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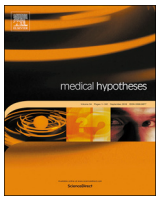

Hypoxia inducible factors as mediators of reactive oxygen/nitrogen species homeostasis in physiological normoxia

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A B S T R A C T

Although once considered by biologists almost exclusively for their toxicity, reactive oxygen (ROS) and nitrogen (RNS) species produced within normal cells under baseline physiological conditions are now appreciated as redox regulators of a wide range of protein functions. Two families of enzymes, the NADPH oxidases (NOXs) and nitric oxide synthases (NOSs), are major sources of ROS/RNS from molecular oxygen. Aquaporins (AQPs) are membrane channels capable of transporting some ROS/RNS, in particular hydrogen peroxide and perhaps nitric oxide. The activities of all these enzymes and channels are sensitive to variations in oxygen levels within the physiological range experienced by cells in the human body. Since ROS/RNS have important physiological roles and their endogenous production is affected by oxygen levels, we hypothesize that the synthesis of these proteins is increased at lower oxygen levels within the physiological range of most human cells *in vivo*, i.e. 2–5%, in order to facilitate the maintenance of ROS/RNS production rates. We further postulate that this is achieved, at least in part, by transcriptional stimulation mediated by the activity of hypoxia inducible factors (HIFs), which are strongly regulated by oxygen levels over the same range of oxygen. Here we survey the evidence supporting this hypothesis, including induction of expression of NOXs, NOSs, and AQPs at lower oxygen levels, presence of hypoxia response elements in the corresponding human genes, and evidence from chromatin immunoprecipitation (ChIP) experiments that HIF-1 and/or HIF-2 bind these regions. We find a significant amount of empirical data supporting the hypothesis that HIFs could function as physiological regulators of ROS/RNS homeostasis in the normoxic range in human cells.

Introduction

Reactive oxygen (ROS) and nitrogen (RNS) species were once viewed as toxic molecules causing macromolecular damage, a view that has inspired extensive research into the chemistry and biology underlying the oxidation of lipids, proteins, and nucleic acids. However, over time this 'toxic' view of ROS/RNS has shifted significantly. It is now appreciated that, at physiologically normal levels, ROS/RNS such as superoxide, hydrogen peroxide, and nitric oxide are important signaling intermediates. These molecules drive redox modifications within specific target proteins that alter structure and function to regulate important biological activities, such as vascular relaxation, neurotransmission, cell cycle progression, and differentiation [1–8]. Redox modifications of cysteines, for example, are widespread [4,7]. Redox modifications of specific cysteines in protein kinase A and protein kinase G alter the functions of these enzymes, thus grafting redox regulation onto well characterized and extensive reversible protein phosphorylation systems [4]. Thus ROS/RNS are fully integrated into cellular physiology and function in the regulation of many important cellular activities (reviewed in [1,7]).

Endogenous cellular ROS and RNS originate from oxygen-consuming reactions in organelles and cytosolic or membrane-bound enzymes. The NADPH oxidases (NOXs), which produce superoxide and hydrogen peroxide, and nitric oxide synthases (NOSs), which produce nitric oxide, are highly sensitive to oxygen levels within the physiological range normally encountered by mammalian cells (see below). Therefore, relatively small changes in ambient oxygen will affect rates of endogenous superoxide, hydrogen peroxide, and nitric oxide synthesis by these enzymes. This has the potential to affect downstream redox regulatory events. It may therefore be important for mammalian cells to defend ROS/RNS production at lower physiological oxygen levels, but how is this accomplished?

Hypoythesis

We hypothesize that superoxide, hydrogen peroxide, and nitric oxide production at lower physiological oxygen levels are maintained by inducing the synthesis of NOXs and NOSs, as well as the aquaporins (AQPs) that mediate the transport of some of these molecules across cellular membranes. We further hypothesize that hypoxia inducible factors (HIFs) play a role in mediating this response.

