

Intracellular energy production and distribution in hypoxia

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The hydrolysis of ATP is the primary source of metabolic energy for eukaryotic cells. Under physiological conditions, cells generally produce more than sufficient levels of ATP to fuel the active biological processes necessary to maintain homeostasis. However, mechanisms underpinning the distribution of ATP to subcellular microenvironments with high local demand remain poorly understood. Intracellular distribution of ATP in normal physiological conditions has been proposed to rely on passive diffusion across concentration gradients generated by ATP producing systems such as the mitochondria and the glycolytic pathway. However, subcellular microenvironments can develop with ATP deficiency due to increases in local ATP consumption. Alternatively, ATP production can be reduced during bioenergetic stress during hypoxia. Mammalian cells therefore need to have the capacity to alter their metabolism and energy distribution strategies to compensate for local ATP deficits while also controlling ATP production. It is highly likely that satisfying the bioenergetic requirements of the cell involves the regulated distribution of ATP producing systems to areas of high ATP demand within the cell. Recently, the distribution (both spatially and temporally) of ATP-producing systems has become an area of intense investigation. Here, we review what is known (and unknown) about intracellular energy production and distribution and explore potential mechanisms through which this targeted distribution can be altered in hypoxia, with the aim of stimulating investigation in this important, yet poorly understood field of research.

The provision of sufficient biochemical energy is a vital requirement for any cell to carry out the active reactions necessary to maintain homeostasis. The energy needed for mammalian cell reactions is provided primarily through nucleoside triphosphate complexes. These complexes are comprised of a nitrogenous base combined with a ribose sugar to form the respective nucleoside structure. A phosphoester bond then allows binding of an inorganic phosphate group to the nucleoside, and further additional inorganic phosphate groups are bound to the preceding phosphate groups by phosphoric anhydride bonds, for example X-monophosphate, X-diphosphate, and X-triphosphate where X is the respective nucleoside structure. Energy is provided by these molecules

through hydrolysis of the phosphoric anhydride bonds. With five naturally occurring nitrogenous bases, five separate natural nucleoside triphosphate complexes are possible. ATP is the main energy source of the cell, and it fuels most active processes (1–3) in addition to its role in intracellular signaling activities. These involve functions as diverse as activation of kinases (4) and signaling pathways (5) as well as cell-specific functions ranging from the activation of ion channels (6, 7) to the propulsion provided by flagella (8, 9). Hydrolysis of ATP typically yields a Gibbs free energy of -7.3 cal/mol (10) (Fig. 1).

GTP is involved in iron homeostasis (11), assembly of microtubules (12), involvement in GTP-binding signal transducing proteins (13), and energy homeostasis in some cancers (14). Cytidine, thymidine, and uridine triphosphate (CTP, TTP, and UTP, respectively) also have important roles (15–17), namely the formation of DNA and RNA synthesis through their deoxynucleotide form. While each nucleoside triphosphate has important functions in the mammalian cell, it is unclear how and why nucleosides have developed to play distinct roles during evolution with respect to cellular bioenergetics and metabolism.

However, the reason as to why ATP arose to become the universal energy currency in cells remains unknown. It is hypothesized that prebiotic synthesis of ATP is chemically favourable in aqueous solution in prebiotic conditions and thus led to the universal conservation of this molecule as a source of energy (18). Alternatively, a recent review paper has expertly discussed the relevant molecules needed to synthesise ATP and have postulated that the prebiotic synthesis of ATP could have been volcanism-dependent and became a direct energy source as enzyme-catalysed reactions replace abiotic ones (19). Yet, while acknowledging other nucleoside triphosphates and their contribution to normal physiological function, ATP is utilised as the main energy source of mammalian cells and will be focus of this review.

ATP synthesis

The synthesis and production of ATP occurs mainly through the oxidative metabolism of glucose *via* respiration. This well-described model of respiration is highly efficient in yielding ATP with 38 molecules being produced per glucose molecule consumed (20–25). Respiration is the principal mechanism of ATP generation in mammalian cells. This

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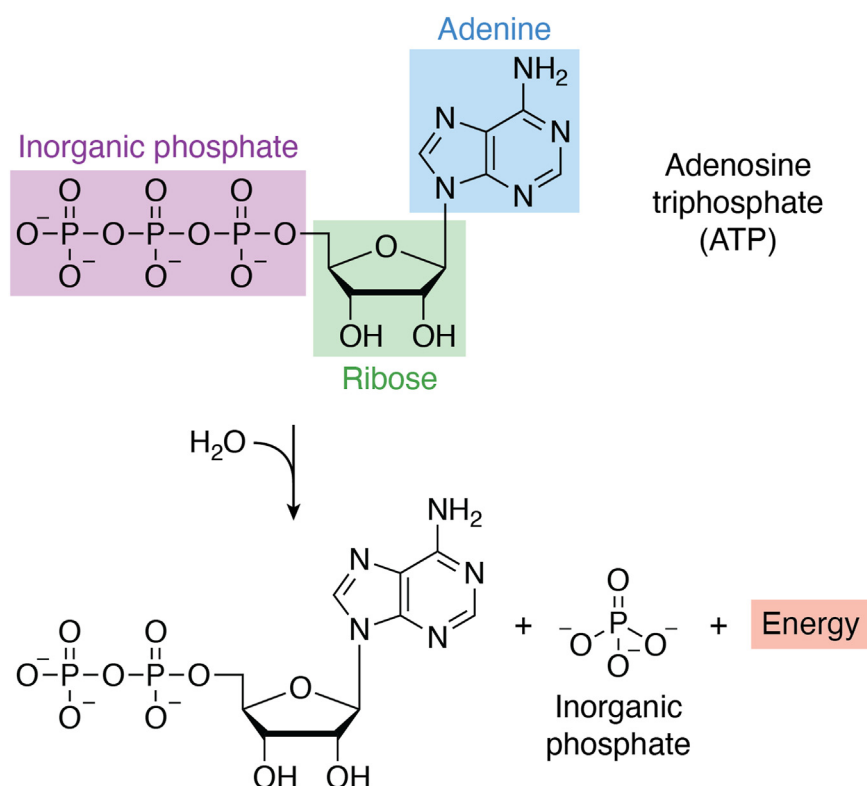


