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Mitochondria control acute and chronic responses to hypoxia

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Abstract

There are numerous mechanisms by which mammals respond to hypoxia. These include acute changes in pulmonary arterial tone due to smooth muscle cell contraction, acute increases in respiration triggered by the carotid body chemosensory cells, and chronic changes such as induction of red blood cell proliferation and angiogenesis by hypoxia inducible factor targets erythropoietin and vascular endothelial growth factor, respectively. Mitochondria account for the majority of oxygen consumption in the cell and have recently been appreciated to serve as signaling organelles required for the initiation or propagation of numerous homeostatic mechanisms. Mitochondria can influence cell signaling by production of reactive oxygen species and metabolites. Here we review recent evidence that mitochondrial signals can imitate acute and chronic hypoxia responses.

Keywords

Hypoxia; Mitochondria; Reactive Oxygen Species; Metabolism

INTRODUCTION

Effective cellular oxygen delivery is a major factor in human physiology and alterations of hypoxia-related pathways have been implicated as key drivers of multiple pathological states including cancer, cardiovascular disease, stroke, and pulmonary hypertension (Semenza 2011). The vast majority of eukaryotic cells use molecular oxygen as the terminal electron acceptor in the process of mitochondrial ATP production. In addition to their role in ATP production, mitochondria are an important source of metabolic intermediates that are required for anabolic reactions and maintenance of post translational protein modifications. Mitochondria also have a broad impact on the redox state of the cell since the mitochondrial electron transport chain complexes are major sites of reactive oxygen species (ROS) production and the NAD/NADH ratio is intimately tied to electron transport chain function. The redox state of the cell can impinge on cellular signaling pathways in multiple ways. As the largest consumers of cellular oxygen, the mitochondria may be uniquely positioned to

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sense oxygen levels and initiate some of the well-established cellular and organismal adaptations to hypoxia (Hamanaka 2010). Three main acute and chronic changes occur in mammals in response to low oxygen; acute pulmonary smooth muscle vasoconstriction, acute carotid body chemoreceptor-induced hyperventilation, and chronic cellular hypoxia inducible factor (HIF) mediated activation of key downstream targets such as erythropoietin (EPO) and vascular endothelial growth factor (VEGF) (Weir 2005, Ward 2008, Haase 2013). Here we discuss recent progress on the role of mitochondrial signals, such as ROS and metabolite concentrations, in hypoxic responses.

SOURCES OF MITOCHONDRIAL SIGNALING

Oxygen utilization in the mitochondria primarily occurs at ETC complex IV (cytochrome c oxidase). At this site, O₂ serves as the terminal electron acceptor of the electron transport chain to form H₂O. However, there are multiple sites along the ETC at which electron leak can occur (Murphy 2009). In this process a single electron is accepted by O₂ forming a superoxide radical which is rapidly converted to H₂O₂ through action of the enzyme superoxide dismutase (SOD). There are at least 12 putative sites of superoxide production in the mitochondria including sites within ETC complexes I, II, and III, and certain non-ETC mitochondrial dehydrogenases (Brand 2016). These sites contribute different amounts of ROS depending on the presence or absence of specific metabolic substrates and inhibitors, however sites at complex I and complex III provide the majority of mitochondrial ROS (Quinlan 2013). Different sites of ROS production have been implicated in a wide variety of physiological and pathophysiological processes (Holmstrom 2014). Mitochondria are critical for the maintenance of biosynthetic and bioenergetic pathways mediated by the tricarboxylic acid (TCA) cycle and the mitochondrial membrane potential. The TCA produces important biosynthetic precursors in the mitochondria that are shuttled to the cytosol. Examples include the production of cytosolic acetyl Co-A from TCA-metabolite citrate for fatty acid synthesis, and the production of cytosolic aspartate from TCA-metabolite oxaloacetate for pyrimidine synthesis. Normal function of the TCA cycle produces NADH and FADH₂, which are reducing equivalents that transfer electrons into the ETC. The mitochondrial membrane potential that is established by ETC complex proton pumping is used to generate ATP at complex V. The mitochondrial membrane potential is required for ROS production (Martinez-Reyes 2016). Mitochondria serve as signaling organelles through the release of ROS and the release of TCA cycle metabolites (Chandel 2015). These two key mitochondrial signals can impinge on cytosolic signaling pathways. ROS can oxidize susceptible thiols in cysteine residues of signaling proteins. For example, the enzymatic site of protein phosphatases contains a candidate cysteine that can be modified by ROS oxidation (Holmstrom 2014). The TCA-metabolite citrate is exported to the nucleus to create cytosolic acetyl CoA which is required for protein acetylation which can influence signaling pathways and the epigenetic state of the cell (Wellen 2009, McDonnell 2016, Martinez-Reyes 2016). Mitochondrial ROS signals are required for diverse cellular functions such as differentiation, proliferation, and adaptation to stressors including hypoxia. (Schieber 2014).