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Evidence

HIFs are functional within a physiologically normoxic range

HIF-1 was originally discovered and characterized as an inducible transcription factor acting on genes containing hypoxia response elements (HREs) to promote cell survival in the face of low oxygen levels [9]. Chief amongst the target genes are those related to oxygen and metabolic homeostasis. HIF-1, and its orthologue HIF-2, stimulate the expression of vascular endothelial growth factors for promoting vascularization of the hypoxic area and erythropoietin to increase the oxygen carrying capacity of blood. HIF-1/2 also stimulate the transcription of multiple glycolytic enzymes as well as glucose transporters (GLUTs), creating more machinery to support anaerobic glycolysis in the face of low oxygen levels. Via these activities, HIFs play an important role in defending cellular ATP homeostasis.

HIF-1/2 are heterodimers, each with an oxygen-dependent α-subunit (HIF-1α and HIF-2α and a constitutively expressed β subunit. The molecular pathways regulating HIF-1/2α have been covered elsewhere [10,11]. HIF-1/2α protein levels are strongly and negatively correlated with oxygen levels within the physiological range experienced by most tissue cells under normal conditions (i.e. no imposed systemic hypoxia). While the vast majority of experiments involving HIF-1 biology have been performed with cells cultured in atmosphere-saturated media (18–19% O_2) representing 'normoxia', this condition is actually substantially hyperoxic for virtually all human cell types. An extensive survey of oxygen levels measured in human tissues has been provided by Keeley and Mann [12]. Oxygen levels in heart, brain, kidney, digestive system, bone, and other tissues of intact humans are typically in the 2–5% O_2 range under basal conditions in people breathing normal atmosphere. Thus, physiological oxygen levels are far lower than those that have been used to model normoxia in the study of HIF biology, and 'hypoxia' is sometimes used to describe oxygen levels that are actually physiologically normal ('physioxia').

Although HIFs are activated during hypoxia, their stabilization is actually extremely oxygen-sensitive within the physiological range. Bracken et al. [13] studied HIF-1α and HIF-2α accumulation over a range of oxygen levels from 18.5% to less than 1% using six common cell lines. All six cell lines responded similarly in these experiments, so we pooled the data and present the mean values in Fig. 1. Although HIF1α was virtually undetectable at oxygen levels above 5%, in the physiological range from 5% to 2% O_2, HIF-1α protein levels increased by ten times. HIF-2α followed essentially the same pattern, and the transcriptional activities of both HIFs were similarly increased at 2% O_2 (not shown). A similar observation has been reported in rat trophoblast cells, where HIF-1α levels increased four-fold between 5% O_2 and 3% O_2 [14]. In U2OS cells, HIF-1α transcriptional activity was three-fold higher at 1% O_2 compared to 6% O_2 [15]. Thus, although HIF-1/2 are 'hypoxia-inducible' factors, their levels and activities are also highly (negatively) correlated with oxygen levels over the physiologically normoxic range. If we define hypoxia as a state in which oxygen levels are lower than cells normally experience *in vivo,* then it is clear that HIFs are also highly responsive to oxygen levels within a physiologically normoxic range that does not represent hypoxia.

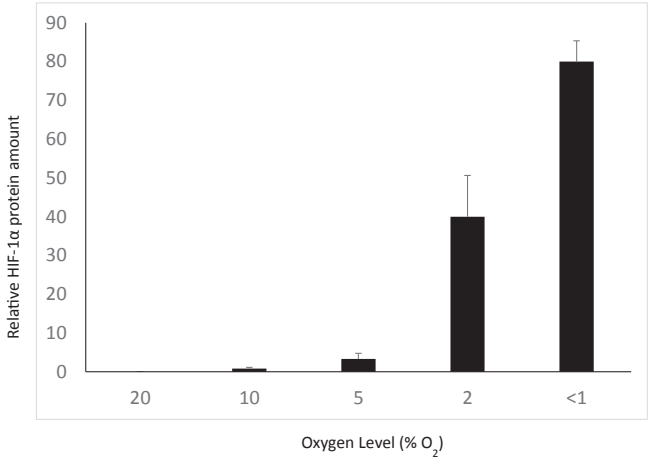

Fig. 1. Relationship between O_2 and HIF-1α protein levels. Data are modified from Bracken et al. [13] and represent the means ± SEM of values from six different cell lines: HeLa, 293 T, Cos-1, PC-12, CACO2, and HepG2.