Figure 1. Diagram showing the hydrolysis of adenosine triphosphate (ATP) into adenosine diphosphate (ADP), energy, and inorganic phosphate. Upon hydrolysis of ATP with H₂O, the phosphoric anhydride bond is broken and releases energy causing the formation of ADP and an inorganic phosphate molecule.

process first involves the transport of glucose into the cell *via* glucose transporters (GLUTs). There have been 14 identified GLUTs all of which play a role in glucose transport between compartments in mammalian cells (26). Upon transport of glucose into the cell, respiration begins with glycolysis and yields a net gain of two ATP molecules per molecule of glucose consumed. Alongside this ATP production, glycolysis also produces two molecules of pyruvate which can be utilized in the tricarboxylic acid (TCA) cycle and electron transport chain (ETC). The TCA cycle and ETC are located within the mitochondria and yield a further 36 molecules of ATP, provided that there is sufficient oxygen available as the final electron acceptors of the ETC.

Ketone body metabolism and beta oxidation are also important pathways which result in the production of ATP, as both pathways produce acetyl-coA which then feeds into the TCA cycle within the mitochondria (27, 28). While ketone body metabolism and beta oxidation are recognized mechanisms of ATP production, they will not be further explored in the context of this review. Instead, the focus will be on mitochondria and the glycolytic pathway as the main sources of cellular ATP.

Research regarding ATP has been heavily revolved around the synthesis, functions, and processes that ATP is involved in. However, there remain major questions concerning whether and how ATP is distributed inside the cell to fuel active cellular processes efficiently and effectively in subcellular microenvironments.

The “ideal” state of intracellular energy distribution

The distribution/trafficking of intracellular ATP remains incompletely understood and with a lack of published studies concerning this topic, this review aims to highlight what is known thus far regarding intracellular energy production and distribution and then propose potential mechanisms by which directed transport can occur.

With the physiological intracellular concentration of ATP being approximately 4.4 mM (29), one theory is that this concentration exceeds the amount needed at local sites for normal cellular processes. According to this “idealized model” or “ideal state”, which is the simplest manner in which a dynamic system can be represented (30), the distribution of ATP occurs through passive diffusion across intracellular cytoplasmic space. In this model, as ATP is consumed in subcellular microenvironments, the concentration in that area is depleted but soon restored through passive diffusion of ATP from subcellular regions of high ATP concentration. The “ideal state” allows cells to continue all ATP-dependent tasks with any rise in bioenergetic demand being met with an intracellular ATP increase to compensate. This allows the passive distribution of ATP to continue and maintain energy homeostasis across the cell, as ATP concentration remains in excess of cellular demand. It is possible that this may be an effective process for cells in physiological conditions. However, as cells undergo hypoxia, ATP production is reduced due to reduced mitochondrial metabolism. Hypoxia then presents a “non-ideal state” in which passive distribution of ATP is

insufficient to provide subcellular components with the energy required for their respective processes. While the “ideal state” *versus* the “nonideal state” paradigm is used to understand the basic concept of cellular energy distribution in this review, it is likely more complex as it is a dynamic system which fluctuates physically, spatially, and temporally between the “ideal” and “nonideal” states (Fig. 2).

The hypothesis of distinct intracellular microenvironments in the ideal and nonideal state is another complex phenomenon to consider. This can arise due to the diffusion restriction that organelles and other cellular structures impose on enzymes and molecules such as ATP. As the creation of ATP-depleted microenvironments may occur under normal physiological conditions, it is possible (indeed highly likely) that cells possess mechanisms that allow directed distribution of ATP-producing systems to regions of high ATP requirement to increase the availability and utilization of energy.

Mathematical studies have modeled the dynamics of enzyme-enzyme interactions, enzyme-substrate interactions, and substrate channeling (30–35) in which most agree upon the principle that intracellular distribution of these interactions, intermediates, and products cannot rely solely on passive diffusion. It is therefore likely that this concept applies to the distribution of ATP-producing systems to sites of high energy demand. This could include the possible formation of metabolic complexes involving the glycolytic enzymes and/or movement of mitochondria. Yet, there remains a deficit in our understanding of intracellular energy distribution. Forms of

cellular stress which inhibit the respiratory capabilities of the cell and reduce the production of ATP, provide a model for investigations into these proposed interactions and systems relating to energy distribution. Metabolic stress can be induced by hypoxia and can bring upon a marked reduction in ATP production *via* inhibition of mitochondrial respiration. In mammalian cells, hypoxia causes a decrease in ATP concentration by up to 30% (36). This review will focus on the cellular response to hypoxia and how the cell may adapt to overcome this nonideal state of ATP distribution. Currently there is a lack of evidence to support the targeted distribution of intracellular energy. Despite this, by understanding hypoxia and the concept of the “nonideal state” of energy distribution, potential mechanisms may be identified for further investigation. We believe this is an area of fundamental biologic importance which remains poorly understood and is in need of further investigation.