There has been significant debate about in the literature about whether mitochondrial ROS increase or decrease during hypoxia. The evidence is reviewed in detail elsewhere (Sabharwal 2014, Waypa 2010). Oxygen is a necessary substrate for the creation of

mitochondrial ROS and early evidence indicated that ROS levels within the mitochondria decreased during hypoxia. One hypothesis was that this decrease in ROS was also matched by a decrease in the redox state or energy state of the cell which could be a trigger for the cell to sense a low oxygen state. Indeed experiments with mitochondrial inhibitors can mimic hypoxia, however studies in multiple cell types prove that cells maintain their redox and energy states within the normal range until they are exposed to oxygen tensions much lower than those to which the cells can induce a hypoxia response (Gnaiger 2001). However, recent studies indicate that the mitochondria of specialized acute oxygen sensing cells may be uniquely sensitive to small changes in oxygen availability (Buckler 2013). Furthermore, the initial observations of decreased ROS were due to a change in ROS production specifically in the mitochondrial matrix, while ROS produced in the intermembrane space and throughout the cytosol were actually paradoxically increased during hypoxia. Subsequently, genetic and antioxidant methods have been used to demonstrate the requirement of ROS elevation for oxygen sensing (Waypa 2010).

ACUTE HYPOXIC PULMONARY VASOCONSTRICTION

Acute organismal responses to environmental hypoxia begin in the lung. In order to prevent ventilation-perfusion mismatch during normal respiration, pulmonary arterial smooth muscle cells (PASMC) contract to shunt blood from poorly ventilated alveoli with low oxygen tension to regions of the lung with higher alveolar oxygen tension. The hypoxic contraction of PASMC is caused by a biphasic increase in intracellular $[Ca^{++}]$ (Aaronson 2006). The first phase of contraction occurs within seconds of exposure to hypoxia. The events that occur during the first phase involve calcium release from the sarcoplasmic reticulum, decreased opening of voltage gated potassium channels, and influx of extracellular calcium through multiple calcium channel subtypes. This calcium influx activates myosin light chain kinase allowing for contraction via myosin and actin interactions (Weir 2005). The second phase, which will not be discussed in detail here, occurs over a timeframe of minutes and involves factors released from the endothelium that alter the sensitivity of PASMC contractile apparatus to $[Ca^{++}]$ through modulation of Rho kinase activity (Wang 2001). It is important to note that this PASMC hypoxic contraction is unique, since most other vascular smooth muscle cells demonstrate a reduction in contractile force in response to hypoxia which functions to increase blood flow and oxygen delivery to hypoxic tissues in the periphery (Weir 2005).

Recent *in vivo* mouse genetic evidence points to an important role of mitochondrial ROS in acute PASMC hypoxic contraction. Using an inducible cre-lox system, the Rieske iron-sulfur protein (RISP) of electron transport chain Complex III was conditionally depleted in PASMC (Waypa 2013). This led to a decrease in mitochondrial ROS production in response to hypoxia and abolished the hypoxia-induced calcium ion influx. Furthermore, isolated pulmonary arteries from these mice were able to contract in response to exogenous stimulation with H_2O_2 indicating that this acute vasoconstriction is ROS dependent. This study included the first demonstration of genetic ETC complex disruption blocking hypoxic pulmonary vasoconstriction in intact animals (Waypa 2013). The mechanism by which ROS can increase cellular $[Ca^{++}]$ involves activation of the sarcoplasmic reticulum ryanodine receptors. ROS may activate these specialized calcium channels directly through oxidation

