# Cellular adaptation to hypoxia through hypoxia inducible factors and beyond

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Abstract | Molecular oxygen ($O_2$) sustains intracellular bioenergetics and is consumed by numerous biochemical reactions, making it essential for most species on Earth. Accordingly, decreased oxygen concentration (hypoxia) is a major stressor that generally subverts life of aerobic species and is a prominent feature of pathological states encountered in bacterial infection, inflammation, wounds, cardiovascular defects and cancer. Therefore, key adaptive mechanisms to cope with hypoxia have evolved in mammals. Systemically, these adaptations include increased ventilation, cardiac output, blood vessel growth and circulating red blood cell numbers. On a cellular level, ATP-consuming reactions are suppressed, and metabolism is altered until oxygen homeostasis is restored. A critical question is how mammalian cells sense oxygen levels to coordinate diverse biological outputs during hypoxia. The best-studied mechanism of response to hypoxia involves hypoxia inducible factors (HIFs), which are stabilized by low oxygen availability and control the expression of a multitude of genes, including those involved in cell survival, angiogenesis, glycolysis and invasion/metastasis. Importantly, changes in oxygen can also be sensed via other stress pathways as well as changes in metabolite levels and the generation of reactive oxygen species by mitochondria. Collectively, this leads to cellular adaptations of protein synthesis, energy metabolism, mitochondrial respiration, lipid and carbon metabolism as well as nutrient acquisition. These mechanisms are integral inputs into fine-tuning the responses to hypoxic stress.

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Given the central importance of oxygen in maintaining intracellular ATP levels and serving as an electronic acceptor in a large number of biochemical reactions, it is unsurprising that responses to hypoxia are rapid, important and highly conserved. Examples of oxygen-consuming reactions include aerobic respiration, fatty acid desaturation and those catalysed by a growing number of α-ketoglutarate dioxygenases, which are involved in various metabolic reactions, including RNA, DNA and histone demethylation reactions. Due to vascular insufficiency or overt blood vessel damage and tissue oedema, hypoxia arises in various diseases, including the growth of solid tumours. Once an initially avascular tumour achieves a size extending beyond the natural diffusion limits of oxygen, hypoxic microdomains develop. For the disease to progress, tumours must acquire blood vessels, either through angiogenesis or vessel co-option. However, tumour blood vessels differ from their normal counterparts in various important phenotypes and perfuse tissue poorly. As such, adaptation to oxygen starvation is a key feature of both primary and metastatic neoplasms (BOX 1).

Cellular hypoxia (0.5–2% oxygen) can be transient, owing to temporary mismatches between oxygen supply and cellular metabolic demands, or more chronic because of permanent vascular inadequacy, unresolved tissue oedema and inflammation. Moreover, the degree of oxygen deprivation can result in distinct responses, as certain effects on protein folding or oxygen-consuming biochemical reactions are only observed under severe oxygen depletion (anoxic conditions, <0.5% oxygen). All of these adaptations must be integrated to support essential cellular activities until tissue and organismal responses return cells to appropriate oxygen levels.

A major breakthrough in our understanding of cellular responses to changes in oxygen levels was the discovery of hypoxia inducible factors (HIFs) and their regulation by the von Hippel–Lindau (VHL) tumour suppressor protein (pVHL) and prolyl hydroxylases (PHD1–PHD3 or EGLN1–EGLN3), members of the α-ketoglutarate dioxygenase superfamily. This finding provided a molecular framework for how changes in oxygen levels can mount robust transcriptional responses and provides therapeutic targets for cancer, cardiovascular disease and anaemia. Importantly, this also opened up the burgeoning field of studying α-ketoglutarate dioxygenases that include DNA, RNA and histone demethylases.

Box 1 | **Hypoxia and cancer**

Hypoxic regions (partial pressure of oxygen <10 mmHg) arise in tumours through the rapid proliferation of cancer cells in the absence of an efficient vasculature, resulting in the exhaustion of available nutrient and oxygen supplies. As a consequence, hypoxia induces multiple adaptive pathways and genomic changes that enable tumour cells to adapt to poor nutrition and hostile microenvironments for malignant progression[140–142]. The upregulation of hypoxia inducible angiogenic factors from hypoxic tumour sites, such as vascular endothelial growth factor (VEGF), triggers tumour mass vascularization to overcome proliferation limitations[200]. However, the vessels formed during neovascularization are often poorly organized and dysfunctional, either being blunt ended or having variability in flow velocity or direction. In addition, endothelial cells in normal vessels create a smooth surface permitting laminar flow; however, endothelial cells of tumour-associated vessels have gaps between them, resulting in vascular leakiness, non-laminar flow making blood prone to clotting and local tissue oedema[200–202]. Overall, most solid tumours retain hypoxic domains throughout disease progression, selecting for aggressive malignant cells that can withstand the ischaemic stresses of adverse tumour microenvironments.

The biology of hypoxic cancer cells is a product of the interplay between the prevailing oxygen tension, hypoxia-induced signalling (including that of hypoxia inducible factors (HIFs)), interacting genetic defects and cellular damage by reactive oxygen species (ROS), as discussed in this Review. As such, a solid tumour has dynamic fluctuations in oxygen from mild to severe hypoxia and necrosis, as well areas of acute hypoxia and reoxygenation. In patients with cancer, tumour hypoxia is a therapeutic problem often leading to poor prognosis owing to the potential of increased malignancy through clonal selection of hypoxia-resistant cancer cells, DNA damage, resistance to chemotherapy and radiation treatment, and an increased likelihood of metastasis[203–205]. For example, HIFs can enhance the expression of both collagen and extracellular matrix remodelling enzymes that promote aberrant collagen-containing extracellular matrix network formation, leading to tumour cell extravasation, survival in the circulation and colonization of distant organs[206]. This makes tumour hypoxia itself an attractive therapeutic target. Over the years, non-toxic hypoxia-activated 'prodrugs' have been developed to meet this need[207,208]. Conceptually, 'trigger' units in hypoxic prodrugs are selectively activated in oxygen-starved cells to release toxic 'effectors', capable of killing surrounding tumour cells. These triggers include nitroaromatics, quinones, N-oxides and transition metals. The N-oxide tirapazamine even entered phase III clinical trials; however, the future translation of tirapazamine and other hypoxic prodrugs remains in doubt[209]. Other strategies encompass targeting the downstream sequelae of tumour hypoxia, such as angiogenesis and the HIFs themselves.

This groundbreaking research was justifiably honoured with the 2019 Nobel Prize in Physiology and Medicine awarded jointly to William G. Kaelin Jr, Sir Peter J. Ratcliffe and Gregg L. Semenza 'for their discoveries of how cells sense and adapt to oxygen availability'. Beyond HIFs, responses to decreased oxygen levels involve changes in the epigenome, non-coding RNAs, the metabolome, signalling pathways, biochemical reactions and diverse homeostatic measures to ensure cell survival during hypoxic stress.

In this Review, we discuss current knowledge on how cells respond and adapt to hypoxia. We briefly consider transcriptional responses to hypoxia, mostly dependent on the HIF–PHD–pVHL axis. However, our primary focus is on adaptive mechanisms, involving the impact of hypoxia on protein homeostasis, the functionality of mitochondria (central oxygen-consuming organelles), metabolism and nutrient uptake in stressful microenvironments, which include important HIF-independent mechanisms. Of note, we largely focus on cellular responses; critical hypoxic adaptations at the organismal level, including changes in carotid body activity, the cardiopulmonary system, neurological behaviours, erythropoietin production and other physiological responses are reviewed elsewhere[1].

Dioxygenases
A group of enzymes that reduce molecular oxygen by incorporating both oxygen atoms into their substrates.

von Hippel–Lindau (VHL) tumour suppressor protein (pVHL). A protein named after the physicians von Hippel and Lindau, who characterized patients with highly vascular neoplasia of the kidney, eye and central nervous system who carried mutations in the *VHL* gene. pVHL is required for the ubiquitylation of hypoxia inducible factor-α and its degradation.

Carotid body
A cluster of peripheral chemoreceptor cells (glomus type I and glomus type II), which sense oxygen, carbon dioxide and pH levels of blood.

## Transcriptional regulation by hypoxia

Hypoxia transcriptionally induces a robust set of genes controlled by HIFs but also a range of other transcription factors, including nuclear factor-κB (NF-κB)[2]. Nevertheless, the vast majority of oxygen-sensitive genes are in fact direct HIF targets[3]. These genes at the cellular and organismal levels help adaptation to diminishing levels of oxygen. However, persistent activation of hypoxia-induced genes can result in pathologies, including pulmonary hypertension.

***HIF transcription factors and their regulation.*** HIF1 and HIF2 are major transcription factors involved in the hypoxic response[4]. HIFs bind to hypoxia response elements in the promoter regions of a large number of targets, including those involved in cell survival, angiogenesis, glycolysis and invasion/metastasis. HIFs are heterodimers consisting of the regulated HIFα protein subunit, which is only expressed during hypoxia, and a constitutively expressed HIF1β protein subunit. During normoxia, HIFα subunits are polyubiquitylated by the pVHL–elongin BC–CUL2 complex (referred to as the VHL complex) and targeted for proteasomal degradation[3] (FIG. 1). The normoxia-dependent interaction between HIFα subunits and the VHL complex requires hydroxylation of two proline residues within the oxygen-dependent degradation (ODD) domain of HIFα[3,5]. This hydroxylation reaction, which is catalysed by PHDs, is coupled to the oxidative decarboxylation of α-ketoglutarate to succinate and carbon dioxide[5] (FIG. 1). Importantly, all three PHD enzymes can hydroxylate both HIF1α and HIF2α and require oxygen, iron ($Fe^{2+}$) and α-ketoglutarate to function[6]. Mice harbouring individual loss of PHD2 are embryonic lethal compared with loss of PHD1 or PHD3, thereby highlighting distinct functions of PHDs in vivo[7]. PHD2 is the primary enzyme responsible for HIFα hydroxylation and subsequent degradation. Hypoxia prevents the hydroxylation of HIFα subunits and their ubiquitin-mediated proteasomal degradation. As a result, HIFα subunits dimerize with HIF1β to form transcriptionally active complexes (FIG. 1). The transcriptional activity of HIFs is fine-tuned by another member of the $Fe^{2+}$ and α-ketoglutarate-dependent dioxygenase family, HIF asparaginyl hydroxylase or factor inhibiting HIF1 (FIH1)[3,5]. FIH1 hydroxylates an asparaginyl residue within the C-terminal transactivating domain of HIF1α and HIF2α under normoxia to prevent recruitment of the transcription co-activators p300 and CBP, thereby curbing transcriptional output of HIFs[8] (FIG. 1). It is important to note that FIH1 has other substrates beyond HIFα protein subunits and their physiological functions continue to be investigated[9,10]. By contrast, a recent report by Ratcliffe and colleagues indicates that, at least in vitro, PHDs have exquisite specificity towards HIFs[11]. However, PHDs have been shown to control multiple biological processes independent of HIFs, perhaps by enzyme activity-independent mechanisms[12]. Moreover, other potential PHD targets might require structures, such as molecular scaffolds, to be efficiently hydroxylated in living cells. It will be important to generate mice harbouring mutations within the PHD hydroxylase C-terminus to render

Erythropoietin
A glycoprotein cytokine secreted by the kidney in response to hypoxia to stimulate erythropoiesis.

