].

3. Mitochondrial transfer

mtDNA encoded proteins are essential for OXPHOS and also influence the synthesis of TCA cycle metabolites linked to epigenome modifications. In the extreme example of cells without mtDNA, called rho null (ρ^0) cells, respiration is terminated, concentrations of succinate and 2HG are elevated, and α -KG, fumarate, malate, and citrate levels are reduced [79]. These changes in TCA cycle intermediates in mtDNA-deficient cells can reduce H3K9, H3K18, and H3K27 histone acetylation [80]. Additionally, mtDNA depletion increases one-

carbon cycle serine metabolism and transsulfuration, and leads to both hypo- and hyper-methylation of CpG islands [81–84]. Although not thoroughly investigated, mtDNA depletion does not result in consistent changes across the epigenome. Rather, these studies suggest that dramatic changes to mitochondrial metabolite concentrations in $\rho 0$ cells may activate and repress gene expression with variability. Further studies are needed to elucidate which metabolites drive patterns of epigenetic modification of specific genes and pathways in response to altered mtDNA levels.

Artificially transferring isolated, functional mitochondria into $\rho 0$ cells rescues OXPHOS and restores the metabolic and transcriptional profiles of the recipient hybrid cells (Figure 2). Wu *et al.* transferred mitochondria from a donor cell line into $\rho 0$ recipient cells using a large cargo delivery device called a photothermal nanoblade and generated three rescue clones that reestablished OXPHOS. Mitochondrial transfer did not fully restore all metabolites, such as 2HG and citrate, to wild-type levels [79]. Furthermore, one of three examined rescue clones still resembled the $\rho 0$ recipient line's metabolomic profile and TCA cycle gene expression pattern, suggesting that incomplete remodeling of the epigenome occurred despite restoring ETC activity. Latorre-Pellicer *et al.* recently reported that mice with identical nuclear genomes but different mtDNA genotypes exhibit significant differences in metabolic profiles, key cellular pathways, and lifespan [85]. These data suggest that different mtDNA haplotypes may be more or less compatible with different nuclear genotypes, influencing mitochondrial function, altering metabolism in whole organisms, and leading to epigenetic and phenotypic changes [86].

In addition to haplotype incompatibility, results from these two studies may also be from epigenetic modifications to the mtDNA itself. Although controversial and initially determined to have no methylation [87] or associated histone proteins, interest in mtDNA epigenetics has increased with evidence suggesting mtDNA contains CpG sites that may be methylated to low levels [88,89] and that DNMT and TET enzymes localize to mitochondria [90–92]. Several publications suggest the accumulation of mtDNA methylation as biomarkers in a wide variety of pathologies involving mitochondrial function, from environmental pollution to aging and cardiovascular disease [88,93,94]. Although mtDNA methylation could modulate transcription like methyl groups in nuclear chromatin, such methylation marks may also regulate the cellular compatibility and tolerance of mtDNA, similarly to how the immune system identifies self from non-self. Further work is needed to determine whether these sporadically reported marks are not artifacts and quantify their biological relevance as any mitochondrial replacement therapy would need to overcome mitochondrial-nuclear compatibility issues.

The transfer of mitochondria between cells is also a biological phenomenon that occurs by endocytosis, tunneling nanotubes, and potentially other uncharacterized pathways in tissue culture and in vivo [3] (Figure 2). When non-tumorigenic $\rho 0$ cancer cells are injected into a mouse, they receive mitochondria and mtDNA from the microenvironment, regain OXPHOS, and recover their tumorigenicity [8,9]. Whether this transfer also occurs between the stroma and cancer cells with their endogenous mtDNA intact, which is more relevant to human disease, remains to be clarified. This question has been addressed in non-cancer models showing that mitochondrial transfer modulates metabolism [95], tissue damage

repair [96], and disease progression [3,97]. However, these studies often focus on ATP production, ROS levels, or cell viability associated with mitochondrial transfer rather than alterations to global gene expression and the epigenome that may be driving these biological changes.

4. Brief remarks

Mitochondria are metabolite-generating factories that support biomolecule and epigenome modification. The metabolites formed within mitochondria are substrates for and regulators of the writers and erasers of the epigenome (Table 1). This regulation may extend beyond altering chromatin structure and gene expression in the nucleus. A more complete understanding of mitochondrial-associated metabolism as a driver of epigenome modifications and its relationship to cell-to-cell mitochondrial transfer may provide mechanistic insight for treating a wide variety of diseases, including mtDNA-disorders.