of susceptible cysteine thiols, or indirectly through ROS-induced metabolite cyclic ADP-ribose (Waypa 2008). Further evidence for the involvement of ETC function in PASMCMC hypoxic contraction is provided by studies focusing on complex II, or succinate dehydrogenase (Sdh). Genetic loss of mitochondrial complex II in PASMCMC resulted in a partial reduction of hypoxic PASMCMC specific to intra-acinar arteries (Paddenbergh 2012). However this phenotype was not as widespread or severe as complex III loss.

The mitochondria are not the only source of hypoxia-induced ROS in the cell. The NADPH oxidase system has also been proposed to be involved in hypoxia induced ROS production in pulmonary artery vasoconstriction (Weissmann 2000). The evidence that RISP depletion blocks hypoxia induced ROS suggests that perhaps mitochondrial ROS is the upstream ROS producer and that the cytosolic NADPH oxidases can help to amplify this signaling. It has been suggested that perhaps a small ROS signal produced by the mitochondria can produce cell-wide ROS signaling and that this mechanism may prevent high levels of ROS at the mitochondria which could be damaging for mitochondrial structures (Waypa 2010). This hypothesis of ROS-mediated ROS production requires further investigation, but it is now more appreciated that ROS induction upon hypoxia is necessary for pulmonary arterial vasoconstriction via modulation of calcium release.

ACUTE HYPOXEMIA SENSING IN THE CAROTID BODY

A second acute response to organismal hypoxia occurs via chemoreceptors in the carotid body and peripheral circulatory system. Specialized glomus cells (also known as type 1 cells) in the carotid body sense acute decreases in arterial oxygen concentration and rapidly induce compensatory increases in ventilation (Peers 2005). Hypoxemia-induced hyperventilation occurs has recently been shown to require appropriate mitochondrial function in the chemoreceptor cells.

Glomus cells contain a subset of cell membrane potassium channels that are rapidly inhibited in response to hypoxemia. This inhibition leads to depolarization of the cell which allows opening of voltage-gated calcium channels and influx of extracellular calcium. This increase in $[Ca^{++}]$ leads to neurotransmitter release that activates afferent sensory fibers. These sensory fibers, in turn, rapidly transmit this hypoxemic signal to the brainstem respiratory center leading to a reflexive increase in ventilation and sympathetic tone to counteract the drop in arterial oxygen tension (Peers 2010). The mechanism by which potassium channels are inhibited in response to hypoxia requires further clarification. Heme-oxygenase-2, hydrogen sulfide, nitric oxide, lactate, AMPK, and ROS have all been proposed to be involved in the oxygen sensing mechanism of glomus cells (Prabhakar 2014, Chang 2015, Lopez-Barneo 2016).

A major challenge to the idea that acute oxygen sensing occurs in the mitochondria has been the fact that cytochrome c oxidase (Complex IV) has a very high affinity for oxygen and would therefore be insensitive to the relatively small variations in oxygen concentrations that occur during physiological hypoxia. However, recent work has demonstrated that glomus cells have unique cytochrome c oxidases with relatively low oxygen affinity. This property of glomus cells allows the mitochondrial electron transport chain to be uniquely sensitive to

oxygen (Buckler 2013). These studies validate the necessity for a functional mitochondrial ETC in specialized acute oxygen sensing cells *in vivo*. The importance of the rapid response to changes in oxygen clearly may require overlap of mitochondrial and cytosolic sensors and potentially may involve redundant pathways to orchestrate effective oxygen responses. Therefore we and others propose a general theory that cytosolic mechanisms of oxygen sensing can be triggered by mitochondrial signals under physiological conditions. Acute sensing of oxygen by specialized PAMSC and glomus cells both require intact electron transport chain complexes and work through a mechanism of redox-induced increases in intracellular $[Ca^{++}]$.