HIFs in the physiological regulation of ROS/RNS levels

To examine the evidence for our hypothesis that HIFs regulate ROS/RNS homeostasis by tuning the expression of NOXs, NOSs, and AQPs to prevailing oxygen levels, we review five categories of experimental evidence: (1) evidence that the expression of NOXs, NOSs, and AQPs is increased at lower oxygen levels; (2) evidence that the effects of lower oxygen levels on expression of these enzymes are attenuated or abolished when HIF1/2 is knocked out or expression is knocked down; (3) the presence of consensus HREs in the genes encoding NOXs, NOSs, and AQPs; (4) evidence from chromosome immunoprecipitation assays that HIFs bind to these HREs; (5) evidence for reduced expression of NOXs, NOSs, and AQPS when the HREs have been mutated or deleted. Using this approach, we systematically summarize the substantial evidence supporting the hypothesis that HIFs can function in ROS/RNS homeostasis over the physiological range of oxygen by regulating the expression of ROS/RNS producing enzymes and the channels that facilitate the diffusion of these molecules within and outside of cells. Note that the vast majority of available data is for HIF-1, which may reflect an existing research bias rather than a real difference in the involvement of HIF-1 versus HIF-2 in these processes.

Transcriptional regulation of NOXs by HIF

NOXs are widely expressed in mammalian cells and tissues [16]. They produce superoxide and/or hydrogen peroxide from NADPH and O_2, and thus regulate a wide range of redox-sensitive processes as fundamental to cell biology as the cell cycle and proliferation. In mammals, there are five NOX isoforms that differ in tissue/cell type expression, subcellular localization, oxygen sensitivity, and product (superoxide or hydrogen peroxide). We have sufficient data from three of these isoforms to examine our hypothesis.

NOX4 is a highly oxygen-sensitive enzyme that produces primarily H_2O_2 from O_2 both intracellularly and extracellularly [17]. NOX4 has a $K_m(O_2)$ in the range of 16–18% and produces H_2O_2 at a rate that is three times higher at 6% O_2 than at 1% O_2 [17]. There is abundant evidence from a wide range of cell types and experimental contexts that NOX4 expression increases at lower oxygen levels (Table 1). For example, in pulmonary artery smooth muscle cells, NOX4 mRNA levels double within 30 min of switching media from 20% to 1% O_2 and by 8 h NOX4 mRNA levels increase by 5-fold [18]. This mRNA trend is paralleled by increases in NOX4 protein levels, which are more modest but also double by 2 h. Very similar increases in NOX4 are reported for other cell lines in similar experimental scenarios (Table 1), and in mouse lung tissue (*in vivo*) upon switching mice from normal atmosphere (21% O_2) to a 10% O_2 atmosphere. While there are multiple regulators of NOX4 transcription, HIF-1 appears to play a key role under these conditions. The NOX4 promoter contains an ideal HRE at position −387 to −391 from the transcriptional start site, with flanking sequences similar to those found in well-characterized HIF-1 regulated genes [18]. ChIP assays show HIF-1 binding to this NOX4 promoter and knockdown of HIF-1α prevents the induction of NOX4 expression during hypoxia [18].

Although NOX4 is the most oxygen-sensitive of all NOX isoforms within the physiological range, ROS (typically superoxide) production

Table 1
Effects of O_2 levels and HIF-1 on the expression of NADPH oxidases.