Elongin BC–CUL2
Additional complexes that interact with von Hippel–Lindau (VHL) tumour suppressor protein (pVHL). The Elongin BC complex acts as an adaptor connecting Cullin (Cul) proteins.

Michaelis constant
($K_m$). The substrate concentration at half of the maximum reaction velocity.

Acidosis
The process or condition where there is increased acidity in the blood and other body tissues.

them catalytically inactive to decipher their enzymatic and non-enzymatic roles in vivo.

***Regulation of PHDs.*** PHDs are the best-characterized oxygen-sensitive proteins, although various other oxygen sensors have been described (BOX 2). An intrinsic property of all three PHDs is low affinity for oxygen (high Michaelis constant ($K_m$)), making them primed to sense oxygen levels and control HIFα stabilization[13]. Tissue oxygen levels vary, with levels as low as 0.5% oxygen in the large intestine and up to 13% oxygen in the lungs. However, many major organs have tissue oxygen levels at ~3–7%[14]. The $K_m$ values of oxygen for all three PHDs are similar (230–250 μM) and close to the concentration of dissolved oxygen in blood in atmospheric air[13]. By contrast, FIH1 has a relatively higher affinity for oxygen ($K_m$ value ~90 μM)[15]. Thus, any decrease in oxygen levels below atmospheric air (~21% oxygen) will decrease PHD enzymatic activity[5].

Experimentally in cell culture, HIF1α protein levels demonstrate a small increase in stability between atmospheric air and 6% oxygen followed by an exponential rise as oxygen levels approach 0.5% oxygen[16]. There are likely multiple inputs into PHDs when oxygen levels are below 5% that explain the exponential rise in HIF1α protein levels, including other sensors of intracellular oxygen levels and the production of reactive oxygen species (ROS)[17]. Manipulating intracellular oxygen levels can control PHD activity and, thus, HIFα protein stabilization during hypoxia[18]. Furthermore, the mitochondrial intermembrane space protein coiled-coil helix domain containing protein 4 (CHCHD4; Mia40 in yeast) is necessary for hypoxic induction of HIF1α protein stabilization by regulating mitochondrial oxygen consumption[19]. ROS are generated during hypoxia by mitochondrial complex III (FIG. 1), and their production exponentially increases starting at 5% oxygen. Decreasing ROS levels genetically or pharmacologically during hypoxia has been shown to diminish HIFα protein levels[20–25]. How ROS inactivate PHDs to stabilize the HIFα protein subunit is not understood. One new hypothesis is that mitochondrial ROS generated during hypoxia promote the oxidation of cysteine residues within PHD2, resulting in oxidative PHD2 homodimerization and inactivation, leading to HIFα protein stabilization[26]. Indeed, oxidative modification of cysteine residues is one well-characterized mechanism by which ROS act as signalling molecules[27]. In response to ROS, redox-sensitive cysteine thiol groups (R–SH) can be oxidized to form disulfide bonds (R–S–S–R) that can mediate structural and functional changes within proteins and thereby regulate their activity. Importantly, PHD2 has several reactive cysteine residues in its C-terminal catalytic domain that may be oxidized by ROS[26,28]. Interestingly, PHD2 activity requires high intracellular levels of free cysteine, which is regulated by cysteine dioxygenase (CDO1)[28]. Free intracellular cysteines may compete with the reactive cysteine residues of PHD2 for ROS-mediated oxidation. Thus, when free intracellular cysteine levels are high, PHD2 cysteine oxidation is prevented; PHD2 is then active, and HIFα protein levels are low. By contrast, limiting the amount of free intracellular cysteine would trigger HIFα protein accumulation. Currently, the significance of these PHD2-reactive cysteines in the stabilization of HIFα under physiological hypoxic conditions remains unknown.

Metabolites are a second input that inhibits PHD2 activity (FIG. 1). Mutations in the tricarboxylic acid (TCA) cycle components succinate dehydrogenase or fumarate hydratase result in the accumulation of the metabolites succinate and fumarate, respectively, and are linked to rare neuroendocrine and renal tumours[29]. Succinate and fumarate accumulation inhibits PHD2 activity by competing with its substrate α-ketoglutarate, causing an accumulation of HIFα protein under normoxia[30,31] (FIG. 1). Independent of these mutations, succinate and fumarate can accumulate and trigger HIFα stabilization in response to innate immune signals in monocytes to increase the mRNA expression of cytokines, such as IL-1β[32]. Another competitive antagonist of α-ketoglutarate is L-2-hydroxyglutarate (L-2HG). Hypoxia (0.5% oxygen) concomitant with acidosis can

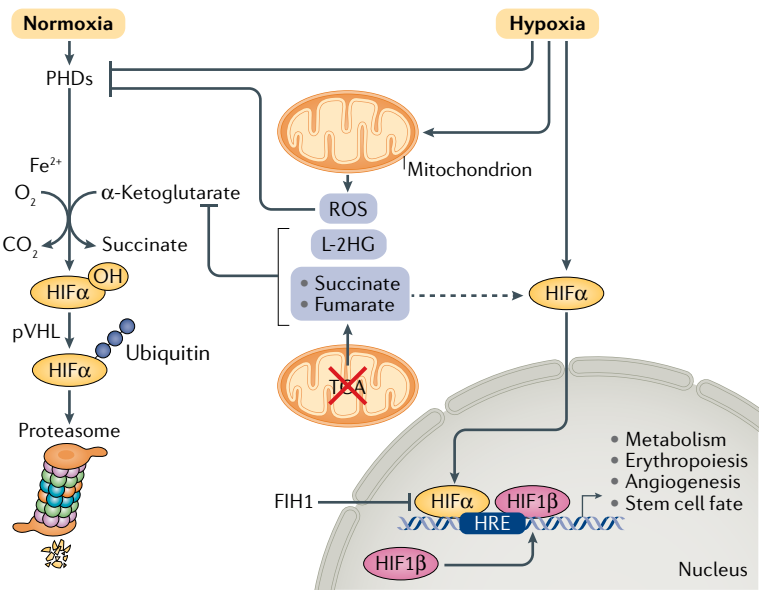


Fig. 1 | **Transcription regulation induced by hypoxia.** The oxygen-dependent interaction between the hypoxia inducible factor-α (HIFα) subunits and the von Hippel–Lindau (VHL) tumour suppressor protein (pVHL) complex requires hydroxylation of two HIFα proline residues by a family of α-ketoglutarate-dependent dioxygenases termed prolyl hydroxylases (PHDs), which requires oxygen ($O_2$), iron ($Fe^{2+}$) and α-ketoglutarate to function. Following hydroxylation, HIFα subunits are polyubiquitylated by pVHL and targeted for proteasomal degradation. Hypoxia prevents the hydroxylation of the HIFα protein subunits and their ubiquitin-mediated proteasomal degradation. As a result, the HIFα protein subunits are allowed to dimerize with the HIF1β protein subunits to form transcriptionally active complexes that bind to hypoxia response elements (HREs) to coordinate the induction of a large network of genes involved in metabolism, erythropoiesis, angiogenesis and cell fate. Factor inhibiting HIF1 (FIH1) hydroxylates HIFα subunits under normoxia to prevent recruitment of the transcription co-activators. Various mitochondrial products can also influence the hypoxic response. The production of reactive oxygen species (ROS) by mitochondrial complex III and L-2-hydroxyglutarate (L-2HG) under hypoxia can promote the stabilization of HIFα protein levels. ROS likely inhibit PHDs by Cys oxidation, whereas L-2HG competes with α-ketoglutarate. Mutations in tricarboxylic acid (TCA) cycle components result in the accumulation of succinate and fumarate, which also inhibit PHD activity by competing with α-ketoglutarate, thereby causing an accumulation of the HIFα protein even under normoxia. $CO_2$, carbon dioxide; OH, hydroxide.

Box 2 | **Cellular oxygen sensing beyond the PHD–pVHL–HIF pathway**

As described in this Review, the transcriptional response to hypoxia is canonically dependent on the axis comprising prolyl hydroxylases (PHDs), von Hippel–Lindau (vHL) tumour suppressor protein (pVHL) and hypoxia inducible factors (HIFs). Although the PHDs are the best-characterized oxygen-sensitive proteins controlled by the family of α-ketoglutarate-dependent dioxygenases (now including ≥70 members), other members of this superfamily could putatively be regulated by variable oxygen levels as well as hypoxia-induced changes in reactive oxygen species (ROS) and metabolites. Proteins in this superfamily include collagen prolyl 4-hydroxylase I, Jumonji-C domain-containing histone lysine demethylases (JmjC-KDMs), the ten–eleven translocation (TET) family of enzymes involved in DNA demethylation as well as the AlkB homologue 5 (ALKBH5) and fat mass and obesity-associated (FTO) RNA demethylase enzymes[6]. Interestingly, these proteins are all inhibited by the accumulation of succinate, fumarate and L-2-hydroxyglutarate (L-2HG) — metabolites produced by mitochondria upon perturbation of the tricarboxylic acid (TCA) cycle. Furthermore, most of these proteins have a high affinity for oxygen and, thus, would be active in the hypoxic range. However, there are notable exceptions that demonstrate high Michaelis constant ($K_m$) values for molecular oxygen (that is, low oxygen affinities) and are therefore likely to be sensitive to tumour hypoxia, such as the JmjC-KDMs[210]. Recombinant KDM4B, KDM5A and KDM6A/UTX exhibit low oxygen affinities like the PHD enzymes, whereas KDM4A, KDM5B, KDM5C, KDM5D and KDM6A have high oxygen affinities[211,212]. Thus, hypoxia-induced inhibition of KDM4B, KDM5A or KDM6A/UTX in cell culture results in an increase in various histone methylation marks, including trimethylation of K4 on histone H3 (H3K4me3), H3K9me3, H3K27me3 and H3K36me3 (REF.[211]), which is independent of the PHD–pVHL–HIF axis. It is important to note that hypoxia induces the transcription of many of the genes encoding JmjC-KDMs, likely to compensate for their decreased activity under low oxygen conditions by simply increasing the cellular abundance and output of JmjC-KDM enzymes[210].

Recently, the mammalian cysteine oxidase, cysteamine (2-aminoethanethiol) dioxygenase (ADO), was reported to be an oxygen-sensing enzyme that functions similarly to plant cysteine oxidases (PCOs)[213]. These enzymes add oxygen atoms to N-terminal cysteine thiol groups on target proteins to form sulfinic acid, marking them for ubiquitin-mediated degradation. In plants, this PCO-mediated pathway, the N-degron pathway, acts as an oxygen-sensing system for hypoxic adaptation[214–216]. Like PCOs, ADO is not dependent on α-ketoglutarate, but is an iron-dependent and oxygen-dependent enzyme. Interestingly, ADO can complement the loss of PCO in plants. Under normoxia, ADO promotes proteasomal degradation of RGS4 and RGS5, proteins involved in attenuating G protein signalling, and IL-32, a pro-inflammatory cytokine linked to gastric inflammation. ADO has a low affinity for oxygen (apparent $K_m$ of oxygen >500 μM) like the PHDs; thus, hypoxia suppresses ADO activity, resulting in RGS4, RGS5 and IL-32 protein stabilization[213]. It will be of interest to elucidate the oxygen-regulated target proteins of ADO to see whether, beyond oxygen, metabolites or ROS are able to suppress ADO activity to control physiology or disease. Collectively, the past 25 years of hypoxia research have revealed that multiple oxygen-sensing mechanisms have evolved to fine-tune diverse responses to hypoxia. Beyond the large transcriptional programmes regulated by HIFs in most eukaryotes, additional highly conserved oxygen sensors must coordinate numerous adaptations required for cell survival under hypoxic stress.