The high K_m for oxygen in the glomus cell cytochrome c oxidase would result in increased levels of lactate under physiological hypoxia. Lactate rapidly accumulates in the blood and tissues during acute hypoxia as lower levels of oxygen as a substrate for oxidative phosphorylation lead to an increased flux of pyruvate to lactate to regenerate cytosolic NAD⁺. Increased lactate levels in the blood can induce a hyperventilation response. Olfr78, initially described as an olfactory receptor, is expressed in glomus cells at high levels and can be stimulated by lactate (Chang 2015). Recent work identified Olfr78 as a candidate oxygen sensor through comparison of the expression levels of genes in the carotid body versus the adrenal medulla (Chang 2015). Olfr78 deficiency impairs the glomus cell-mediated acute hypoxia response *in vivo* without impairing the carotid body response to hypercapnia (Chang 2015). As a G-protein coupled receptor, the precise mechanisms by which Olfr78 activation leads to increased $[Ca^{++}]$ require further investigation.

An alternative but not mutually exclusive mechanism is that mitochondrial ETC through ROS during hypoxia are necessary for hypoxic increase in calcium within glomus cells. A recent report demonstrated that a defect in hypoxemia-induced ventilation induction in tissue specific NDUFS2-(NADH Dehydrogenase Ubiquinone Iron-Sulfur Protein 2)-deficient mice. NDUFS2 is a key mitochondrial complex I protein that is directly involved in ubiquinone binding and electron transfer (Fernandez-Aguera 2015). NDUFS2-deficient carotid bodies were still able to induce respiratory reflexes in response to hypercapnia, but the NDUFS2-deficient glomus cells exhibited a defect in the hypoxic response. These cells were able to maintain normal ATP levels in the absence of complex I function through a succinate and FADH₂ dependent pathway utilizing ETC complexes II-III-V. Their results suggest a mechanism by which normal glomus cells signal decreases in oxygen tension through hypoxia induced changes in complex I function. Due to the specialized succinate-dependent metabolism of these glomus cells, the Q-pool is rapidly saturated with reduced QH₂. This prevents complex I from passing electrons forward into the Q-pool. Therefore, electron flow from the overly saturated Q-pool can shuttle electrons backwards through the ubiquinone binding site of complex I to induce an abnormal complex I state, referred to as reverse electron transport (RET). In this state, complex I produces ROS at high levels and NADH rapidly accumulates due to inhibition of complex I's NADH dehydrogenase function. Both ROS and NAD(P)H can directly lead to inhibition of cell membrane potassium channels and trigger the increased $[Ca^{++}]$ needed for neurotransmitter release. (Fernandez-Aguera 2015). The dependency of this mechanism on the ubiquinone binding site of complex I is supported by evidence that inhibitors that bind to complex I sites outside of the ubiquinone binding site do not lead to impairment of hypoxia sensing, while inhibitors

like rotenone and 1-methyl-4-phenylpyridinium (MPP), which directly disrupt ubiquinone binding, do prevent this specialized hypoxic response (Lopez-Barneo 2016). Furthermore loss of NDUFS4, a non-ubiquinone binding subunit of complex 1, does not display impaired carotid body oxygen sensing (Fernandez-Aguera 2015). These studies indicate that mitochondrial ETC is likely to be the site of oxygen sensing in glomus cells.

CHRONIC HYPOXIC ADAPTATION THROUGH HIF

The canonical hypoxia inducible factor pathway (HIF) is perhaps the most well-studied physiological oxygen response. HIF is a hetero-dimer of basic helix-loop-helix/PAS proteins that create a transcription factor. The hetero-dimer consists of a constitutively present beta subunit (HIF- β) and an alpha subunit (HIF- α) that is constitutively transcribed and translated but is rapidly degraded under normal oxygen conditions (normoxia) (Kaelin 2008). HIF- α subunits accumulate during hypoxia to generate the active transcription factor which induces the expression of a host of genes involved in angiogenesis, cell survival, proliferation, inhibition of mitochondrial respiration, and induction of glycolysis. Two important HIF targets at the organismal level are erythropoietin (EPO), which is a glycoprotein hormone that binds EPO receptors on erythrocyte precursors leading to expansion of red blood cell numbers, and vascular endothelial growth factor (VEGF) which is a key activator of angiogenesis. Both EPO and VEGF are essential for long term increases in oxygen delivery to tissue regions experiencing hypoxia (Semenza 2012).