	Condition	mRNA level	Protein level	HRE	Effect of HRE mutation	HIF-1 binding (ChIP)	Cell or tissue type	Ref.
NOX1	1% O_2	Increase	Increase	NA	NA	NA	A549 cells	[64]
	1% O_2	Increase	Increase	NA	NA	NA	Human pulmonary artery endothelial cells	[22]
	1%O2	Decrease	NA	NA	NA	NA	Rat pulmonary artery smooth muscle cells	[65]
NOX2	Urotensin-II	Increase	Increase	Present	NA	Yes	Mice and HMEC-1 cells	[21]
	Intermittent 1.5% O_2	Increase	Increase	NA	NA	NA	PC12 rat cells	[20]
NOX4	1% O_2	Increase	Increase	NA	NA	NA	Rat pulmonary artery smooth muscle cells	[65]
	1% O_2	Increase	Increase				Mouse lung tissue	[65]
	1% O_2	Increase	Increase	NA	NA	NA	Human papillary thyroid carcinoma cell lines	[66]
	Cycling hypoxia	Increase	Increase	NA	NA	NA	GBM8401 and U87 cells	[67]
	1% O_2	Increase	Increase	Present	No activity	Present	Pulmonary artery smooth muscle cells, HEK 293 cells, and HepG2 cells	[18]
	1% O_2	Increase	Increase	NA	NA	NA	Mouse lung tissue	[18]

NA = data not available.

Table 2
Effects of O_2 levels and HIF-1 on the expression of nitric oxide synthases.

	Condition	mRNA Level	Protein Level	HRE	Effect of HRE mutation	HIF-1 binding (ChIP)	Cell or Tissue Type	Ref.
nNOS	21% to 1% O_2	NA	increase	present	NA	NA	Human arterial smooth muscle cells	[32]
	21% to 8% O_2	Increase	Increase	NA	NA	NA	Rat (multiple cells)	[32]
	Atmosphere to 0.4 atm	increase	Increase	NA	NA	NA	Rat nodose ganglion and cerebellum	[30]
	$CoCl_2$ treatment	NA	NA	Present	Present	Present	Human neuroblastoma SK-N-SH cells	[33]
	9% O_2	Increase	NA	NA	NA	NA	Rat lungs, heart and kidney cells	[31]
	21% to 10% O_2	No effect	No effect	NA	NA	NA	Fetal guinea pig hearts	[38]
iNOS	20% to 1% O_2	Increase	NA	Present	Present	NA	Mouse macrophage	[35]
	20% to 1–2% O_2	Increase	NA	Present	Present	NA	Rat cardiac myocytes	[68]
	21% to 10% O_2	Increase	Increase	NA	NA	NA	Fetal guinea pig hearts	[38]
	21% to 1% O_2	NA	NA	Present	Present	NA	A549 lung epithelial cells	[36]
	9% O_2	Decrease	NA	NA	NA	NA	Rat heart cells	[31]
	9% O_2	No effect	NA	NA	NA	NA	Rat lung cells	
eNOS	21% to 2% O_2	Increase	NA	Present	Present	NA	Human umbilical vein endothelial cells / HMEC-1	[37]
	1% O_2	Increase	NA	Present	NA	NA	Human umbilical vein endothelial cells	[28]
	Acute exercise- increased HIF-1a	NA	NA	NA	NA	Present	Vastus lateralis muscles in rats	[40]
	0% O_2	Decrease	Decrease	NA	NA	NA	Human umbilical vein endothelial cells	[39]
	21%–10% O_2	Increase	Increase	Present	NA	NA	Rat lungs	[69]
	9% O_2	Increase	Increase	NA	NA	NA	Rats (multiple cells)	[31]
	21%−12% O_2	NA	Increase	NA	NA	NA	Fetal guinea pig arteries and adult and fetal guinea pig hearts	[38]

NA = data not available.

by other NOXs is also sensitive to oxygen levels. The rate of superoxide production by NOX2, the predominant isoform expressed in polymorphonuclear cells, at 1% O_2 is less than half that at 6% O_2 [17]. Again, there is evidence that reduced NOX2 activity at lower oxygen levels is compensated by increased NOX2 expression. NOX2 protein levels in macrophages are more than three-fold higher at 3% versus 21% O_2 [19]. Intermittent switching of PC12 cells between ~20% and 1.5% O_2 increases NOX2 mRNA and protein levels by up to five-fold [20]. Diebold et al. [21] identified a HRE in the mouse NOX2 promoter and demonstrated HIF-1 binding to this region using ChIP. The stimulation of NOX2 expression in PC12 cells by HIF-1α is prevented by pharmacological or genetic interference with HIF-1α, while overexpression of HIF-1α increases NOX2 expression and activity [20].