Acknowledgments

The authors regret any missing references due to length constraints. ANP and AJS are supported by NIH NRSA grants CA009120 and GM007185, respectively. MAT is supported by AFOSR grant FA9550-15-1-0406; NIH grants GM114188, GM073981, and CA185189; and by a California Institute for Regenerative Medicine grant, RT3-07678. We also acknowledge the Library of Science and Medical Illustrations (<http://www.somersault1824.com/>).

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Highlights

- The mitochondrion is an energy and metabolite-producing hub
- Mitochondrial metabolites regulate the activity of epigenome modifying enzymes
- Metabolism-driven epigenetic changes influence cell fate, longevity, and pathology
- Cell-to-cell mitochondrial transfer may impact the epigenome

Figure 1. Mitochondrial metabolites influence the epigenome

Mitochondrial metabolites from many pathways, including the one-carbon (1C) and TCA cycles, are cofactors and regulators of the writers and erasers of epigenetic marks. Arginine (PRMT), lysine (KMT), and DNA (DNMT) methyltransferases require *S*-adenosylmethionine (AdoMet) to generate methylation marks, which can either activate or repress transcription. Likewise, histone acetyltransferases (HATs) require acetyl-CoA to acetylate histones and activate transcription. Both methylation and acetylation can be removed by demethylases (KDMs, JmjCs, and TET enzymes) and histone deacetylases (HDACs), respectively. Abbreviations: β-HB, beta-hydroxybutyrate; 2HG, 2-hydroxyglutarate; αKG, alpha-ketoglutarate.

Figure 2. The biological and epigenetic impact of mitochondrial transfer

Transferring mitochondria with specific mtDNA sequences into cell lines of interest helps our understanding of metabolic control of the epigenome and could be developed into therapies for mitochondria disorders. The transfer of mitochondria from healthy (labeled orange) to metabolically impaired (labeled grey) cells can occur in vivo via tunneling nanotubes and endocytosis. Additionally, isolated mitochondria can be transferred using artificial methods, such as the photothermal nanoblade. Several MRTs show promise for treating heritable mtDNA disorders, including somatic cell nuclear transfer followed by in vitro fertilization. Both biological and artificial mitochondrial transfer methods have been shown to alter respiration and the functions of astrocytes, cardiomyocytes, lung epithelial and corneal epithelial cells after tissue injury, and tumorigenicity in mouse models. However, mitochondrial transfer results in cells with new mtDNA sequences (highlighted yellow) that may change metabolic processes and the epigenome of cells. MRTs that completely replace a cell's mitochondrial population and initially give rise to disease free children may result in longer term metabolic disturbances, potentially due to non-evolved incompatibilities between nuclear DNA and mtDNA encoded proteins.

Figure 3. TCA cycle-associated metabolites, epigenetics, and cell fate

The fates of stem cells, T cells, and macrophages are regulated by cellular concentrations of αKG and succinate. These outcomes are at least partially mediated by αKG-dependent dioxygenases, which remove methyl epigenetic marks to alter nuclear gene expression. Activation or inhibition of these enzymes is dictated by high levels of αKG or succinate, respectively, and the resulting changes to the epigenome alters the differentiation and development of cells. Key: ESCs, embryonic stem cells; hPSCs, human pluripotent stem cells; EpiSCs, epiblast stem cells.

Table 1

Mitochondrial metabolites and their effect on epigenome marks

Metabolite	Enzyme(s) Affected	Enzyme Substrate	Effect on Enzyme Activity	Transcription Change
TCA cycle				
α KG	Demethylases	DNA, histones	+	+ or –
fumarate	Demethylases	DNA, histones	–	+ or –
succinate	Demethylases	DNA, histones	–	+ or –
acetyl-CoA	HATs	Histones	+	+
TCA cycle associated				
2HG	Demethylases, acetyltransferases	DNA, histones	– +	+ or – +
Respiration				
NAD ⁺	Sirtuin Deacetylases	Histones	+	–
FAD	Demethylases	Histones	+	+ or –
Fatty acid beta-oxidation				
D-beta-hydroxybutyrate	Deacetylases	Histones	–	+
One-carbon cycle associated				
AdoMet	Methyltransferases	DNA, histones	+	+ or –

+ Enhanced; – Repressed