During normoxia, HIF alpha subunits are hydroxylated by a group of 2-oxoglutarate-dependent dioxygenase enzymes named prolyl hydroxylase 1, 2, and 3 (PHD 1,2,3), with PHD2 serving as the dominant HIF hydroxylase (Bruick 2001, Epstein 2001). This hydroxylation occurs in the HIF- α oxygen-dependent degradation domain. Factor inhibiting HIF (FIH) also can hydroxylate HIF alpha subunits at a different site preventing transcriptional activation. PHD-mediated hydroxylation is required for HIF- α ubiquitination by the ubiquitin ligase Von Hippel-Lindau tumor suppressor protein (VHL) which targets HIF- α for rapid proteasomal degradation (Ivan 2001, Jaakkola 2001). The PHDs use ferrous iron as a cofactor to catalyze the hydroxylation of HIF by donating one oxygen atom of split molecular O₂ to HIF and donating the other oxygen atom to 2-oxoglutarate forming succinate and CO₂. Since molecular oxygen is a direct reactant in this regulatory step and the inhibition of this PHD-mediated HIF hydroxylation leads to downstream hypoxic signal activation, it has logically been proposed that PHDs serve as the proximal oxygen sensors that activate the HIF pathway (Hagen 2003). However it has also been demonstrated that activity of the PHDs can be influenced by mitochondrial function through the redox state of the cell and through changes in metabolite and ROS concentrations (Pan 2007). Recent work has shed light on the possible mechanisms by which mitochondrial signals influence HIF signaling.

The idea that HIF signaling requires functional mitochondria stems from the early observation that ETC-depleted Hep3B cells fail to stabilize HIF or induce EPO expression under hypoxic conditions (Chandel 1998). Exogenous antioxidant treatment prevents hypoxic HIF signaling, suggesting that ROS are required. Furthermore, exogenous ROS supplementation is sufficient to induce HIF stabilization and signaling during normoxia

(Chandel 2000). Desferrioxamine (DFO), an iron chelator, is also capable of inhibiting PHD2 due to the necessity of the ferrous iron as a cofactor for HIF hydroxylation and still can activate HIF signaling with ROS inhibition indicating that the effect of ROS on the HIF pathway is at the level of the PHD enzymes. Genetic validation came from the observation that loss of complex III protein Reikse-iron sulfur protein or cytochrome c prevents hypoxic stabilization of HIF- α (Mansfield 2005, Guzy 2005, Brunelle 2005). Further studies using pharmacological inhibitors or genetic impairment of ROS production at ETC electron leak sites suggested that complex III is the primary site of HIF-inducing ROS (Bell 2007). The Q binding site of complex III (Site III_Q) has the unique ability to generate ROS at relatively high levels in the intermembrane space rather than the mitochondrial matrix, which positions this site to produce ROS that can most directly influence cytosolic signaling pathways (Brand 2016). In support of this hypothesis, this site of electron leak has been implicated in the widest variety of mitochondrial ROS dependent signaling changes (Orr 2015). Therefore Orr et al. recently sought to identify specific inhibitors of ROS production at site III_Q without inhibiting oxidative phosphorylation. High throughput screening of small molecules identified multiple suppressors of site III_Q electron leak (S3QELs). These inhibitors were validated to prevent release of ROS from site III_Q, were nontoxic, and did not alter oxidative phosphorylation. Use of these inhibitors significantly blunted the HIF-mediated response to hypoxia providing support for the hypothesis that complex III ROS are required for cellular oxygen sensing (Orr 2015). Furthermore, a recently described small molecule inhibitor of complex I which abolished the mitochondrial membrane potential also prevented hypoxic HIF stabilization (Bastian 2016).