NOX1 expression is also affected by oxygen levels. NOX1 mRNA, protein level, assembly into functional enzymes, and activity are all increased in human pulmonary artery endothelial cells at 1% versus ~20% O_2 [22]. Similarly, NOX1 mRNA and protein levels are increased in A549 cells and PC12 cells exposed to 1% O_2 (versus ~20% O_2) for several hours. Deep et al. [23] showed that 24 h exposure to 1% O_2 increased NOX activity (isoform not specified) upon return to 21% O_2 in multiple prostate cancer cell lines. We are not aware of reports demonstrating HREs or the involvement of HIF-1 or HIF-2 in NOX1 expression. However, we located a putative HRE sequence at −2,316 bp in the promoter region of the human NOX1 gene using the UCSC genome browser at genome.ucsc.edu.

Transcriptional regulation of NOSs by HIF

NOSs catalyze the production of nitric oxide (NO) and L-citrulline from O_2 and L-arginine [24]. Nitric oxide participates in protein redox modifications and contributes to the maintenance of cardiovascular homeostasis via vasodilation [24] and to neurotransmission [25]. There are three NOS isoforms in mammals: neuronal NOS (nNOS or NOS1), inducible NOS (iNOS or NOS2) and endothelial NOS (eNOS or NOS3). These isoforms have tissue-specific expression patterns: nNOS is expressed in kidney, skeletal muscles, pancreatic islet cells, adrenal glands, central and peripheral nervous system. iNOS is expressed in vascular smooth muscle cells, cardiomyocytes and hepatocytes. eNOS is found in endothelial cells, fibroblast, type II alveolar cells and skeletal muscles [26]. NOSs also participate in an 'uncoupled' reaction to produce superoxide in the absence of L-arginine [27,28].

Estimates of $K_m(O_2)$ for nNOS range from 2.3 to 40.0% O_2 for nNOS

Table 3
Molecules transported by Aquaporins (AQPs).

	Molecule	Cell/ Tissue type	Transport	Ref.
AQP1	NO	Mouse thoracic aorta	Yes	[47]
		CHO-K1 cells	Yes	[41]
AQP1	O_2	Yeast cells	Yes	[46]
		Synthetic phospholipid bilayer	Yes	[42]
		Synthetic phospholipid bilayer	Yes	[43]
		KO mice under anesthesia	No	[70]
		AQP KO mice	No	[71]
AQP3	H_2O_2	HEK 293, HT29 human colon cancers	Yes	[48]
		CHO-K1 cells)/ human hepatoma (HepG2)	Yes	[45]
		Mouse T cells	Yes	[49]
		Keratinocytes	Yes	[50]
AQP4	NO	Mouse retina	Yes	[57]
		Synthetic phospholipid bilayer	Yes	[43]
	O_2	Synthetic phospholipid bilayer	Yes	[43]
AQP5	H_2O_2	Rat AQP5 expressed in yeast	Yes	[44]
AQP9	H_2O_2	Chinese hamster ovary cells (CHO-K1 cells)/ human hepatoma (HepG2)	Yes	[45]