Cap-dependent protein synthesis
Eukaryotic mRNAs contain a modified guanosine (the cap) at their 5′ ends. Cap-dependent translation requires the binding of an initiation factor, eukaryotic translation initiation factor 4E (eIF4E), to the cap structure.

PERK
(Protein kinase RNA (PKR)-like endoplasmic reticulum (ER) kinase). A sensor of ER stress and stress-induced protein misfolding.

favour the production of L-2HG and promote the stabilization of the HIFα protein subunit[33,34] (FIG. 1). A unifying model to explain HIF activation is the intrinsic decrease in PHD2 activity owing to declining oxygen levels coupled with added inputs, such as ROS or metabolites, that further diminish PHD2 activity to maximally increase HIFα protein levels.

## Adaptation of protein accumulation

Although transcriptional components of hypoxic responses have been relatively well studied, changes in protein synthesis rates under low oxygen have been less well characterized. Downregulating translation suppresses energy-expensive processes, such as 'unnecessary' protein synthesis, and prevents the accumulation of stress-induced unfolded and/or misfolded proteins. Hypoxia-induced targeted repression of cap-dependent protein synthesis occurs predominantly at the level of translation initiation, which is normally accomplished by the recruitment of 40S ribosome subunits and initiation tRNAs at the mRNA AUG start codon[35,36]. Under low oxygen levels, this repression is mainly driven by two pathways: downstream of protein kinase RNA (PKR)-like endoplasmic reticulum (ER) kinase (PERK) and mechanistic target of rapamycin (mTOR) complex 1 (mTORC1). In addition, there are mechanisms to suppress translation elongation and termination during hypoxia. At the same time, translation of certain transcripts that encode proteins essential for survival in hypoxic environments is increased, thereby allowing adaptation of cells to this stressful condition[37].

***Inhibition of translation initiation.*** Under physiological conditions, eukaryotic initiation factor 2 (eIF2; α, β and γ components) promotes translation initiation, which is associated with eIF2α being underphosphorylated[36]. Oxygen depletion, which can lead to oxidative stress, nutrient deprivation and signalling disruption, can interfere with protein folding and, subsequently, result in an accumulation of misfolded as well as unfolded proteins. This has been shown to result in ER stress[38]. In order to alleviate ER stress, cells trigger the unfolded protein response (UPR) (Supplementary Box 1), which serves as a critical cell survival mechanism, involving various adaptations, importantly including reduction of the protein load via decreasing protein synthesis (see next section). In this context, one UPR sensor, PERK (Supplementary Box 1), functions as a kinase to phosphorylate eIF2α and inhibit translation initiation. This causes a general suppression of mRNA translation initiation and global protein synthesis to limit the detrimental effects of proteotoxicity[39] (FIG. 2). The protective role of PERK during hypoxic stress has been shown using mouse embryonic fibroblasts isolated from PERK-null mice, where PERK-deficient mouse embryonic fibroblasts are more sensitive to hypoxic stress than their wild-type counterparts[40,41]. In addition, initiation of cap-dependent translation requires the binding of the eIF4F complex — comprising eIF4A, eIF4G and eIF4E — to the mRNA cap. This is regulated by mTORC1, which phosphorylates eIF4E-binding proteins (4E-BPs). 4E-BPs prevent eIF4E from assimilating into the eIF4F complex, and this phosphorylation releases eIF4E, allowing eIF4F assembly[36]. mTORC1 activity is also negatively regulated by hypoxia (BOX 3). Accordingly, in hypoxia, eIF4E remains bound to 4E-BPs, resulting in the inhibition of eIF4F assembly and a decrease in global translation rates[42] (FIG. 2). Prolonged hypoxic exposure activates a second, eIF2α and mTORC1-independent pathway that maintains translation repression, where eIF4F complex breakdown results in eIF4E becoming sequestered in the nucleus by its transporter 4-ET[43]. This eIF2α regulation shows how varying states of oxygen deprivation regulate mRNA translation through distinct mechanisms, each with important contributions to hypoxic gene expression.

mTOR
(Mechanistic target of rapamycin). A protein kinase that regulates protein synthesis and cell growth in response to growth factors, nutrients, energy levels and stress.

Eukaryotic initiation factor 2
(eIF2). A complex comprising α, β and γ components that integrates a diverse array of stress-related signals to regulate both global and specific mRNA translation.

***Inhibition of translation elongation and termination.*** Alongside regulation at the initiation level, elongation of mRNA translation is also regulated during hypoxia. Peptide elongation is mediated by eukaryotic elongation factors (eEFs), and eEF2 has so far been shown to be regulated by variable oxygen levels[44,45]. eEF2 is negatively regulated by eEF2 kinase (eEF2K). eEF2K is normally subject to proteasomal degradation via mTORC1, but mTORC1 inhibition in hypoxia (BOX 3) results in eEF2K stabilization, increased eEF2K levels, increased eEF2 phosphorylation and associated eEF2 inhibition[44] (FIG. 2). In addition, PHD2 inhibits eEF2K to promote eEF2 activity[46,47]. Therefore, in low oxygen conditions, when PHD2 activity is impaired, eEF2 becomes phosphorylated and inactivated (FIG. 2). Finally, post-translational hydroxylation of eukaryotic release factor 1 (eRF1) by oxygen-sensitive JMJD4 (Jumonji domain-containing 4) (BOX 2) is required for efficient translation termination rates during normoxia[48], and during hypoxia, decreased eRF1 hydroxylation leads to inefficient termination (FIG. 2).

***Adaptive protein synthesis in hypoxia.*** Despite global shutdown of translation, it is important to note that not all mRNA translation is halted under hypoxia, which is crucial for cell survival when oxygen levels are low. Overcoming translational repression during oxygen starvation is needed for de novo synthesis of