Cellular response to hypoxia

As described above, hypoxia occurs when oxygen demand exceeds supply. Due to the importance of molecular oxygen in respiration, adaptation to hypoxia is vital for metazoan cell survival. A primary mechanism underpinning adaptation to hypoxia are transcription factors which induce adaptive gene expression. Primary among these hypoxia-induced transcription factors is the hypoxia-inducible factor (HIF) (37).

HIF is a heterodimeric protein which is comprised of a labile, oxygen-sensitive α -subunit, and a stably expressed

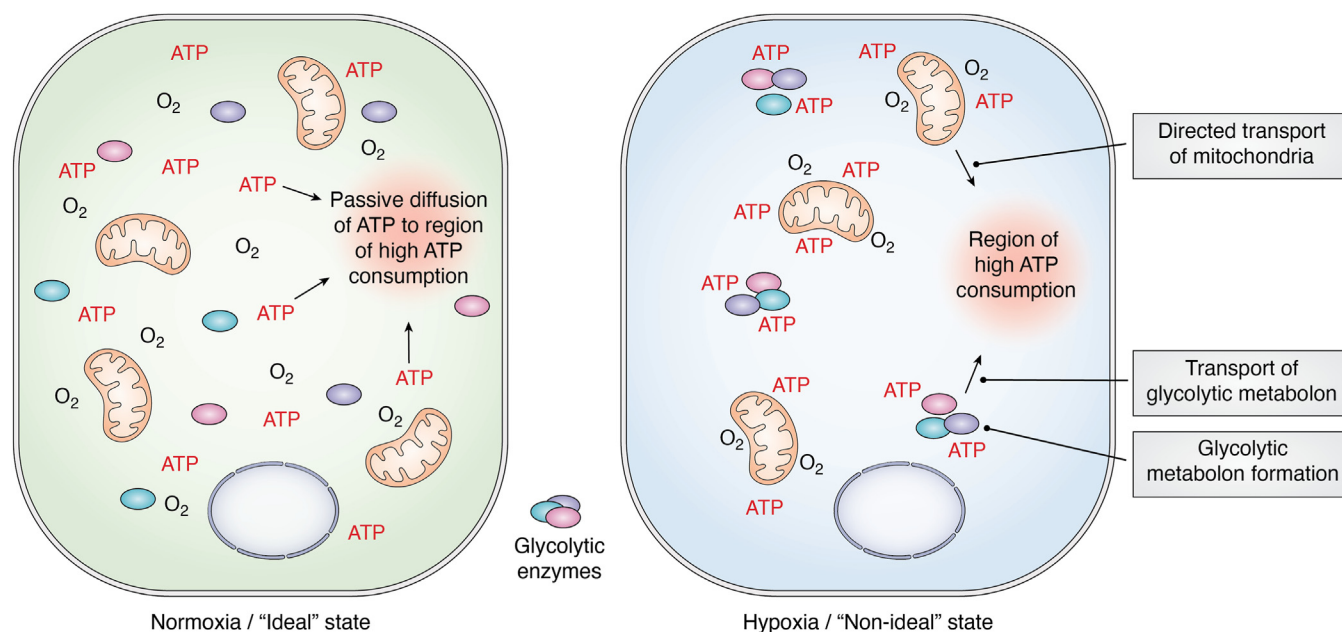


Figure 2. Schematic demonstrating the ATP concentrations in the ideal state *versus* the hypoxia-induced nonideal state in a cell. Mitochondria and glycolytic enzymes can be seen in both states as the ATP-producing systems which create the homogenous ATP concentration seen in the ideal state and the localization of regions of high ATP concentrations in the hypoxia-induced nonideal state. Decreases in ATP concentration due to hydrolysis are quickly restored *via* passive diffusion of ATP from regions of high concentration to low in the ideal state. The hypoxia-induced nonideal state section of the schematic demonstrates a hypothetical situation where decreases in ATP production or increases in consumption can result in regions of low ATP concentration. ATP is then localized to sites of production such as the mitochondria and glycolytic enzymes. Decreased ATP production results in a decreased intracellular concentration and so compromises the ideal state of passive diffusion. Regions of insufficient ATP concentrations are created which requires targeted intracellular energy trafficking. These regions of low intracellular ATP could be equilibrated *via* the formation and transport of a glycolytic metabolon.

β -subunit otherwise known as the aryl hydrocarbon receptor nuclear translocator or HIF-1 β . There are three HIF- α isoforms (HIF-1 α , -2 α , and -3 α) which have been described (38–40). This review will focus on HIF-1 α which is widely expressed, whereas HIF-2 α has been shown to be more cell-type specific with restricted tissue expression patterns (41).

In the presence of oxygen, Fe²⁺, 2-oxoglutarate, and ascorbate (42–44), prolyl hydroxylases (PHD1, PHD2, and PHD3) cause the degradation of HIF-1 α by hydroxylation at two proline residues (Pro402 and Pro564) located within the oxygen-dependent degradation domain of HIF-1 α (42, 45, 46). The hydroxylation of these residues promotes binding to von Hippel Lindau protein which is a component of the E3 ubiquitin ligase complex that tags the HIF-1 α -subunit for proteasomal degradation (47–50). Factor inhibiting HIF is another hydroxylase enzyme which hydroxylates an asparaginyl residue (Asn803) which is in the C-terminal transactivation domain of HIF-1 α (51, 52). This prevents the interaction of the C-terminal transactivation domain with other transcriptional co-factors while in the presence of oxygen, 2-oxoglutarate, Fe²⁺, and ascorbate (53). As a result of these oxygen-dependent modifications, HIF-1 α is degraded *via* the ubiquitin-dependent proteasomal degradation pathway (54–59).