(reviewed in [29]). Therefore, nitric oxide production by nNOS in, for example, brain tissue, where oxygen is typically in the range of 2–6% O_2 [12], will be affected by prevailing oxygen levels. Since brain NO is involved in many different functions, from learning and memory formation to movement (reviewed in 4), significant deviation from baseline NO production will have consequences. There is abundant evidence for stimulation of nNOS transcription in hypoxia (see Table 2). Prabhakar et al. [30] showed time-dependent increases in nNOS mRNA and protein levels in nodose ganglion and cerebellum tissue of adult rats subjected to hypobaric hypoxia. Similar results were shown by Gess et al. [31] and Ward et al. [32]. In the latter study, rats subjected to hypoxia at 10% O_2 for 48 h had elevated nNOS mRNA levels in mesenteric arteriole, kidney and aorta cells [32]. In this same study, hypoxia similarly increased protein levels in the brain, aortae, mesenteric arterioles, pulmonary arteries, and diaphragm. Increased nNOS transcription in hypoxia appears to be mediated by HIF-1 (Table 2). For example, Li et al. [33] showed that $CoCl_2$ treatment of human SK-N-SH neuroblastoma cells, a common model for pseudohypoxia via inhibition of prolyl hydroxylases, caused the concomitant accumulation of HIF-1a protein and nNOS mRNA and protein. Ward et al. [32] reported the presence of multiple HREs in the human nNOS gene promoter region, and Li et al. [33] used ChIP analysis to demonstrate binding of HIF-1 to this HRE. Mutation or deletion of these HREs abolished nNOS transcriptional stimulation during hypoxia.

Similar observations have been made with iNOS, for which estimates of $K_m(O_2)$ range from 0.6 to 11.0% O_2 (see 30 for summary). The effects of hypoxia on iNOS activity and iNOS mRNA and protein expression have been summarized comprehensively by Robinson et al. [34]. Hypoxia acutely decreases iNOS activity in a wide variety of cell types, followed by increased iNOS expression. Melillo et al. [35] and Lee et al. [36] have described HREs in the murine and human iNOS gene promoter regions, respectively. HIF-1 appears to play a role in this, though the effect is complex due to interactions with multiple transcriptional regulators.

The $K_m(O_2)$ of eNOS is estimated to be 0.3–0.8% (reviewed in 30), making it less sensitive to changes in oxygen levels around physiologically relevant values, particularly in arterial endothelia since O_2 levels are close to 13%. Nonetheless, eNOS expression is also stimulated by low O_2. mRNA levels are higher in human umbilical vein endothelial cells (HUVEC) and human dermal microvascular endothelial cells (HMEC-1) cells exposed to 2% versus 18–19% O_2 [37]. Gess et al. [31] also demonstrated an increase in eNOS mRNA levels in the heart, lung, liver and kidney cells of rats under hypoxia. eNOS protein levels in guinea pigs arteries were increased by exposure to 12% O_2 for 14 h [38]. However, contradictory findings have also been reported (eg. 40). Coulet et al. [37] identified an HRE on the human eNOS gene at −5375 to −5366, which was transcriptionally active. Transfection of HUVEC cells with a luciferase reporter construct containing this region demonstrated HIF-mediated expression that was strongest with HIF-1. Mutation of the HRE reduced the HIF-mediated increase in expression. Rodriguez-Miguelez et al. [40] used ChIP analysis to show HIF-1α binding of this HRE.

Taken together, the results of these studies support the hypothesis that activities of NOX and NOS isoforms are sensitive to changing oxygen levels within the physiological range, and that HIF-1/2 stimulate transcription of multiple NOX and NOS isoforms, perhaps as a homeostatic mechanism, as intracellular oxygen levels are reduced.

Transcriptional regulation of AQPs by HIF

In addition to effects on the expression of ROS/RNS-producing enzymes, there is evidence that HIFs regulate the expression of various AQPs isoforms. The rate at which O_2, H_2O_2 and NO traverse phospholipid membranes is slow, thus potentially limiting biological activities that are regulated by these molecules. AQPs are integral membrane proteins that, although first characterized as water transporters [41], also contribute to transport of other molecules. AQPs are homotetramers with all four individual monomers capable of transporting water; they also contain a central pore that has been hypothesized to mediate the transport of gases, including O_2 and NO [42,43]. In mammals there are thirteen distinct AQP isoforms with tissue-specific expression patterns and sequence/structural differences that relate to their substrate specificity [44,45].