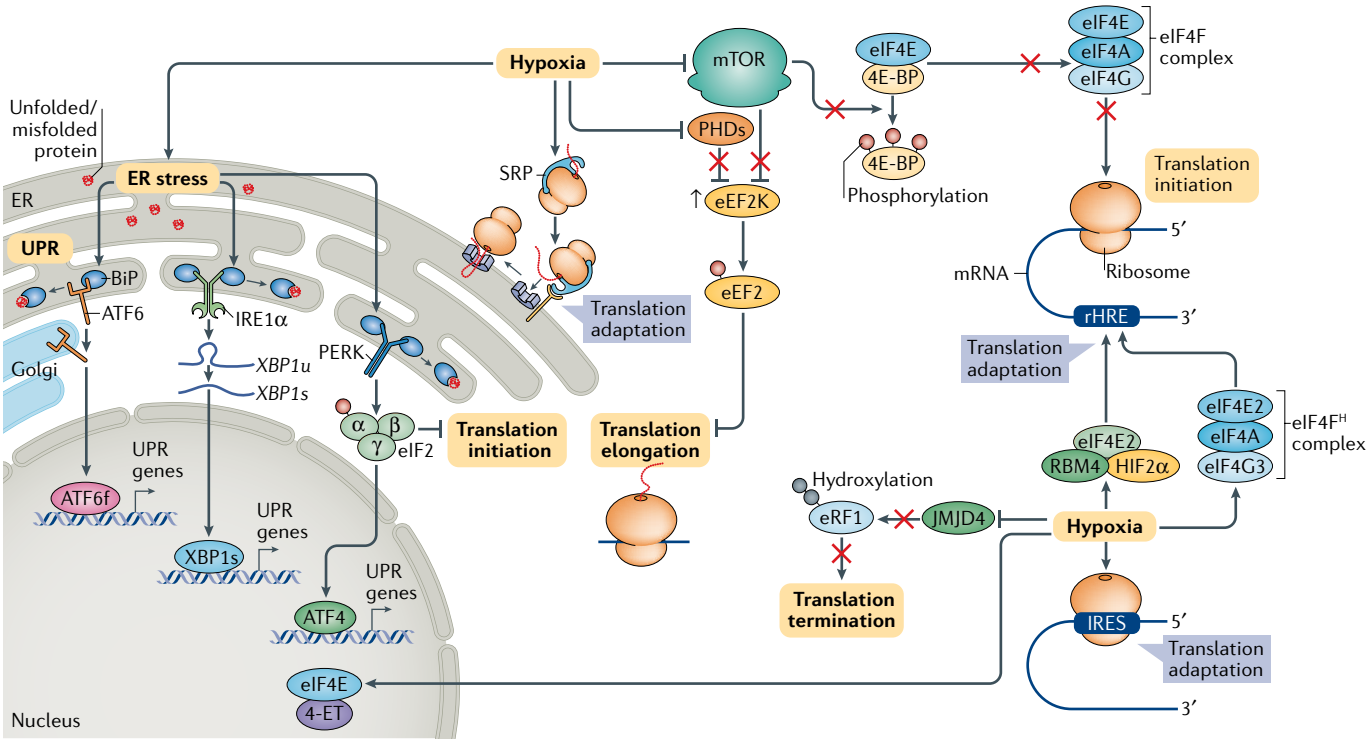


Fig. 2 | **Hypoxic adaptations in proteostasis.** Low oxygen can induce endoplasmic reticulum (ER) stress and the unfolded protein response (UPR; Supplementary Box 1). Misfolded peptides bind to binding immunoglobulin protein (BiP) and cause it to activate the stress sensors activating transcription factor 6 (ATF6), inositol-requiring protein 1α (IRE1α) and protein kinase RNA (PKR)-like ER kinase (PERK) to initiate responses to restore ER homeostasis. ATF6 is transported to the Golgi apparatus, where it is processed to release its active transcriptional form (ATF6f). IRE1α activation and dimerization trigger its RNase activity, which processes unspliced X box-binding protein 1 (*XBP1u*) to produce an active transcription factor, spliced XBP1 (XBP1s). Upon activation, PERK phosphorylates the initiation factor eukaryotic translation initiator factor 2α (eIF2α) to attenuate general peptide translation and promote the expression of transcription factor ATF4. Activation of the UPR alleviates the burden of misfolded and/or unfolded proteins, whereas translation inhibition reduces energy expenditure. Hypoxia also negatively regulates translation initiation by controlling the formation of the mRNA cap-binding eIF4F complex, comprising eIF4E, eIF4A and eIF4G. Formation of this complex is promoted by the release of eIF4E from its inhibitors, eIF4E-binding proteins (4E-BPs). This release is regulated by phosphorylation of 4E-BPs by mechanistic target of rapamycin (mTOR). Hypoxia inhibits mTOR activity, thereby interfering with eIF4E release. Hypoxia also promotes nuclear sequestration of eIF4E by its transporter, 4-ET. Elongation of mRNA translation is also regulated during hypoxia by modulating the activity of eukaryotic elongation factor 2 (eEF2) kinase (eEF2K), an inhibitor of eEF2. eEF2K is negatively regulated by prolyl hydroxylases (PHDs) and mTOR, and inhibition of both during hypoxia increases eEF2K activity, which, by phosphorylating eEF2, prevents mRNA elongation. Finally, translation termination is negatively affected by hypoxia owing to decreased hydroxylation of eukaryotic release factor 1 (eRF1) by Jumonji domain-containing 4 (JMJD4), an α-ketoglutarate and $Fe^{2+}$-dependent oxygenase that, like PHDs, is inhibited in hypoxia. Importantly, some mRNAs need to overcome the translation repression induced by hypoxia, prominently including those encoding mediators of hypoxia, such as hypoxia inducible factor (HIF)-responsive genes. HIF-responsive mRNAs contain reverse hypoxia response elements (rHREs). Low oxygen stimulates the formation of a complex including HIF2α, RNA binding motif protein 4 (RBM4) and eIF4E2 (eIF4E homologue) that assembles at these rHREs to promote translation initiation. Hypoxia also promotes formation of hypoxia-specific eIF4F complex ($eIF4F^H$) that binds rHREs. Selective hypoxia-responsive mRNA translation can also occur by direct binding of ribosomes to internal ribosome entry sites (IRES) encoded within the 5′ untranslated region (UTR) of certain mRNAs (such as *VEGF*, *eIF4G* and *MYC*). Adaptive protein synthesis during hypoxia is further regulated by the partitioning and recruitment of mRNAs to the ER by signal recognition particles (SRPs), which deliver mRNAs, such as those encoding *VEGF*, *HIF1* and *P4HA1*, to SRP-binding proteins on the ER membrane.

Box 3 | **Hypoxic regulation of mTOR**

mTOR (mechanistic target of rapamycin) controls biomass accumulation and metabolism by modulating key cellular processes. One of the two mTOR complexes, mTOR complex 1 (mTORC1), is inhibited by hypoxia, usually in conjunction with other cell stress stimuli, predominantly through its repressors, tuberous sclerosis protein 1 (TSC1) and TSC2. Acute hypoxic exposure rapidly and reversibly triggers hypophosphorylation of mTORC1 and its effectors, eukaryotic translation initiation factor 4E (eIF4E)-binding proteins (4E-BPs), p70 S6 kinase and eukaryotic translation initiation factor 4G (eIF4G), which is independent of AKT (a serine–threonine kinase involved in multiple processes, including metabolism, cell survival and growth) and AMP-activated protein kinase (AMPK) phosphorylation, ATP levels and hypoxia inducible factor 1α (HIF1α)[42,135]. In addition, chronic hypoxia exposure results in repression of mTORC1 signalling via two pathways, one being AMPK-dependent and the other through regulated in development and DNA damage response 1 (REDD1). Upregulation of REDD1 (REFS[217,218]) liberates the TSC2 complex from the chaperone protein 14–3–3, allowing it to associate with TSC1 and repress mTORC1 signalling. Certain mutations in TSC2 confer a growth advantage to cells by repressing hypoxic mTORC1 inhibition and hypoxia-induced cell cycle arrest[135]. Under conditions of aberrant mTORC1 activation, hypoxia causes dephosphorylation of 4E-BP1/4E-BP2 and increases their association with eIF4E to suppress translation as a means of hypoxia tolerance[219]. The 3′ untranslated region of REDD1 possesses microRNA (miRNA) binding sites that further contribute to its post-transcriptional regulation, with miR-7 identified as a repressor of REDD1 expression. Under hypoxia, miR-7 expression is downregulated, resulting in elevated REDD1 and consequent inhibition of mTORC1 signalling[220].

Hypoxia may also negatively regulate mTORC1 through proteins that interfere with the interaction between mTORC1 and RHEB — a G protein that activates mTORC1 (REFS[221,222]). In this case, mitochondrial protein Bcl-2/adenovirus E1B 19 kDa-interacting protein 3 (BNIP3), which is induced in hypoxia by HIF1 activation, directly binds to RHEB to inhibit the mTORC1 pathway[223]. The sequestering of RHEB away from mTORC1 is also observed when hypoxic cells acidify their microenvironment owing to the release of lactate, driving the redistribution of perinuclear lysosomes away from perinuclear RHEB. This suppresses lysosome-bound mTORC1 activity (lysosome is the activation site for mTORC1)[65]. In addition, hypoxia results in ataxia telangiectasia mutated (ATM)-dependent HIF1α phosphorylation, which was shown to be required for REDD1 upregulation and downregulation of mTORC1 signalling[224]. Although HIF1α stability and transcriptional activity are directly regulated by oxygen levels, its translation is heavily influenced by mTOR. Inactivating mutations in TSC1/TSC2 or PML — which negatively regulates mTORC1 association with RHEB — and activating mutations in mTOR increase HIF1α expression to influence the hypoxia-induced transcriptional landscape[221,225–227].

proteins essential for adaptive responses, such as ATF4 (activating transcription factor 4)[49–51]. ATF4 directly induces genes involved in protein synthesis, antioxidant response, amino acid transport (*AAAT* and *SLC3A2* (REF.[49])) and metabolism (*ASNS*[49]), as well as autophagy, as part of the integrated stress response[49,52]. Concurrently, translation of the HIF family of transcription factors and other proteins necessary for hypoxic responses is maintained to ensure cells have the correct repertoire of stress-responsive factors. Selective hypoxia-responsive mRNA translation can occur by the direct binding of ribosomes to internal ribosome entry sites encoded within the 5′ untranslated region (UTR) of mRNAs, which allows specific mRNAs to bypass the requirement for eIF4F formation at the 5′ UTR cap structure[53,54] (FIG. 2). Genes utilizing internal ribosome entry site-dependent translation in hypoxia include *VEGF*[55], *eIF4G*[56] and *MYC*[57]. Preferential translation of HIFα subunits during hypoxia was originally attributed to an internal ribosome entry site as well[57,58], but it was later shown that HIF UTRs bind regulatory non-coding RNAs and/or RNA-binding proteins to regulate translation rates by interacting with HIF subunit mRNAs, potentially by creating mRNA loops to facilitate ribosome recycling[59,60].

Oxygen levels have also been shown to impact the composition of the eIF4F complex, switching from eIF4A–eIF4E–eIF4G to eIF4A–eIF4E2–eIF4G3 (termed eIF4F$^{H}$, where eIF4E2 and eIF4G3 are homologous to eIF4E and eIF4G), which was shown to selectively recruit mRNAs regulated by HIFs to the ribosome for translation, irrespective of the overall cellular transcription competency[61] (FIG. 2). Interestingly, along with its function as a transcription factor, HIF2α is also a component of a cap-dependent translation initiation complex. Low oxygen tension stimulates the formation of a complex that includes HIF2α, the RNA-binding protein RNA binding motif protein 4 (RMB4) and cap-binding eIF4E2 (REFS[62,63]). This complex assembles at the reverse hypoxia response elements in RNA, allowing for evasion of hypoxia-induced protein synthesis repression (FIG. 2). Furthermore, other physiological stresses associated with hypoxia, such as acidosis due to lactate accumulation, have been shown to affect gene expression changes in a hypoxia-independent manner[64,65]. This includes the upregulation of genes involved in translation, such as *eIF4A2* and ribosomal protein L37 (*RPL37*). Of note, acidosis alone significantly suppresses peptide synthesis, much like hypoxia[64,65].

Adaptive protein synthesis during hypoxia is further regulated by the partitioning and recruitment of mRNAs to the ER, a critical site of peptide synthesis. Signal recognition particles bind to conserved sequences in the UTR and deliver mRNAs to signal recognition particle-binding proteins on the ER membrane[66] (FIG. 2). Examples of mRNAs that specifically localize to the ER in hypoxia for translation include *VEGF*, *HIF1* and *P4HA1*, which further contribute to hypoxia-specific proteomic adaptations[67].

## Activation of ER quality control

Exposure to hypoxia, and other cell stresses, can lead to extensive protein modifications, such as protein oxidation, carbonylation and nitrosylation, and, as discussed above, accumulation of unfolded proteins, particularly at the ER[68]. The control and degradation of misfolded proteins, potentially as a consequence of hypoxia, is crucial to prevent detrimental consequences to cell function. Therefore, in order to alleviate ER stress, the UPR is triggered to serve as a critical cell survival mechanism by reducing the protein load via decreasing protein synthesis and augmented protein degradation through ER-associated degradation and autophagy[68] mediated by the PERK, IRE1α and ATF6 signalling pathways[69] (FIG. 2; Supplementary Box 1).

As described in previous sections, during hypoxia, activated PERK phosphorylates eIF2α to attenuate translation initiation[39]. This transient halt in protein synthesis allows for energy conservation, decreased ER protein load as well as increased ribosomes available for mRNAs encoding UPR adaptive functions, such as ATF4 (REFS[49,50]). Another UPR branch is activated by IRE1α, which, following dimerization and autophosphorylation[70], generates a functional spliced XBP1 (*XBP1s*)[71] that, as a transcription factor, regulates

ER stress
A condition when the capacity of the endoplasmic reticulum (ER) to fold proteins becomes saturated due to impaired protein glycosylation or disulfide bond formation, or by overexpression of or mutations in proteins entering the secretory pathway.

eIF4F
The cap-binding eukaryotic translation initiation factor 4F (eIF4F) complex consists of three subunits, eIF4A, eIF4E and eIF4G. eIF4G strongly associates with eIF4E, the protein that directly binds the mRNA cap.

ATF4
(Activating transcription factor 4). A cAMP-response element binding protein that belongs to the cAMP response element-binding protein 2 (CREB2) family of transcription factors.

Integrated stress response
An adaptive pathway to restore cellular homeostasis by optimizing the cellular response to stress. Its activity is dependent on the cellular context and the type as well as intensity of the stress stimuli.

Signal recognition particles
Universally conserved ribonucleoproteins that recognize and target specific proteins to the endoplasmic reticulum.

ER-associated degradation
The cellular pathway that targets misfolded proteins of the endoplasmic reticulum (ER) for ubiquitylation and subsequent degradation by the proteasome.

Electron transport chain
(ETC). A series of complexes within the inner mitochondrial membrane that shuttle electrons from NADH and $FADH_2$ to molecular oxygen.

Enzymatic maximal velocity
($V_{max}$). The reaction rate when an enzyme is fully saturated by substrate.

Iron–sulphur (Fe–S) clusters
Molecular ensembles of iron and sulfide, often found as components of electron transfer proteins. The ferredoxin proteins are the most common iron–sulfide clusters in nature.