In hypoxia, the oxygen-dependent hydroxylation of HIF-1 α cannot occur, and this promotes the stabilization of HIF-1 α and its accumulation in the cytoplasm. Upon stabilization and accumulation, HIF-1 α is then translocated to the nucleus of the cell where dimerization with HIF-1 β /aryl hydrocarbon receptor nuclear translocator occurs (60). Once the dimer is

formed, HIF-1 then binds to hypoxia response elements located in promoter or enhancer regions of >500 genes including vascular endothelial growth factor (61) and erythropoietin (62). These genes are then transcribed to facilitate hypoxic adaptation (60, 63) (Fig. 3).

Further information regarding the HIF pathway and its implications in health and disease has been reviewed elsewhere recently (64). While HIF is the primary transcription factor that mediates responses to hypoxia, there are other responses which are also mediated by hypoxia. Other transcription factors which are sensitive to hypoxia include nuclear factor kappa-B (65–69), cyclic AMP response binding element (70, 71), activating protein 1 (72–74), and others which have been reviewed in detail elsewhere (75–77).

Other oxygen-sensitive pathways which are independent of transcription factors have also been described. AMP-activated protein kinase (AMPK) signaling is a primary oxygen sensor in mammalian cells. AMPK provides cells with a mechanism to adapt to low energy situations signaled by an increase in AMP to ATP ratio. Increases in the AMP:ATP ratio activates AMPK and allows for adaptation by decreasing energy expensive anabolic processes and increasing catabolic reactions (78–80). There is also an interconnected relationship between AMPK and HIF which has been reviewed in detail elsewhere (81). Other pathways include the suppression of energy-expensive protein synthesis *via* protein kinase R-like endoplasmic reticulum kinase (82) and mechanistic target of rapamycin complex 1 (83). While these pathways are important in overall energy metabolism, they will not be covered in further detail in this review.

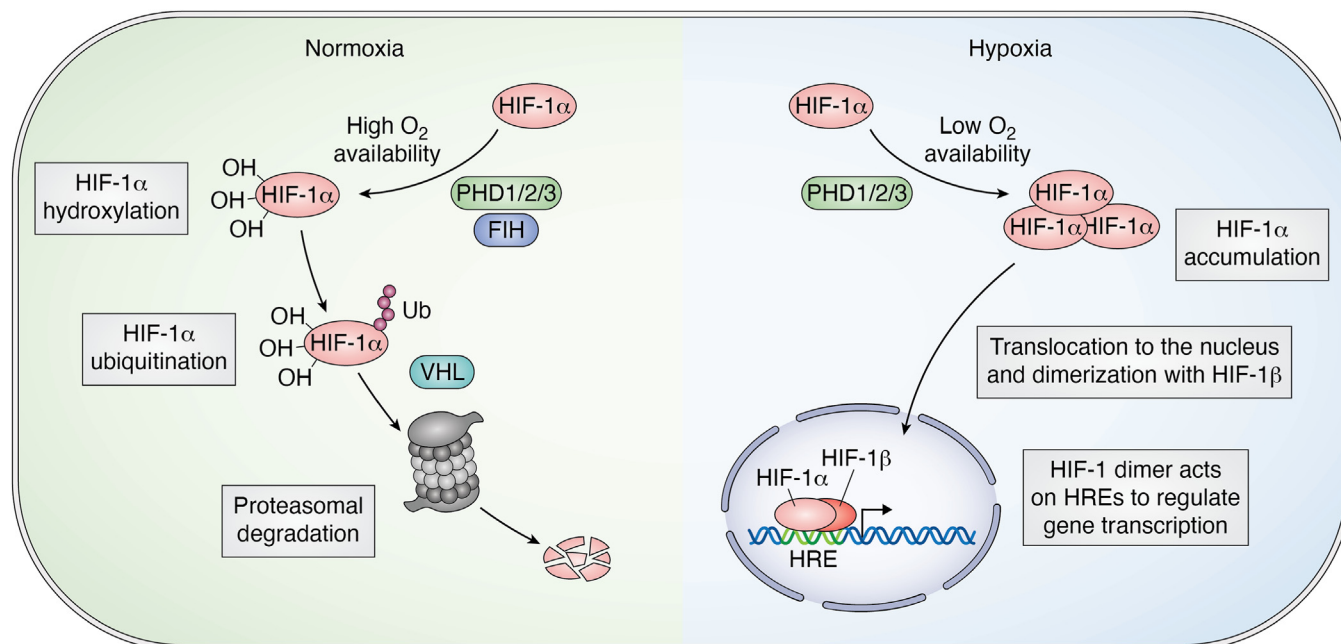


Figure 3. Diagram showing the degradation and stabilization of the HIF-1 α protein in the presence or absence of oxygen, respectively. HIF-1 α is degraded in the presence of oxygen *via* ubiquitin-dependent proteasomal degradation pathway upon hydroxylation, through FIH and PHDs, and ubiquitination *via* VHL. In hypoxic conditions, lack of oxygen prevents proteolytic degradation and dimerization of HIF-1 α with HIF-1 β /ARNT then allows for binding to hypoxia response elements on DNA and elicits a transcriptional effect to upregulate the production of the protein as a response to hypoxia. ARNT, aryl hydrocarbon receptor nuclear translocator; FIH, factor inhibiting HIF; HIF, hypoxia-inducible factor; PHD, prolyl hydroxylases; VHL, von Hippel Lindau.