Experimental and molecular modeling evidence suggests that AQPs transport O_2 and NO (Table 3). A limited O_2-transporting activity of AQP1, primarily through the central pore, has been demonstrated in synthetic lipid bilayers [42], and more recently in a yeast model expressing whale myoglobin as reporter [46]. Molecular dynamics simulations also suggest that AQP1 is an O_2 transporter though they predict that AQP4 is a better conductor of both O_2 and NO [43]. Both AQP1 and AQP4 have been suggested to transport NO. Herrera et al. [41] showed increased NO influx into Chinese hamster ovary (CHO) K1 cells transfected with hAQP1, which was prevented by addition of (admittedly non-specific) AQP inhibitor $HgCl_2$. This activity of hAQP1 was recapitulated in a lipid vesicle model. Subsequently, Herrera and Garvin [47] showed reduced rates of NO diffusion from endothelial cells of AQP1 KO mice. Thus, AQP1 and perhaps AQP4 could contribute to NO and perhaps O_2 transport.

There is stronger evidence that AQP3, AQP5, and AQP9 transport H_2O_2 (Table 3). Using HEK293 embryonic kidney and HT29 colon cancer cells loaded with a fluorescent reporter, Miller et al. [48] showed that hAQP3 overexpression increased H_2O_2 uptake rates. This finding was corroborated by Hara-Chikuma et al. [49,50] who showed reduced H_2O_2 transport in T cells and keratinocytes from AQP3 KO versus wildtype mice. An $HgCl_2$ inhibitable increase in H_2O_2 transport rates has been shown for rat AQP5 [44]. Similarly, hAQP9 overexpression in CHO-K1 cells increases H_2O_2 transport, while siRNA-mediated knockdown of hAQP9 expression reduces H_2O_2 transport rates in HepG2 human hepatoma cells [45]. Taken together, there is strong evidence that AQP3, AQP5, and AQP9 (and perhaps other AQPs) transport H_2O_2 across mammalian cell membranes.

We suggest that increasing the H_2O_2 and NO permeability of membranes at lower O_2 levels is part of the same homeostatic strategy as increasing NOX and NOS expression. If, despite transcriptional up-regulation, higher levels of NOX and NOS enzymes nonetheless fail to fully maintain ROS/RNS production at lower oxygen levels, increasing their ability to move between cellular compartments could work to offset this. There is indeed evidence that hypoxia stimulates AQP expression (Table 4). In prostate cancer (PC3-M) cells, density-induced pericellular hypoxia or $CoCl_2$-induced pseudo-hypoxia increase hAQP1

Table 4
Effects of reduced O_2 levels and HIFs on the expression of aquaporins (AQPs).

	mRNA Level	Protein Level	HRE	Effect of HRE mutation	HIF-1 binding (ChIP)	Cell or tissue type	Ref.
AQP1	Increased	Increased	Present	NA	NA	PC-3 M human prostate cancer cells	[51]
AQP1	No effect	No effect	Present	No effect	Yes	Nucleus pulposus cells of intervertebral disc	[53]
AQP1	Increased	Increased	NA	NA	NA	Human retinal vascular endothelial cells	[52]
AQP1	Increased	NA	Present	NA	Yes	Human retinal vascular endothelial cells	[72]
AQP1	NA	Increased	NA	NA	NA	RSC96 (Schwann cells)	[73]
AQP3	Increase	NA	Present	NA	Yes	L929 mouse fibrosarcoma	[54]
AQP4	increased	increased	NA	NA	Yes	Mouse retina	[57]
AQP4	Increased	Increased	NA	NA	NA	Rat astrocytes/microglia	[59]
AQP4	Decreased	NA	NA	NA	NA	Rat brain (cerebral cortices)	[58]
AQP4	Variable	Variable	NA	NA	NA	Rat cerebellar astrocytes	[55]
AQP5	No effect	No effect	Present	Variable	Yes	Nucleus pulposus cells of invertebral disc	[53]
AQP5	Decrease	Decrease	NA	NA	NA	MLE12 mouse lung epithelial cells and mouse lung tissue	[60]
AQP5	Increase	Increase	Present	Abolished effect	NA	Rat alveolar epithelial cells/MLE – 15 cells	[61]
AQP9	decrease	decrease	NA	NA	NA	Rat astrocytes	[74]
AQP9	Increase	Increase	NA	NA	NA	Rat retinal ganglion cell line	[63]
AQP9	Decrease	NA	NA	NA	NA	Brain (Cerebral Cortices from rats)	[58]
AQP9	Increase	Increase	NA	NA	NA	Adult male Sprague-Dawley rats	[56]
AQP9	Decrease	Decrease	Present	NA	NA	Placental tissue	[62]