Pyruvate
The conjugate base, $CH_3COCOO^-$, of pyruvic acid is a key intermediate in several metabolic pathways throughout the cell.

the expression of numerous genes maintaining ER and metabolic homeostasis[72–78] (FIG. 2). The XBP1s transactivation domain interacts with RNA polymerase II, and XBP1 can also physically interact with many other transcription factors, such as HIF1α, where it regulates the expression of HIF1α targets via the recruitment of RNA polymerase II[79]. Hypoxia induces XBP1 mRNA expression and splicing in an HIF1α-independent manner, resulting in increased levels of activated XBP1 protein, and XBP1 loss severely inhibits tumour growth owing to a reduced capacity for tumour cells to survive in hypoxic microenvironments in vitro and in vivo[79,80].

In contrast to the other two branches, ATF6, following its activation in the ER, translocates to the Golgi, where it is cleaved by two Golgi-resident proteases[81,82]. Subsequently, the N-terminal domain (ATF6f), comprising a transcriptional activation domain, a basic leucine zipper (bZIP) domain, a DNA-binding domain and nuclear localization signals, translocates to the nucleus, where it induces UPR target gene expression to increase ER folding and load capacity, including genes encoding XBP1 and C/EBP homologous protein (CHOP), the latter playing an important role in promoting UPR-induced apoptosis[83–87] (FIG. 2, Supplementary Box 1). Chronic ER stress mediates a pro-death response — likely via positive feedback loops allowing stabilization of pro-apoptotic transcripts, which are generally unstable[88] — indicating that UPR activation plays a role in both adaptive and apoptotic responses under hypoxia[40,89,90]. Of note, cells with a compromised UPR, such as those with abrogated PERK and eIF2α signalling, are substantially more sensitive to ER-induced cell death compared with their wild-type counterparts, presumably owing to proteotoxicity[91]. Altogether, activation of the UPR plays a major role in the response and adaptation of cells to hypoxia.

## Changes to mitochondrial function

The mitochondrial electron transport chain (ETC) in most cell types is the largest consumer of intracellular oxygen for generation of ATP (that is, oxidative phosphorylation). Thus, it can be expected that changes in oxygen levels will affect mitochondrial ETC activity. Hypoxia controls ETC function at multiple levels, including the regulation of different mitochondrial ETC complexes and the availability of TCA cycle-reducing equivalents NADH and $FADH_2$.

***Modulation of ETC activity and the TCA cycle under hypoxia.*** It is important to note that intracellular oxygen levels at 0.3% begin to become rate limiting for ETC activity[92]. Complex IV (also known as cytochrome *c* oxidase or COX), the terminal complex within the ETC, donates four electrons to oxygen, producing two molecules of water. COX has a high affinity for oxygen and an apparent $K_m$ value close to 0.1% oxygen; thus, the ETC can function at near anoxic levels[93], and cells largely maintain their ATP levels during hypoxia. Although hypoxia does not acutely inhibit ETC function, prolonged hypoxia — lasting over hours — can decrease ETC function. Surprisingly, reduction in ETC efficiency is independent of the hypoxic limitation of oxygen as a substrate for COX activity, but is mediated through the activation of HIF1-dependent and HIF1-independent mechanisms[94]. Of note, hypoxia has been shown to diminish the enzymatic maximal velocity ($V_{max}$) of isolated COX, suggesting an intrinsic oxygen dependence of COX during prolonged hypoxia[95]. However, to ensure that the hypoxia-mediated suppression of the COX $V_{max}$ does not prevent cells from meeting their metabolic demands, hypoxia induces switching between COX subunits, which is mediated by HIF1 activation[96] (FIG. 3). COX is composed of 13 different subunits: ten regulatory nuclear-encoded subunits and three catalytic subunits encoded in the mitochondrial DNA. Hypoxia-mediated HIF1 activation induces the expression of the nuclear-encoded COX4 isoform 2 (COX4I2) subunit and the mitochondrial protease LON, which targets the alternative COX4 isoform 1 (COX4I1) for proteasomal degradation[96]. The incorporation of the COX4I2 subunit into COX allows for a more efficient transfer of electrons to oxygen during hypoxia.

Another HIF1-dependent protein that enhances COX activity through unknown mechanisms is the hypoxia inducible gene domain family member 1A (HIGD1A)[97]. By contrast, hypoxia diminishes the activity of the other ETC complexes (I, II and III). Hypoxia induces the mitochondrial NADH dehydrogenase (ubiquinone) 1α subcomplex, 4-like 2 (*NDUFA4L2*) gene in a HIF1-dependent manner to decrease complex I activity and respiration through unknown mechanisms[98]. Moreover, hypoxia induces several microRNAs (miRNAs), including *mir-210* through HIF1, which represses ISCU1 and ISCU2, two assembly factors for iron–sulphur (Fe–S) clusters, ultimately disrupting the correct assembly of iron–sulphur clusters within ETC complexes I, II and III[99]. *mir-210* also represses the expression of the complex I subunit NDUFA4, the complex II subunit succinate dehydrogenase subunit D (SDHD) and the COX assembly protein COX10 (REFS[100,101]) (FIG. 3).

Beyond ETC modulation, hypoxia also controls pyruvate entry into the TCA cycle through HIF1 induction of lactate dehydrogenase A (*LDHA*) and pyruvate dehydrogenase kinase 1 (*PDK1*)[102–104]. LDHA converts pyruvate to lactate, thus diminishing pyruvate entry into the mitochondrial matrix. Pyruvate dehydrogenase (PDH) converts pyruvate into acetyl-CoA to initiate TCA cycle flux. PDK1 phosphorylates and inactivates the catalytic subunit of PDH, thus preventing conversion of pyruvate to acetyl-CoA. This results in decreased TCA cycle flux, which limits the generation of mitochondrial NADH and $FADH_2$. The decrease in the generation of reducing equivalents diminishes electron flux through the ETC (FIG. 3). Another important consequence of reduced TCA flux during hypoxia is the diminished generation of aspartate from the TCA cycle metabolite oxaloacetate[105]. Aspartate is necessary for nucleotide synthesis and cell proliferation. Hypoxia decreases aspartate levels, which was shown to impair cell proliferation in vitro as well as tumour growth in mouse models. Aspartate levels negatively correlate with the markers of hypoxia in primary human tumours.

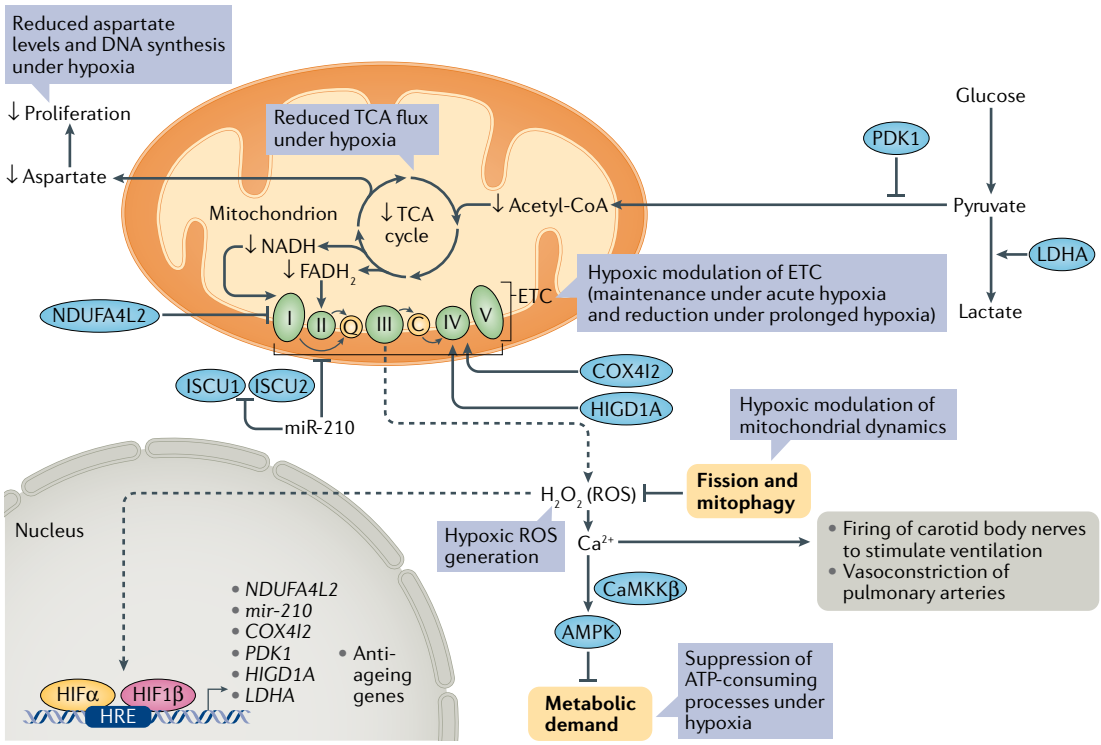


Fig. 3 | **Impact of hypoxia on mitochondrial function.** Hypoxia is characterized by decreased flux through the tricarboxylic acid (TCA) cycle in mitochondria, leading to the reduction of metabolites, such as acetyl-CoA and aspartate, required for anabolic processes. Specifically, hypoxia inducible factor 1 (HIF1) induces the expression of lactate dehydrogenase A (*LDHA*) and pyruvate dehydrogenase kinase 1 (*PDK1*), which then negatively regulate the entry of pyruvate to the TCA cycle (by promoting lactate generation and inhibiting its conversion to acetyl-CoA, respectively). Hypoxia also impacts on the activity of the electron transport chain (ETC). Under acute hypoxia, ETC activity is maintained by hypoxia-induced expression of the complex IV (COX) subunit COX4I2, which substitutes the COX4I1 subunit, allowing for a more efficient transfer of electrons to oxygen during hypoxia. Another HIF1-dependent protein that enhances COX activity through unknown mechanisms is the hypoxia inducible gene domain family member 1A (HIGD1A). This allows cells to maintain energy homeostasis in the event of short-term stresses. However, under prolonged hypoxia, ETC activity is diminished by inducing NADH dehydrogenase (ubiquinone) 1α subcomplex, 4-like 2 (*NDUFA4L2*) in an HIF1-dependent manner to decrease complex I activity and respiration, as well as several microRNAs (miRNAs), including *mir-210*, which represses ETC complex assembly. This repression of ETC activity has the potential benefit of decreasing the production of reactive oxygen species (ROS), which are generated by complex III under hypoxia (although mechanisms of hypoxic induction of ROS remain poorly understood) and can be potentially toxic by mediating damage to macromolecules. Nevertheless, hypoxia-induced ROS, and in particular hydrogen peroxide ($H_2O_2$), can also have various signalling roles. One of the consequences of mitochondrial ROS increase in hypoxia is the elevation in calcium ($Ca^{2+}$), which activates $Ca^{2+}$/calmodulin-dependent protein kinase (CaMKK). CAMKK then activates AMP-activated protein kinase (AMPK) to suppress ATP-consuming processes (metabolic demand). $Ca^{2+}$ is also a signal for firing of carotid body nerves to stimulate ventilation and for vasoconstriction of pulmonary arteries. Moderate amounts of mitochondrial ROS were also shown to have anti-ageing functions. In addition to metabolism, hypoxia modulates mitochondrial dynamics (fission), likely to enhance quality control of damaged mitochondria via mitophagy, which can aid in limiting ROS generation. I–V, respiratory complex I–V; C, cytochrome *c*; HRE, hypoxia response element; ISCU1/2, iron–sulphur cluster assembly scaffold protein 1/2; Q, ubiquinone.