Hypoxic adaptations in the “nonideal state”

Hypoxia presents the cell with a unique challenge in that it does not allow the maintenance of the “ideal state” model of energy distribution reliant on passive diffusion of ATP due to diminished mitochondrial ATP production and results in the development of the proposed “nonideal state” model of intracellular bioenergetics. Induction of the “nonideal” state can allow for exposure of the mechanisms by which intracellular energy is distributed more efficiently through the formation or movement of ATP-producing systems to subcellular microenvironments of high ATP consumption. These systems which yield energy in the form of ATP can be influenced directly or indirectly by transcription factors such as HIF-1 and by other nontranscriptionally mediated adaptations to hypoxia. During hypoxia, important HIF-dependent adaptations are those of ATP-producing systems including the mitochondria and the glycolytic pathway.

Mitochondrion—the powerhouse

The mitochondrion is the “powerhouse” of the cell producing 36 molecules of ATP per molecule of glucose metabolised. In hypoxia, mitochondrial ATP production is reduced due to the lack of available oxygen and as a result aerobic ATP is decreased (36). Additionally, further hypoxic adaptations which decrease the activity of the mitochondria are present in the cell including the HIF-1 dependent upregulation of pyruvate dehydrogenase kinase 1. Pyruvate dehydrogenase kinase 1 promotes the phosphorylation of pyruvate dehydrogenase, mediating the inhibition of pyruvate as a substrate for the TCA cycle, causing a reduction in the consumption of oxygen by the mitochondria (84, 85). In addition, HIF-1 inhibits mitochondrial biogenesis through the suppression of c-Myc leading to decreases in PCG-1 β expression which is responsible for a loss in mitochondrial mass (86). While reductions in mitochondrial biogenesis and oxygen consumption can be seen as a protective mechanism against oxidative stress by reducing the production of reactive oxygen species (87, 88), it likely challenges the cell to overcome an intracellular energy deficit due to the reduction in activity of the mitochondria.

Although hypoxic adaptations of the mitochondria exist primarily to reduce oxidative stress (but resulting in decreased ATP production) a lower level of oxygen-dependent respiration could remain in the cell depending on the degree of hypoxia. Considering this, additional adaptations increase the output of mitochondrial ATP production despite the low oxygen availability. One such adaptation is the HIF-1-dependent enhancement of mitochondrial respiration efficiency *via* changes to the cytochrome oxidase subunits from the predominant normoxic COX4-1 isoform, to the hypoxia-induced COX4-2 isoform which improves the efficiency of respiration (89). Alongside this increase in COX4-2 expression, HIF-1 also promotes the degradation of COX4-1 through LON protease (90) allowing for the efficient COX4-2 isoform to become predominant throughout hypoxic periods.

With limited ATP production by the mitochondria, ATP distribution and utilization quickly becomes a vital factor in

maintaining bioenergetic homeostasis. Directed localization of the mitochondria to regions of high ATP demand within the cell could improve the usage and production rather than relying on passive diffusion to these regions in need of energy. Spatial reorganization of mitochondria has been demonstrated to involve the HIF-1-dependent upregulation of a mitochondrial protein termed hypoxia-upregulated mitochondrial movement regulator (HUMMR). HUMMR is localized to the mitochondria of astrocytes to influence the anterograde transport of mitochondria, and upon loss of HUMMR or HIF-1 function, a significant decrease in mitochondrial number was observed in axons which were exposed to hypoxia (91). Furthermore, studies investigating the antiapoptotic protein survivin, demonstrated that survivin is increased by hypoxia (92–94), through HIF-1 α (95). Survivin also mediates subcellular mitochondrial trafficking to the cortical cytoskeleton in periods of hypoxia in prostate cancer PC3 cells (96). The reorganization of the mitochondria to the cortical cytoskeleton near focal adhesion complexes was proposed to aid in fueling the energy-intensive movements of tumor cells and regulate tumor cell invasion and metastasis (96).

In combination with the altered localization of mitochondria, mitochondrial network morphology must also be considered. As it is known that the mitochondria can exist as highly branched tubular networks (97, 98) the effects of this on energy production and distribution is the key. These mitochondrial networks can differ in morphology and are dependent on tissue-type, cell-type, and the energetic needs of the cell and can exist in heterogeneous states (97, 99–103). In hypoxia, the mitochondria can appear donut-shaped, shortened, demonstrate perinuclear redistribution (104) and mitochondrial fission mediated by a mitochondrial protein Fun14-domain protein 1 (105). However, the effects and implications of the mitochondrial network or mitochondrial autophagy on the distribution of intracellular ATP to intracellular microenvironments of high demand are not yet fully understood.

Overall, in response to hypoxia, the mitochondrial adaptations mediated by HIF-1 primarily decrease activity of these organelles to prevent the production of reactive oxygen species. Along with this, other adaptive responses can increase the efficiency of respiration during periods of hypoxia and to spatially rearrange mitochondria which may optimize the mitochondrial network as well as the distribution of ATP to sites of high demand. In summary, hypoxia leads to a reduced but more efficient and spatially directed mitochondrial ATP production.

Glycolysis

Due to net mitochondrial ATP production in hypoxia being reduced, the cell turns toward other energy-producing systems such as glycolysis to compensate for decrease in the intracellular levels of ATP.

These glycolytic hypoxic adaptations include the HIF-directed transcriptional upregulation of ATP-producing and rate-limiting glycolytic enzymes including phosphoglycerate

kinase, aldolase, lactate dehydrogenase-A, enolase, and phosphofructokinase live-type as well as GLUT1 and 3 (106–110). The increased HIF-1 α -dependent transcription of glycolytic enzymes and GLUT drive increased the uptake of glucose and activity of the glycolytic pathway during periods of hypoxia. While this upregulation of glycolytic enzymes increases the production of ATP, glycolysis may still be considered as an inefficient method of ATP production due to the relatively low ATP yield and the intermediate substrates produced relying on passive diffusion in the “ideal state” of energy distribution to allow for pathway activity. Directed channeling of glycolytic intermediates to the correct enzymes may provide a solution to increase the efficiency of glycolysis in terms of ATP production.