NA = data not available.

mRNA and protein levels [51]. A HRE was located at position −1293 to −1286 of the hAQP1 gene, and mutation of this site abolishes the $CoCl_2$-induced increase in hAQP1 expression [51]. Kaneko et al. [52] demonstrated a similar hypoxia-induced upregulation of hAQP1 mRNA and protein in human retinal vascular epithelial cells. When the HRE was mutated or deleted this hypoxia-induced effect on hAQP1 transcription was abolished [52]. Using ChIP, Tanaka et al. [52] showed HIF-1 binding to hAQP1. Johnson et al. [53] showed that knockdown of HIF-1α expression resulted in a dramatic reduction in AQP1 protein levels in nucleus pulposus cells. Taken together, these results strongly suggest a HIF-1 dependent stimulation of AQP1 expression in hypoxia. Hypoxia and HIF-1 also stimulate expression of other AQPs. HREs have been identified in the murine AQP3 gene [54], and ChIP assay confirmed HIF-1 binding of these sites. Hypoxia increased AQP3 expression in L929 fibrosarcoma cells derived from mice that was abolished by HIF-1α knock down. In rat brain [55,56], retina [57], and cultured astroctyes and microglial cells [58,59] AQP 4 mRNA and protein levels are increased by hypoxia. On the other hand, results for AQP5 are more equivocal. For example, while Johnson et al. [53] identified two HREs in the hAQP5 gene and evidence for weak HIF-1 binding and increased hAQP5 expression in human intervertebral disc tissue during hypoxia exposure, neither mutation of the HREs nor HIF-1 knockdown affected AQP5 expression in hypoxia. Kawedia et al. [60] showed no increase in AQP5 in MLE12 mouse lung epithelial cells and mouse lung tissue exposed to hypoxia. On the other hand, HIF-1 increased rat AQP5 mRNA and protein levels in rat alveolar epithelial cells, and this effect was prevented by knockdown of HIF-1α expression [61]. Fourteen putative HRE sites have been identified in hAQP9 gene, but in cells treated with $CoCl_2$ AQP9 expression decreased [62]. In contrast, Dibas et al. [63] showed an increase in AQP9 mRNA and protein levels in rat retinal ganglion cells (RGC-5) exposed to hypoxia. Taken together, there is a significant amount of experimental evidence supporting the hypothesis that the expression of AQPs is negatively correlated to oxygen levels and that HIF-1 participates in the stimulation of transcription.

Conclusions and interpretation

Endogenous production of ROS and RNS is characteristic of normal cell physiology and these molecules have increasingly well-defined roles in the redox regulation of key pathways involved in critical biological processes. Since the production of ROS/RNS by NOXs and NOSs is sensitive to oxygen levels, a mechanism to maintain ROS/RNS homeostasis over the physiological range of O_2 experienced by many human cell types is likely important. HIFs are well positioned to contribute to the mediation of this response. The levels and transcriptional activities of HIF-1/2 are strongly affected by oxygen levels within the physiologically normoxic range, which give them the ability to transcriptionally regulate the levels of NOXs, NOSs, and AQPs to promote ROS/RNS synthesis and transport at the lower end of this range. We therefore suggest that an over-looked function of HIFs is in the maintenance of endogenous ROS/RNS homeostasis within the range of oxygen levels typically experienced by most human cell types.

Declaration of Competing Interest

The authors have no conflicts of interest to report.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mehy.2019.109249.

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