Therefore, aspartate may be a limiting metabolite for tumour growth, and aspartate availability could be targeted for cancer therapy.

A major benefit of hypoxia diminishing respiratory rates is a resultant decrease in the production of mitochondrial ROS[103]. Importantly, ROS at low levels initiate cellular signalling events (see below, Impact on carbon and lipid metabolism), but at high levels ROS can initiate cell injury[27]. Thus, cells exposed to prolonged hypoxia activate HIF1-dependent transcriptional targets, including *HIGD1A*, *PDK1*, *COX4I2* and *NDUFA4L2*, to repress mitochondrial ROS production[96,98,103,106]. Accordingly, HIF1-deficient cells that are exposed to hypoxia increase mitochondrial ROS to levels that might induce cell death[103].

***Changes in mitochondrial morphology in response to hypoxia.*** Beyond effects on the TCA cycle and ETC function, hypoxia also controls mitochondrial morphology and quality control. Hypoxia promotes HIF-independent but CHCHD4-mediated perinuclear localization of mitochondria[107,108]. Mitochondria form tubular networks under normoxia, but undergo fission (fragmentation) under hypoxia[109]. During hypoxia, the GTPase dynamin-related protein 1 (DRP1) is recruited from the cytosol to the outer mitochondrial membrane

by mitochondrial protein fission 1 (FIS1). FIS1, a protein localized to the outer mitochondrial membrane, serves as a receptor for DRP1. Importantly, the scaffolding protein A kinase anchoring protein 121 (AKAP121) can direct cAMP-regulated PKA-mediated phosphorylation of DRP1, resulting in disruption of its association with FIS1 (REF.[110]). In order to relieve DRP1 inhibition by PKA, hypoxia induces the activity of SIAH2, an E3 ubiquitin ligase that promotes the degradation of AKAP121, allowing for DRP1 to interact with FIS1, resulting in mitochondrial fission[110]. It is possible that hypoxia promotes mitochondrial fission in order to induce mitophagy, the selective, autophagic elimination of mitochondria that are dysfunctional, to limit ROS production during hypoxia. Indeed, hypoxia can activate mitophagy in certain cell types through Bnip3-like/NIP3-like protein X (BNIP3L/NIX), Bcl-2/adenovirus E1B 19 kDa-interacting protein 3 (BNIP3) and FUN14 domain-containing protein 1 (FUNDC1)[111,112]. These proteins localize to the outer mitochondrial membrane and serve as receptors for the mitophagy machinery. Currently, the mechanisms underlying how hypoxia is sensed to trigger mitochondrial fission and mitophagy are not understood.

***Hypoxia-induced production of ROS by mitochondria.*** For decades, production of superoxide by the mitochondrial ETC and subsequent generation of hydrogen peroxide by superoxide dismutases were viewed as a major culprit in causing age-related degeneration of organisms[113]. One of the pillars to support a mitochondrial free radical theory of ageing was the observation that hypoxia extended the lifespan of mammalian cells in vitro by decreasing ROS[114]. However, antioxidants (that is, ROS scavengers) have consistently failed to provide any benefit to normal ageing or age-related diseases in humans or model organisms[115]. In addition, prolonged hypoxia as well as agents that cause an increase in mitochondrial ROS have now been shown to extend the mammalian cell replicative lifespan in vitro, as well as in *Caenorhabditis elegans* and mice[116–118]. Indeed, hypoxia increases mitochondrial ROS to induce HIF-dependent induction of human telomerase (*hTERT*) gene expression to extend the lifespan of mammalian cells[119]. Moreover, mitochondrial ROS can activate HIF1 to increase the lifespan of *C. elegans*[120]. These data have led to a model where generation of superoxide by mitochondrial ETC can result in hydrogen peroxide-dependent signalling to trigger adaptation to hypoxia. However, as discussed above, this response has to be finely tuned as high rates of mitochondrial ETC generation of superoxide can eventually result in an overproduction of ROS to incur damage, and cells exposed to prolonged hypoxia will decrease the activity of ETC to limit ROS.

An important physiologic consequence of hypoxia-mediated increases in mitochondrial ROS is the accumulation of $Ca^{2+}$ observed in cells possessing specialized oxygen-sensing properties, for example carotid body glomus type I cells and human pulmonary smooth muscle cells[121]. Recent studies demonstrate that these specialized cells express atypical mitochondrial ETC subunits, including NDUFA4L2, COX4I2 and COX8B, which contributes to their sensitivity to hypoxia and allows rapid responses to counteract the hypoxic state[122,123]. In this manner, hypoxia potently induces the firing of carotid body nerves to stimulate ventilation and, simultaneously, cause vasoconstriction of pulmonary arteries. Conditional loss of *NDUFS2* (complex I component) or *COX4I2* in carotid body glomus cells diminishes hypoxic increases in mitochondrial ROS production that are necessary to stimulate minute ventilation[122,124]. Interestingly, *Ndufs2*-deficient mice show an increased ventilation upon exposure to hypercapnia (5% carbon dioxide), demonstrating that a lack of ventilatory response upon impairment of mitochondrial ROS generation is specific to hypoxia[124]. Genetic deletion of the Rieske iron–sulphur protein (RISP), a subunit of mitochondrial complex III, in pulmonary smooth muscle cells similarly attenuated both hypoxia-induced increases in ROS production and intracellular $Ca^{2+}$ levels, which are necessary for hypoxia-induced pulmonary artery vasoconstriction[125]. Moreover, mice with either a smooth muscle-specific deletion of a complex I subunit (*NDUFS4* loss), a complex III subunit (RISP loss) or a complex IV subunit (*COX4I2* loss) lack a hypoxia-induced increase in pulmonary arterial pressure[122,126]. Thus, mitochondrial ETC-dependent ROS production is required for regulating the function of cells dedicated to oxygen sensing for organismal responses.

Interestingly, starting at 3% oxygen, when oxygen is not limiting for ETC function, cells have developed mechanisms to decrease their cellular metabolic demand[94]. This response does not occur within seconds but instead takes minutes to hours, involving a combination of transcriptional and post-translational mechanisms. By decreasing demand for ATP by suppressing ATP-consuming processes, cells lower their rate of oxygen consumption and delay the development of anoxia, which, unlike hypoxia, can cause cell death. Work from the 1950s proposed that cellular ATP utilization is a major determinant of the cellular respiratory rate, controlled by cellular ATPases[127]. Indeed, a plasma membrane-localized sodium–potassium ATPase (Na/K-ATPase) can account for 20–70% of the oxygen expenditure of mammalian cells[128]. The Na/K-ATPase, a heterodimer composed of a catalytic α-subunit and a β-subunit, transports sodium ($Na^+$) and potassium ($K^+$) ions across the plasma membrane to maintain an essential electrochemical gradient. Multiple investigators have reported that hypoxia reversibly rapidly suppresses the activity of the Na/K-ATPase by causing endocytosis of the α-subunit from the plasma membrane. This has been shown to depend on the activation of a key cellular energy sensor, AMP-activated protein kinase (AMPK)[129–132]. AMPK is a heterotrimeric serine/threonine protein kinase composed of a catalytic α-subunit and two regulatory β and γ-subunits. Mitochondrial complex III-generated superoxide was shown to potently activate AMPK[130–133]. AMPK is stimulated either by an increase in the AMP:ATP ratio or by an increase in $Ca^{2+}$ through the $Ca^{2+}$/calmodulin-dependent protein kinase (CaMKK)[134]. Multiple studies have demonstrated that acute hypoxia (low oxygen levels for a few minutes to

Superoxide dismutases
A family of enzymes that catalyse the dismutation of the superoxide ($O_2^-$) radical into molecular oxygen or hydrogen peroxide.

Telomerase
A ribonucleoprotein that adds a species-dependent telomere repeat sequence to the 3′ end of a region of repetitive sequences at each end of chromosomes (telomeres).

Essential electrochemical gradient
A gradient of electrochemical potential, usually for an ion that can move across membranes.

Ubisemiquinone
Ubiquinol is the reduced form of coenzyme $Q_{10}$ that is oxidized by mitochondrial complex III to the partially reduced form ubisemiquinone and subsequently to the fully oxidized ubiquinone. Ubisemiquionone is a highly unstable free radical that can donate electrons to molecular oxygen to generate superoxide.

Lipid droplets
Lipid-rich storage organelles that regulate the storage and hydrolysis of neutral lipids. They also serve as a reservoir for cholesterol and acyl-glycerols for membrane formation and maintenance.

several hours) does not alter the AMP:ATP ratio[130,135] but rather increases intracellular $Ca^{2+}$ levels, leading to CaMKK-dependent AMPK activation[131,132] (FIG. 3). AMPK is typically activated under nutrient-limiting conditions to concomitantly suppress ATP-consuming processes, such as protein translation, and promote autophagy to provide intracellular nutrients[134,135]. Thus, hypoxic activation of AMPK is key for reducing cellular metabolic demand under low oxygen conditions.

The mechanisms underlying hypoxic increases in mitochondrial superoxide production are not fully understood. Mitochondrial complex I, II and III have all been implicated in superoxide generation in multiple cell types; however, complex III is implicated in the majority of these studies[20,125,136]. In particular, the ubisemiquinone Qo site within mitochondrial complex III is the only known site in the entire ETC capable of releasing ROS from the mitochondria, producing superoxide into mitochondrial intermembrane spaces as opposed to the mitochondrial matrix[137]. These ROS then enter the cytosol through voltage-dependent anion channels located on outer mitochondrial membranes. A recent report proposed an interesting mechanism by which low oxygen may increase mitochondrial ROS. It was shown that hypoxia-stimulated $Na^+$ entry into the mitochondrial matrix led to reduced mitochondrial inner membrane fluidity via its interaction with lipids[138]. This resulted in the entrapment and accumulation of ubisemiquinone at the Qo site of mitochondrial complex III to increase superoxide production during hypoxia. Importantly, inhibition of the $Na^+/Ca^{2+}$ exchanger NCLX prevented hypoxia-induced import of $Na^+$ to the mitochondrial matrix, resulting in a decrease in mitochondrial ROS production and HIF1α protein stabilization. Going forwards, the advent of new genetic and pharmacologic tools that diminish the production of mitochondrial superoxide at complex I or III should further demonstrate the in vivo importance of mitochondrial ROS in physiology[136,139].