Due to the difficulty of deciphering the many changes in cellular metabolism potentially caused by genetic and pharmacological inhibition of the ETC, a recent study sought to separate the effects of loss of TCA metabolite flux from the effects of mitochondrial membrane potential loss (Martinez-Reyes 2016). Mitochondrial DNA was depleted by inducible deletion of the mitochondrial DNA polymerase which abolished hypoxic HIF-1 α stabilization. Genetic reconstitution of the membrane potential alone was accomplished by knockout of ATP1F1, a physiological inhibitor of F₀F₁-ATPase. ATP1F1 prevents the maintenance of mitochondrial membrane potential in the absence of an intact ETC. With ATP1F1 removed and without mtDNA, the F₁ component of the F₀F₁-ATPase uses glycolytic ATP and maintains the mitochondrial membrane potential through electrogenic ATP⁴⁻/ADP³⁻ exchange via the adenine nucleotide translocator (Martinez-Reyes 2016). Restoration of the membrane potential reestablished ROS production and restored hypoxic HIF stabilization in response to hypoxia in these cells despite the absence of TCA cycle function (Martinez-Reyes 2016).

How ROS inactivate PHDs remains unknown. Initial studies suggested that ROS could oxidize Fe²⁺ thus impairing PHDs (Gerald 2004). Multiple lines of evidence have indicated that the PHDs may be oxidized by reactive oxygen and nitrogen species leading to inhibition in vitro. Candidate cysteine residues required for the PHD oxidation have been identified (Lee 2016). A new proposed mechanism indicates that oxidation of PHD2 leads to the formation of inactive homodimers through disulfide bridge formation (Chowdhury 2011, Lee 2016). There are numerous other methods to activate HIF under normoxic conditions. Notably, loss or mutation of VHL leads to HIF stabilization and is a driving force in

numerous cancers (Shen 2013). HIF induction has also recently been reported as a response to paracrine glutamate accumulation in breast cancer cells. This extracellular glutamate in the tumor microenvironment inhibits the glutamate-cysteine antiporter which leads to cysteine depletion in these cancer cells. Cysteine depletion results in oxidative PHD2 inactivation and subsequent HIF accumulation (Briggs 2016).

Mitochondrial production of metabolites can also inhibit PHD function to increase HIF activation. A systematic screen identified novel genes that led to normoxic HIF stabilization (Burr 2016). This screen identified mitochondrial oxoglutarate dehydrogenase (OGDH) and lipoic acid synthase (LIAS), which both, when mutated, lead to an accumulation of L-2-hydroxyglutarate (L-2-HG). Due to structural similarity to 2-oxoglutarate, L-2-HG is a competitive inhibitor of the 2-oxoglutarate dependent dioxygenase family of enzymes that include the PHDs (Burr 2016). This finding suggests that the TCA cycle dysfunction that leads to 2-HG metabolite accumulation is also a potential mediator of HIF activation in addition to ROS signaling pathways. The TCA cycle enzymes fumarate hydratase and succinate dehydrogenase are tumor suppressors. HIF overexpression in numerous tumors was originally found to be correlated with inactivating fumarate hydratase mutations, and it was suggested that fumarate can act as a competitive inhibitor of PHD enzymes (Isaacs 2005). Buildup of succinate in the cytosol also promotes HIF stabilization through inhibition of PHD enzymes (Selak 2005). Further mechanistic studies characterized the inhibitory effects, physical interactions, and concentrations necessary for succinate and fumarate inhibition of PHDs leading to HIF stabilization (Koivunen 2007, Hewitson 2007). Succinate also promotes an inflammatory response by I κ B activation downstream of HIF-1 α stabilization (Tannahill 2013). Furthermore, fumarate levels have been linked to non-canonical NF κ B activation which can accentuate hypoxia target gene activation (Shanmugasundaram 2014). It is possible that there are multiple redundant pathways that can inhibit PHDs during hypoxia including loss of substrate molecular oxygen, loss of ferrous iron cofactors, buildup of inhibiting metabolites, loss of intracellular cysteine, induction of reactive oxygen species, and potentially others. Perhaps each of these pathways can be the dominant cause of “oxygen sensing” leading to HIF activation based on the context and experimental conditions.