Along with previously described mathematical studies demonstrating models of enzyme-enzyme interactions, enzyme-substrate, and substrate channeling (30–35), data have been presented in several studies that supports the hypothesis that substrate channeling of intermediate products involving glycolytic enzymes exists in various other species such as *Arabidopsis* (111, 112), *Protista* (113), *Saccharomyces cerevisiae* (114), and *Drosophila* (115). More recently, evidence supporting the formation of a glycolytic “metabolon” in mammalian cells such as human cervix adenocarcinoma cells and breast carcinoma cells (116), cardiomyocytes (117) and erythrocytes (118) has been proposed. Furthermore, hypoxia-dependent small ubiquitin related modifier-1 (SUMO-1) posttranslational modifications cause the colocalization of glycolytic enzymes, GAPDH and pyruvate kinase, to facilitate substrate channeling to increase the efficiency of glycolysis (119). Studies carried out on *S. cerevisiae* and human hepatocarcinoma cells have shown a direct link between “metabolon” formation and hypoxic stress in that glycolytic enzymes colocalize under hypoxic stress conditions and form glycolytic bodies or G-bodies (120, 121). Alongside this, a HIF-1-dependent metabolic complex has been recently identified in colonic epithelial cells to increase glycolytic metabolism in the absence of functional transcriptional machinery (122), further adding to the direct link between hypoxic stress and glycolytic “metabolon” formation. Substrate channeling and colocalization of these glycolytic enzymes present a potential mechanism whereby spatial and temporal reorganization of this glycolytic “metabolon” may serve to facilitate the distribution of intracellular energy in hypoxic mammalian cells. Yet, the mechanism by which this colocalization (hypoxia-dependent or hypoxia-independent) occurs remains unclear.

In recent years, many studies have focused on the idea of liquid-liquid phase separated (LLPS) biomolecular condensates which could provide a structured basis for how the glycolytic substrates and enzymes are colocalized. These condensates compartmentalize proteins and nucleic acids into membrane-less bodies with specific functions (123). With the ability to dissociate and reform within the cytoplasm of the cell, this allows the hypothetical “metabolon” to form spatially and temporally where it needs to be within the cell. Further analysis on the regulation of glycolysis *via* hypoxia and HIF-1 α can be found here (124).

Potential mechanisms of transport and spatial reorganization

While studies have identified the interaction of HUMMR and survivin with mitochondria and movement of these organelles (91, 96) alongside the colocalization of glycolytic enzymes in various species (111–115) and mammalian cell types (116–118), further investigation into the mechanisms of transport and spatial reorganization of these ATP-producing systems is warranted. It is important to note that these mechanisms may be mediated *via* adaptations to hypoxia much like the mitochondria and glycolytic enzymes have seen above, due to the reduced availability of intracellular ATP. Cytoskeletal rearrangement could eliminate diffusion restricted enzymes and allow for the transport or relocation of ATP-producing systems. Previously mentioned posttranslational modifications such as SUMOylation could influence the colocalization of glycolytic enzymes or affect other pathways which may prove bioenergetically favorable. Intracellular transport that occurs *via* chaperone proteins is another mechanism which the cell could utilize to allow for efficient ATP homeostasis by facilitating transport of ATP-producing systems both spatially and temporally. Biomolecular condensates or membrane-less organelles that form *via* LLPS have been an intense field of study in the last number of years and could serve as another mechanism in the formation and trafficking of ATP-producing system to sites of high intracellular energetic demand. These potential mechanisms are discussed in further detail below.

Cytoskeleton

The potential role of the cytoskeleton in directing subcellular energy/ATP distribution is poorly understood. The cytoskeleton of mammalian cells provides mechanical support, an intracellular transport system and structure to facilitate cell division and movement. Most important are the reorganization capabilities of the cytoskeleton and transport capabilities the microtubules provide to distribute organelles and other subcellular components. In response to hypoxia, the filamentous actin component of the cytoskeleton reorganizes from a web-like structure into a parallel formation of stress fibers, while also increasing in size and number (125). This long-term reorganization of filamentous actin is also seen when cells are treated with dimethylallyl glycine, a PHD-inhibitor, indicating that the reorganization and formation of stress fibers is HIF-1 α -dependent (126). Potentially, the reorganization of these fibers could alter the distribution of subcellular organelles, enzymes, and proteins within a cell and localize substrates and enzymes as a form of substrate channeling.

Studies into the microtubules have produced conflicting results. While some studies propose that hypoxia results in microtubule stabilization (127, 128) and other studies report that hypoxia results in the depolymerization of microtubules (129–131). This response of the microtubules could be due to cell-type specificity, degree, and duration of hypoxia or various other complex reactions which occur within the microtubular networks of these cells. Microtubule partitioning is another

concept which has been reviewed and indicates that there may be changes in bioenergetics with a shift toward either soluble or polymerized microtubule pools (132). This partitioning hypothesis may allow for substrate channeling or directed transport of ATP producing systems. Though this hypothesis has yet to be fully tested.