***Impact on carbon and lipid metabolism.*** Reprogramming intracellular metabolism is a common feature of oxygen deprivation[140] (FIG. 4). These hypoxic mechanisms are of particular relevance to cancer. Disorganized blood vessel formation as well as mismatched rates of cell proliferation and vascular sufficiency lead to stressful microenvironments in solid tumours, where cells are subjected to both oxygen and nutrient starvation. Hence, cancer cells take advantage of these metabolic adaptations to fuel survival and/or proliferation to ensure tumour progression in these unfavourable conditions. One of the most prominent adaptations in oxygen-starved cells is increased glucose uptake and elevated glycolytic flux to promote glucose catabolism. HIF1 target genes involved in this metabolic reprogramming include those encoding glucose transporter 1 (GLUT1) and GLUT3, hexokinase 1 and 2, enolase 1, phosphoglycerate kinase 1, pyruvate kinase M2 and *LDHA*[141,142]. In addition, as mentioned above, PDK1 inhibits the enzymatic activity of PDH, thereby blocking the conversion of pyruvate to acetyl-CoA for entry into the TCA cycle, favouring generation of lactate. Lactate and $H^+$ generated by glycolysis are exported from the cell through the activity of monocarboxylic transporter 4 (MCT4), sodium hydrogen ($Na^+/H^+$) exchanger (NHE) isoform 1 (NEH1) and carbonic anhydrase 9 (CAR9). Extracellular lactate can then be taken up by other cancer cells as well as stromal cells, where it is used as a carbon source for the TCA cycle (a mechanism known as anaplerosis)[143,144]. Strikingly, human non-small-cell lung cancers not only employ lactate as a fuel source but incorporate more lactate-derived carbons into TCA cycle intermediates than those from glucose, indicating that lactate can be a preferred carbon source in cancer cells[143]. Furthermore, acidification of the microenvironment by secreted lactate and $H^+$ is significant in that it adversely affects the functionality of infiltrating T cells, resulting in tumour immune evasion[140]. Similarly, hypoxia induces the uptake of glutamine, a principle anaplerotic substrate for the TCA cycle, by promoting increased expression of glutamine transporters, such as SLC1A5 and SNAT2/SLC38A2 (REFS[145,146]). In this way, cells are able to continually produce TCA metabolites, such as citrate, which is subsequently converted into cytosolic acetyl-CoA for anabolic reactions, such as lipid synthesis (lipogenesis). This is critical because of enhanced PDK1 activity and reduced entry of pyruvate-derived acetyl-CoA into the TCA cycle in oxygen-starved cells; glutamine anaplerosis can therefore maintain lipid homeostasis. Hypoxia, via HIFs, also induces the gene encoding the E3 ubiquitin-protein ligase SIAH1, which triggers ubiquitylation and degradation of the E1 subunit of α-ketoglutarate dehydrogenase, thereby promoting reductive carboxylation of glutamine-derived α-ketoglutarate to citrate, for acetyl-CoA and lipid synthesis[146]. Finally, HIFs induce the expression of fatty acid synthase (FASN) to stimulate fatty acid synthesis and stearoyl-CoA desaturase (SCD) to drive generation of unsaturated fatty acids[142]. However, as stated below, SCD activity becomes compromised under hypoxic conditions affecting the ratio of saturated to unsaturated fatty acids, plasma and organelle membrane integrity, and cell function. At the same time, HIF1 decreases fatty acid β-oxidation and diversion of fatty acids towards ATP production by repressing the expression of acyl-CoA dehydrogenases[147].

Lipid availability is important for highly proliferating cells as fatty acids and cholesterol support the production of organelle and plasma membranes and modulation of their fluidity. It is noteworthy that SCD is an oxygen-consuming enzyme[148]. Thus, SCD enzymatic activity becomes compromised in low oxygen conditions[148,149] and, to counteract this effect, cancer cells can elevate SCD expression as shown in certain tumours[150]. Otherwise, build-up of saturated fatty acid precursors is potentially toxic and can impair cellular function, which is associated with disruption of ER membranes and apoptosis[148,151]. Saturated fatty acid-induced toxicity can be alleviated by supplying exogenous unsaturated lipids, indicating that lipid uptake is an important mechanism for maintaining homeostasis in hypoxic cells[148]. In addition, recent studies indicate the importance of lipid droplets in alleviating lipotoxicity, including in cancer[152]. Specifically, the composition of triglycerides

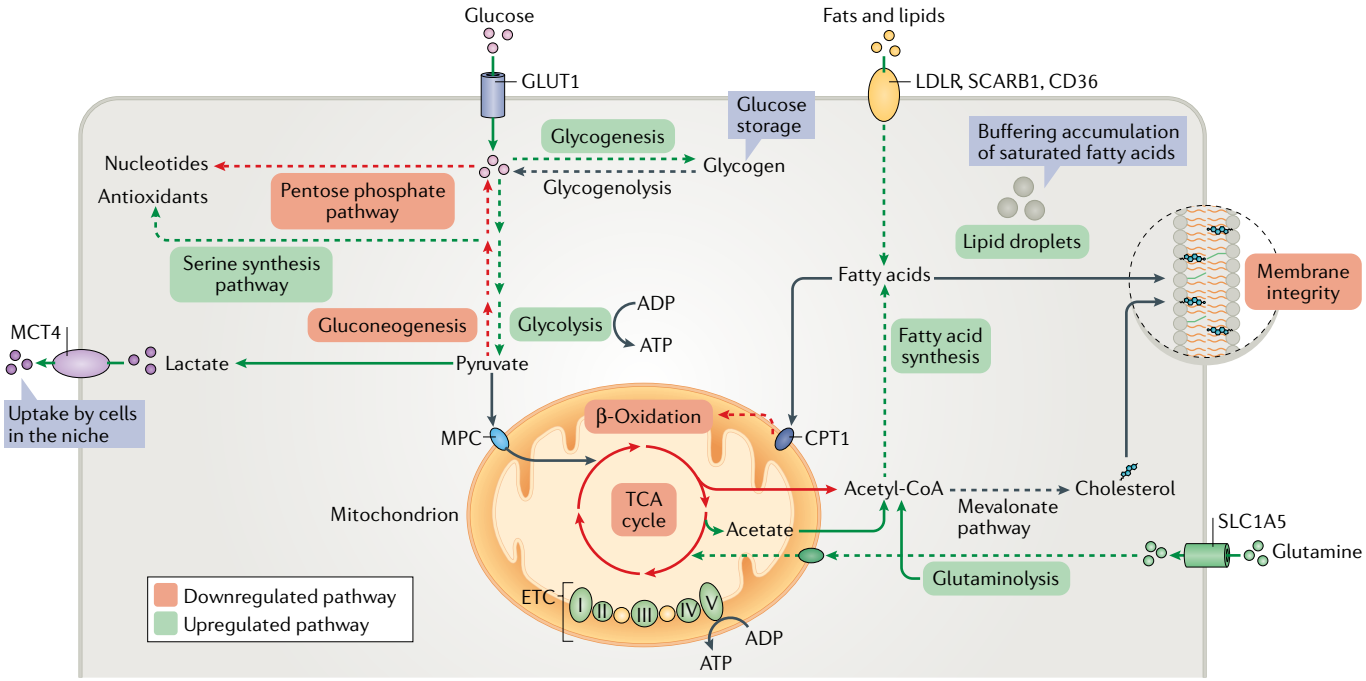


Fig. 4 | **Overview of sugar and lipid metabolic pathways affected by oxygen availability.** The figure highlights the upregulated (green) and downregulated (red) metabolic pathways under hypoxia. Hypoxic cells increase the uptake and utilization of glucose via glycolysis to obtain energy, while reducing their metabolism in mitochondria (FIG. 3). This leads to the generation of large amounts of lactate, which is secreted and can support metabolism of neighbouring cells. As a protective mechanism, glucose is also diverted towards the serine synthesis pathway to overcome the loss of cellular antioxidant capacity, while pentose phosphate pathway activity and nucleotide synthesis are decreased. Hypoxia also promotes glycogenesis, which could provide a mechanism of energy storage to survive prolonged stress. Under hypoxic inhibition of the tricarboxylic acid (TCA) cycle, generation of anabolic metabolites, such as acetyl-CoA, which is key for the synthesis of fatty acids, is largely supported by the increased uptake and metabolism of glutamine. Concomitantly, catabolism of fatty acids via β-oxidation is suppressed, whereas the uptake of lipids from the exterior increased. Lipid desaturation, allowing generation of unsaturated fatty acids, is inhibited in hypoxia (owing to the requirement for oxygen by stearoyl-CoA desaturase). To counteract potential lipotoxicity associated with the accumulation of saturated lipids and disruption of membrane structure and integrity, cells increase the uptake of unsaturated lipids from the environment and increase the formation of lipid droplets, which can act as buffers for saturated lipid species. I–V, respiratory complex I–V; CPT1, carnitine palmitoyltransferase 1; ETC, electron transport chain; GLUT1, glucose transporter 1; LDLR, low-density lipoprotein receptor; MCT4, monocarboxylate transporter 4; MPC, mitochondrial pyruvate carrier; SCARB1, scavenger receptor B1; SLC1A5, solute carrier family 1 (neutral amino acid transporter) member 5.

is altered to buffer changes in fatty acid saturation, by selectively retaining excess saturated fatty acids in lipid droplets[153]. That is, unsaturated oleate is preferentially liberated from existing triglycerides to facilitate sequestration of saturated fatty acids and used to counterbalance increased membrane phospholipid saturation[153]. Transcriptional profiling indicated that the lipid droplet protein perilipin 2 (PLIN2) is elevated in kidney tumours, correlating with increased HIF2α accumulation, and that HIF2α-dependent PLIN2 expression promotes triglyceride and cholesterol ester storage in lipid droplets, needed for ER membrane integrity and suppression of toxic ER stress responses[152]. The ER stress resulting from lipotoxicity also engages the IRE1α–XBP1 pathway through multiple molecular mechanisms as shown in various cancers[75,148,149,152], suggesting that inhibiting the IRE1α–XBP1 pathway is a useful general strategy for treatment of various tumours experiencing oxygen deprivation.

Hypoxic reprogramming of metabolism is also associated with the adaptation to excessive ROS production, which, as discussed above, accompanies mitochondrial changes in hypoxia. In this case, hypoxia decreases the expression of glucose-6-phosphate dehydrogenase, thereby decreasing pentose phosphate pathway activity. This inevitably reduces the generation of nucleotides and cell proliferation. However, at the same time, induction of phosphoglycerate dehydrogenase expression by hypoxia allows diversion of glucose towards serine synthesis for a robust antioxidant response, promoting stress resistance[154]. Glucose can also be diverted towards glycogen synthesis under hypoxia by overexpression of phosphoglucomutase 1 and glycogen synthase 1, which can serve as a preconditioning mechanism that allows the build-up of glucose stores preparing cells for conditions of glucose deprivation[141].

Pentose phosphate pathway
A predominantly anabolic pathway parallel to glycolysis that generates NADPH and precursors for nucleotide synthesis.

## Regulation of nutrient use

Due to various intracellular and extracellular stimuli that a cell encounters, including hypoxia and nutrient deprivation, it is critical to maintain cellular protective and adaptive mechanisms to resist stressful changes.

Adaptation and survival of cells in such a heterogenic microenvironment requires the coordination of several stress response pathways, including regulating nutrient use.

***Activation of autophagy.*** Macroautophagy (hereafter termed autophagy) is a key process whereby cytosolic components, such as proteins and organelles, are captured in double-membrane vesicles (autophagosomes) and fuse with lysosomes to form autolysosomes. These contents are subsequently catabolized by lysosomal degradative enzymes into products, such as amino acids, carbohydrates and fatty acids, that contribute to organelle/protein turnover and nutrient recycling[155–157]. Under physiological conditions, autophagy is maintained at a low basal rate as part of quality-control pathways to remove damaged proteins and organelles[158,159]. However, it potently responds to external cellular microenvironments and can be influenced by nutrient and oxygen availability to promote cell adaptation and survival[157,160].