Mitochondrial regulation of HIF is also necessary for hypoxic induction of EPO at the level of organismal adaptation. While the HIF target hormone EPO is primarily expressed in the kidney, evidence suggests that HIF activation in other tissues is important for this response to be optimal. The skin has been shown to play a key role in the organismal hypoxia response in amphibians and mammals. Deletion of HIF specifically in keratinocytes led to a reduction in the epidermal blood vessel dilation that normally occurs with ambient hypoxia. This change in cutaneous blood flow reduced renal production of EPO in response to hypoxia (Boutin 2008). Furthermore, keratinocyte aberrant activation of HIF by keratinocyte-specific deletion of VHL led to polycythemia due to upregulation of EPO through a mechanism of increased HIF-1 dependent cutaneous blood flow (Boutin 2008). To test the requirement of keratinocyte ETC for organismal adaptation to hypoxia in vivo, the ETC was conditionally and specifically depleted in the mouse epidermis through genetic deletion of the mitochondrial transcription factor A (TFAM) which is required for replication and transcription of mitochondrial DNA. TFAM depletion led to a diminished

HIF response in keratinocytes. Downstream targets of HIF were not induced in the skin during hypoxia which prevented arterial dilation. This led to reduced renal EPO production during prolonged hypoxia. These results indicate that ETC function in the epidermis is required for full organismal adaptation to hypoxic conditions (Hamanaka 2016).

CONCLUSION

It is clear that through multiple mechanisms, the mitochondria, as the primary consumers of cellular oxygen, are highly integrated into oxygen sensing pathways and help to tune cellular and organismal hypoxia responses. Rapid acute responses occur as a result of ROS-induced or metabolite-induced $[Ca^{++}]$ accumulation in specialized oxygen sensitive cells and longer-term hypoxia sensing is possible through ROS-induced or metabolite-induced PHD inhibition due to HIF activation. The detailed delineation of physiological signal transduction mechanisms downstream of the mitochondria remains challenging due to limitations of the detection and manipulation of these transient changes in metabolites and reactive oxygen species, however necessity and sufficiency studies in a variety of models now confirm that mitochondrial function is essential for the hypoxia response.

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Highlights

- Mitochondrial electron transport chain function plays a key role in organismal hypoxia sensing.
- ETC Complex III ROS are necessary for acute hypoxic contraction of pulmonary smooth muscle cells
- ETC Complex I function is essential for acute glomus cell sensing of hypoxemia in the carotid body
- mROS are required for HIF stabilization and organismal adaptation to hypoxia

Figure 1.

Pulmonary Artery Smooth Muscle Cells initiate acute hypoxic contraction. Induction of electron transport chain complex III reactive oxygen species leads to increased intracellular [Ca⁺⁺] which triggers smooth muscle contraction. (Abbreviations: S.R. - Sarcoplasmic Reticulum, TCA - Tricarboxylic Acid, PASMC - Pulmonary Artery Smooth Muscle Cell, ROS- Reactive Oxygen Species, CoQ - Ubiquinone, C Cytochrome C)

Figure 2.

Carotid Body Glomus Cells initiate an acute ventilation reflex in response to hypoxemia. Glomus cell cytochrome c oxidase is uniquely sensitive to hypoxia. Glomus cells exhibit reverse electron transport through complex I leading to increased production of reactive oxygen species and NADH accumulation which in turn lead to membrane depolarization, increased intracellular [Ca⁺⁺], and neurotransmitter release to trigger afferent sensory neurons that initiate the hyperventilation reflex. Lactate production can also stimulate the ventilation reflex in glomus cells. (*Abbreviations: IMS - Intermembrane Space, ROS - Reactive Oxygen Species, CoQ - Ubiquinone, C - Cytochrome C*)

Figure 3.

Mitochondrial function is required for hypoxia inducible factor (HIF) activation via prolyl hydroxylase (PHD) enzyme inhibition. HIF activation requires an intact respiratory chain to maintain mitochondrial ROS. Hypoxia may limit PHD activity directly since PHDs use oxygen to hydroxylate HIF and target HIF for degradation. The PHDs can also be inhibited by oxidation and by the buildup of metabolites such as 2-hydroxyglutarate, fumarate, and succinate. PHD inhibition allows for HIF stabilization and target gene activation.

(Abbreviations: Epo – Erythropoietin, VEGF – Vascular Endothelial Growth Factor, ROS – Reactive Oxygen Species)