The third component of the cytoskeleton is the intermediate filaments which consist of many proteins including vimentin, keratins, desmin, and neurofilaments and glial fibrillary acidic protein. Though intermediate filaments are primarily recognized for their role in withstanding mechanical stress and integrating components of the cytoskeleton, they also influence the organization of the internal structure of the cell (133, 134). Hypoxic effects such as alteration of vimentin distribution (135) and hypoxia-induced degradation of keratin filaments (136) have the potential to impact the subcellular localization of mitochondria and glycolytic enzymes creating microenvironments of enriched concentrations of ATP, although this is currently unknown.

Partitioning of the cell *via* the cytoskeleton, which has already been demonstrated (137) and speculated to play a role in regulating metabolism (132), could create microenvironments of high ATP demand. Alternatively, this feature of the cytoskeleton could aid in biomolecular condensate formation. If cells possess the ability to rearrange these divides, it is a potential mechanism by which energy or energy-producing complexes can be distributed throughout the cell. However, there is a need for more research in this important field of cellular biology.

Posttranslational modifications

Posttranslational modifications drive many intracellular processes and include phosphorylation, ubiquitination, hydroxylation, and SUMOylation. Hypoxia increases the activity of mitogen-activated protein kinases such as Jun-N-terminal kinase, extracellular signal-regulated protein kinase, and p38 kinase (138). With this increase in phosphorylation activity, several studies have demonstrated an accompanied increase in glycolysis (139, 140). A kinase belonging to the proviral integration site for Moloney murine leukemia virus (PIM) family, PIM2, is upregulated *via* NF- κ B (141) which in turn results in the phosphorylation of PFKFB4 by PIM2 promoting anaerobic glycolysis (142).

On the other hand, protein SUMOylation is increased during periods of hypoxia *via* SUMO-1 (119, 143–146). Evidence demonstrating the interaction of SUMO-1 with HIF-1 α promoting its stabilization (145, 146), and enhancement of glycolysis as well as localization of glycolytic enzymes have been associated with SUMO-1 (119). However, the mechanism by which SUMO-1 influences glycolytic activity and the physiological relevance of this is still to be elucidated. mUbc9, a SUMO-conjugating enzyme which links SUMO-1 with various other proteins, can interact with GLUT1 and GLUT4 ultimately modulating glucose transport (147). Overexpression of mUbc9 causes an increase in GLUT4 protein expression, yet conversely reduces the total cellular content of the GLUT1 protein (147). GLUT1 is primarily utilized in basal glucose transport, whereas

GLUT4 is predominantly used to accelerate glucose transport (148), potentially allowing for a SUMOylation-dependent acceleration in glucose transport and an upregulation in overall cell metabolism raising the intracellular concentration of ATP. Alongside this, SUMO-activating enzyme 1 SUMOylation modulates glycolytic metabolism *via* regulation of pyruvate kinase activity (149).

Hypermethylation of promoter regions of genes increases during periods of hypoxia *via* reduction in the activity of oxygen-dependent ten-eleven translocation enzymes, which catalyze DNA demethylation in normoxia (150). Methylation of various proteins, enzymes, and transporters associated with energy metabolism have been implicated in altering glucose transport and ATP synthesis and have been reviewed elsewhere extensively (151).

While the above modifications induced by hypoxia are pertinent to this review in the sense of energy metabolism, their role in altering distribution (if any) remains largely unknown. Other research on hypoxia and HIF relating to post-translational modifications has been expertly discussed elsewhere (152, 153).

Chaperone proteins

Heat shock proteins (HSP) are a highly conserved family of proteins that respond to various stress stimuli and are involved in many intracellular processes such as molecular chaperoning of proteins (154, 155), interactions with the actin cytoskeleton (156), and apoptosis (157). HSP expression is regulated through heat shock factor (HSF), a transcription factor that binds to the promoter region of HSP genes (158). Hypoxia upregulates HSP (159–164) and HSF has been shown to have many conserved hypoxia response elements regions indicating that the hypoxic upregulation of HSP through HSF is HIF-1-dependent (159).

Within the HSP family, HSP70 and HSP90 are of particular interest due to their recent associations with glycolysis and oxidative phosphorylation. HSP90 overexpression enhances glucose consumption, lactate production (165) and drives a modest increase in ATP levels (166). Conversely, inhibition of HSP90 caused by binding with the molecular drug SU086, impairs glycolysis (167). Overexpression of HSP70 also increases glycolytic activity with upregulation of phosphofructokinase and lactate dehydrogenase activity being observed; however, this is met with a decrease in oxidative phosphorylation (168). Despite this converse relationship between both methods of ATP production, overall intracellular ATP concentrations were not significantly different between control cells and those overexpressing HSP70 (168). While the mechanisms by which the increases in glycolysis *via* HSPs described above have not yet been described, it presents an interesting hypothesis considering their involvement in molecular chaperoning.

Biomolecular condensates, mitochondrial networks, and selective ATP use

Liquid-liquid phase separated organelle systems are biomolecular condensates in which proteins and nucleic acids can

compartmentalize and reform temporally and spatially. This is an interesting mechanism due to the possibility of transporting mRNA strands to localized areas in need of ATP. With this, it could then be possible to synthesize proteins related to ATP production at the site of ATP demand, rather than transporting already synthesized proteins. Although this concept is intriguing, there is much to learn about the mechanisms behind LLPS organelle formation. DEAD-box (DDX) ATPases are global regulators of phase-separated condensates (169). While there has been a lack of data connecting DDX ATPases to LLPS organelle formation in stress conditions such as hypoxia, a recent study showed that DDX 1 binds to and protects stress response mRNAs (170). This indicates that DDX proteins play a role in mediating LLPS formation and are possibly involved in the formation of a glycolytic metabolon. Yet, there is no clearly defined role for DDX ATPases in response to hypoxic stress, and this remains an area of investigation.