*BNIP3* emerged as an HIF1α target. Accordingly, it is highly elevated in severe hypoxic conditions (~0.1–1% oxygen) in various cell lines and has pro-survival functions by mediating hypoxia-induced autophagy[161–164]. Closely-related *BNIP3L* is also induced by hypoxia[162,165], indicating that both proteins are necessary for autophagy under these stressful circumstances[166]. HIF1α-dependent expression of BNIP3 has also been described as essential in mitophagy, as previously mentioned[161,167,168].

Severe hypoxic conditions, often accompanied by drastic nutrient depletion, lead to autophagy through HIF-independent mechanisms, such as the UPR (Supplementary Box 1). Upon ER stress, PERK signalling stimulates the translation of ATF4, resulting in the induction of the downstream gene encoding for the transcription factor CHOP, which is responsible for initiation of the apoptotic cascade under prolonged ER stress[50,169]. Furthermore, AMPK, which is activated during metabolic stress and hypoxia as discussed above, is also a regulator of autophagy[170,171]. The best-studied mechanism by which AMPK regulates autophagy is through the suppression of the mTOR signalling pathway[170]. AMPK also targets the unc-51-like kinase 1 (ULK1) complex, an initiator kinase during mammalian autophagy. ULK1 is directly phosphorylated by AMPK to maintain autophagic function and mitochondrial homeostasis[172–174]. Altogether, hypoxia activates autophagy via several mechanisms. This allows the removal of damaged organelles (such as mitochondria through mitophagy, see above, Changes in mitochondrial morphology in response to hypoxia) and the release of nutrients for ongoing cell viability[175], which promote cell survival in stressful environments (FIG. 5).

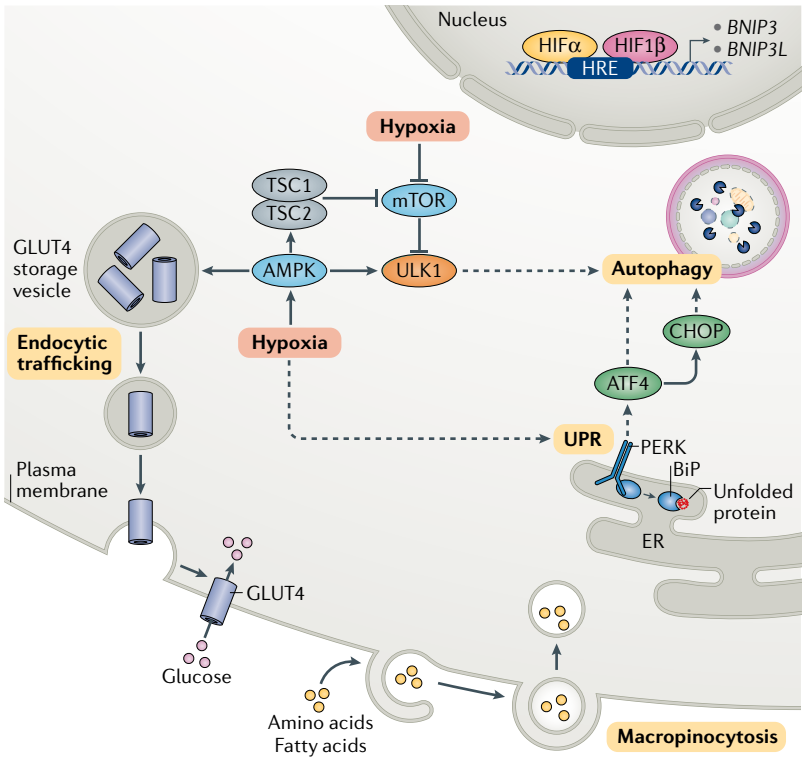


Fig. 5 | **Regulation of nutrient acquisition and use under hypoxia.** Hypoxia induces nutrient recycling via autophagy. In this case, the unfolded protein response (UPR) resulting from hypoxia activates protein kinase RNA (PKR)-like endoplasmic reticulum (ER) kinase (PERK), which induces activating transcription factor 4 (ATF4) and, subsequently, C/EBP homologous protein (CHOP) to activate autophagic machinery. Hypoxia, via activation of hypoxia inducible factors (HIFs), further regulates autophagy by HIF-mediated regulation of expression of two mitochondrial proteins, Bcl-2/adenovirus E1B 19 kDa-interacting protein 3 (BNIP3) and Bnip3-like/NIP3-like protein X (BNIP3L/NIX), that have been linked to autophagy of mitochondria (mitophagy). Autophagy can also be induced during hypoxia via activation of AMP kinase (AMPK), which subsequently activates autophagy directly or indirectly through inhibition of mechanistic target of rapamycin (mTOR), a negative regulator of the Unc-51-like autophagy activating kinase 1 (ULK1). AMPK can also promote the movement of glucose transporter 4 (GLUT4) from its storage vesicles to the cell membrane to promote glucose uptake. Nutrient acquisition is also achieved via macropinocytosis, which is promoted by hypoxia and supports the ingestion of extracellular macromolecules, such as amino acids and fatty acids. BiP, binding immunoglobulin protein; HRE, hypoxia response element; TSC1/2, tuberous sclerosis 1/2.

***Regulation of nutrient uptake.*** The cellular uptake rate of many nutrients and ions is governed primarily by membrane transporters and receptors that show dynamic localization at both the plasma membrane and defined intracellular membrane compartments. Regulation of the rate of endocytosis and the inverse process, recycling of the endocytosed components back to the plasma membrane, controls the amounts of these cell surface proteins, which in many cases determines nutrient uptake or excretion[176]. Here, we discuss the contribution of endocytic trafficking of GLUT4, which has been well characterized in hypoxia (FIG. 5).

GLUT4 is highly expressed in adipose tissue and skeletal muscle, and is responsible for the majority of glucose uptake to maintain blood glucose levels, thus making it a major regulator of systemic glucose homeostasis. GLUT4 membrane localization is normally tightly regulated by an endosomal pathway, whereby GLUT4 is endocytosed and sequestered to specific intracellular compartments[177]. Insulin and exercise stimulate GLUT4 redistribution from intracellular compartments to the cell surface to enhance glucose uptake via the AKT pathway[178,179]. In addition, low-energy signalling, such as decreased ATP levels, leads to increased cell surface GLUT4 via activation of AMPK that promotes GLUT4

Leigh syndrome
A neurological disorder characterized by progressive loss of mental and motor abilities.

recycling to the plasma membrane[180,181] (FIG. 5). Changes in GLUT4 distribution can also be influenced by HIFs and hypoxia. Loss of HIF1α in myocytes impairs GLUT4 vesicle mobilization to the plasma membrane owing to decreased AMPK activation upon insulin stimulation[182]. Furthermore, HIF1α was shown to increase the expression of RAB20, which promotes GLUT4 translocation to the plasma membrane, and to negatively regulate TXNIP expression to promote AKT downstream signalling[183]. In numerous murine studies, long-term and short-term chronic intermittent hypoxia triggers AKT and AMPK pathway activation as well as changes in GLUT4 expression in skeletal muscle[184–186]. Hypoxia can support adaptation of cells to replenish ATP required for cell energetics — such as muscle contraction — by providing ample glucose. By contrast, in adipose tissue, hypoxic response was shown to be linked to obesity and glucose intolerance, as mice with adipose-specific HIF1β knockout were protected from age-induced and diet-induced glucose intolerance, in part owing to decreased GLUT4 expression[187].

In addition to the recycling of membrane proteins, cells also utilize a form of endocytosis known as pinocytosis (hereafter termed macropinocytosis) to ingest extracellular liquid and nutrients. Although macropinocytosis can occur at basal rates, it can also be induced by the GTPase RAS, which has well-studied roles in cell growth, differentiation and survival, and is a potent oncogene[188,189]. Notably, in cancer cells harbouring oncogenic RAS mutations, macropinocytosis serves as a mechanism to take up amino acids for cell growth[190–192]. In addition, hypoxia also promotes the macropinocytosis of unsaturated fatty acids to bypass the inhibition of oxygen-dependent fatty acid desaturases and prevent lipotoxicity as discussed above[148,153] by scavenging serum (FIG. 5). Interestingly, oncogenic RAS mutations can mimic the effects of hypoxia on fatty acid scavenging. Overall, given that tumours must sustain growth under nutrient-deprived and oxygen-deprived conditions, macropinocytosis provides a mechanism for cells to maximize macromolecule uptake from the extracellular space. How RAS mutants and hypoxia regulate macropinocytosis-driven lipid scavenging is currently unknown.

## Conclusions and perspective

In summary, acute and chronic hypoxia induce a myriad of responses on a cellular level, and our understanding of how they are coordinated will continue to be investigated. The discovery of HIFs and their regulation by the PHD–pVHL axis has solidified our understanding of how cells autonomously respond to hypoxia at the transcriptional level. However, although the bases of hypoxic gene expression have been described in some detail, new areas of investigation include further dissection of how oxygen availability influences oxygen sensing and how ROS and metabolite levels affect multiple distinct but integrated adaptations. We highlight here a few specific areas that, in our view, are now ripe for investigation. First, which of the >70 α-ketoglutarate-dependent dioxygenases are truly affected by fluctuating oxygen levels encountered in normal and disease states, and in which context these sensors are important, should be addressed. In light of links between metabolism and chromatin regulation[193], it is warranted to further address how oxygen-regulated metabolic adaptations affect the epigenome and whether these changes are functional. Another important question relates to the close integration of oxygen and nutrient deprivation, and it would be interesting to study how these two stress signals impact on each other and cooperate. As cells in vivo do not reside in isolation, it will also be crucial to understand interactions between multiple cell types in hypoxic microenvironments, which could shed new light on the mechanisms of inflammation, cancer and other pathologies. Understanding the transcriptional regulation by HIFs has provided therapeutic targets currently under clinical evaluation[194,195]. Notably, HIF2α inhibitors are being tested in renal cell carcinoma and the pharmacologic inhibition of PHDs is being explored for the treatment of anaemia[196,197]. However, as conveyed in this article, cellular responses to hypoxia go much beyond the transcriptional networks governed by HIFs. Over the next decade, as more of these mechanisms are elucidated, it is very likely that more clinical opportunities for various diseases will arise. For example, hypoxic exposure increases the survival of mice resembling paediatric diseases linked to mitochondrial dysfunction, such as Leigh syndrome, through poorly understood HIF-independent mechanisms[198,199]. Here, HIF activation is insufficient to rescue disease, and alternative strategies that actually reduce oxygen delivery or modulate more specific pathways downstream of hypoxia are needed. Finally, it would be interesting to study mechanisms of response to increased oxygen levels. For example, age-dependent reduction in whole-body oxygen consumption can result in tissue hyperoxia[198]. This raises the question of mechanisms mediating adaptations to this state, particularly in the brain. Addressing these questions will greatly contribute to our understanding of cellular responses to oxygen — an integral molecule of aerobic life.

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