Furthermore, it has been implicated that c-Myc-responsive long noncoding RNA has the capability to functionally contribute to the formation of a metabolon comprised of glycolytic enzymes which is accompanied with an increase in cell survival as well as ATP production (171). Therefore, RNA could act as the backbone of LLPS condensate formation and transportation of the translation machinery could prove cost-efficient for cells rather than transporting a higher quantity of individual enzymes.

Alongside temporal and spatial LLPS organelle formation, mitochondrial networks can differ between cell types and tissues and is reliant on the energetic demands of the cell. It is possible that this network plays some role in the distribution of intracellular ATP despite the effects of hypoxia. While the hypoxic effects on mitochondria are mostly inhibitory, it is still a site of ATP production. The mitochondrial network could potentially have a role in effectively distributing the

intracellular ATP that is produced by the mitochondria throughout this period of metabolic stress.

Interestingly, in differentiated podocytes inhibition of glycolysis reduced the formation of lamellipodia, decreased cell migration, and induced apoptosis of these cells whereas inhibition of the mitochondria had only minor effects on cell shape and migration (172). Alongside this, in prostate cancer, cells utilize ATP production from glycolysis for cytoskeletal remodeling and cell motility (173). The idea that cells can preferentially use methods of ATP production for specific cellular processes is intriguing considering the nonideal state of energy distribution where insufficient ATP is being produced by the cell. This begs the question: can processes that preferentially use mitochondrial ATP or glycolytic ATP resort to an alternative source of ATP or will the cell adapt to upregulate or increase the efficiency of the preferred method of ATP production? (Fig. 4).

However, methods in identification of these pathways have been insufficient. Emerging new strategies to monitor these ATP dynamics, which have been expertly discussed elsewhere recently (174–177), could allow for the future identification and analysis of these mechanisms.

Conclusion

In summary, the nonideal bioenergetic state induced by hypoxia presents the metazoan cell with a unique challenge in that ATP production becomes limited due to the decrease of available oxygen. While hypoxic adaptations are introduced, the reliance of the cell on the passive diffusion of ATP is likely to be insufficient to fuel important cellular processes. While this review highlights changes in energy production and potential mechanisms by which the mammalian cell may distribute ATP or ATP-producing organelles and complexes in

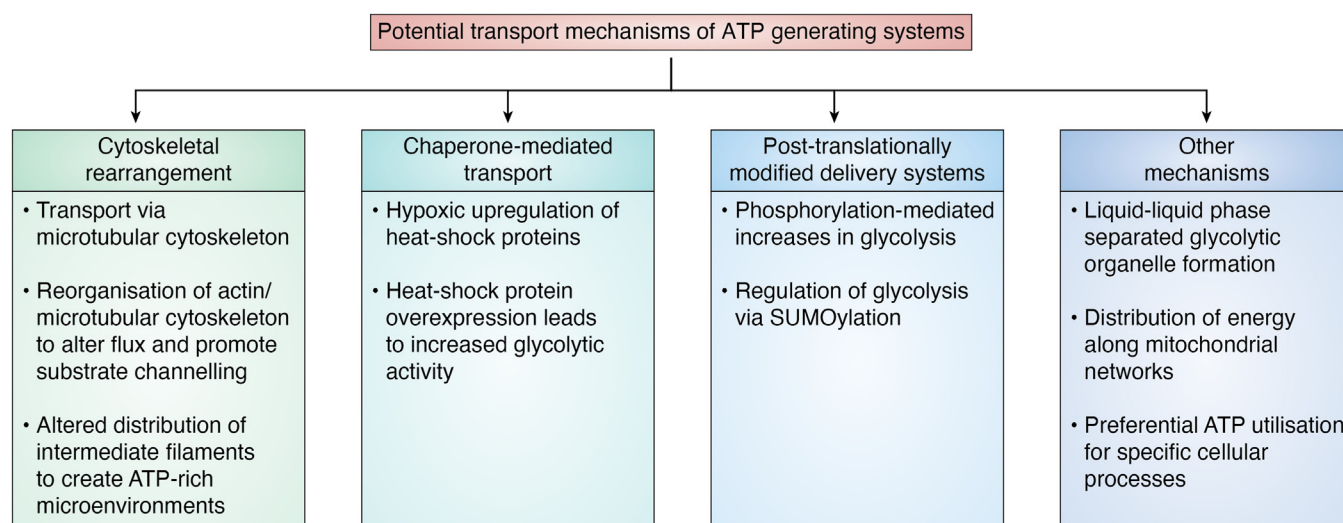


Figure 4. Schematic represents a visual representation of possible mechanisms by which ATP-generating systems are directed throughout the cell. This diagram highlights the key potential mechanisms by which a cell may distribute ATP in a targeted manner. These mechanisms include cytoskeletal rearrangement, chaperone-mediated transport, posttranslationally modified delivery systems, and others including liquid-liquid phase separated organelle formation, distribution along mitochondrial networks and preferential ATP utilization.

hypoxia, there is still much research to be carried out before a full understanding of the intracellular distribution of ATP is uncovered.

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Abbreviations—The abbreviations used are: AMPK, AMP-activated protein kinase; DDX, DEAD-box; ETC, electron transport chain; GLUTs, glucose transporters; HIF, hypoxia-inducible factor; HSE, heat shock factor; HSP, heat shock proteins; HUMMR, hypoxia-upregulated mitochondrial movement regulator; LLPS, liquid-liquid phase separated; PHD, prolyl hydroxylases; PIM, proviral integration site for Moloney murine leukemia virus; SUMO-1, small ubiquitin related modifier-1; TCA, tricarboxylic acid.

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