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REVIEW

Intracellular Mechanisms of Oxygen Sensing

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Abstract—The review describes molecular mechanisms for sensing oxygen levels in various compartments of animal cell. Several pathways for intracellular oxygen sensing are discussed together with details of functioning of the near-membrane and cytoplasmic pools of molecular components in hypoxic cells. The data on the role of mitochondria in cell sensitivity to a decreased oxygen content are presented. Details of mutual influence of the operational and chronic intracellular mechanisms for detecting the negative gradients of molecular oxygen concentration and their relationship with cell metabolism response to the oxidative stress are discussed.

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Oxygen is the main factor affecting cell metabolism in aerobes. Individual cells and small groups of cells incubated under natural or artificial conditions receive vital ingredients via diffusion. In multicellular organisms, specialized respiratory and circulatory transport systems provide the supply of atmospheric oxygen to the cells.

The quality of oxygen distribution cannot be not controlled at the system, regional, or cellular levels. In the latter case, one can expect the existence of specialized cellular oxygen sensors or cell-centered mechanisms involved in the control of cell homeostasis. The majority of data on the cell sensitivity to the variations in the oxygen concentration have been produced for the cellular components of tachytrophic tissues during their transition from normoxia to hypoxia *in vitro*.

The principles of cell oxygen homeostasis have been proposed over the last three decades. The molecular platform for the studies in this area has been established in the works of Lopez-Barneo et al. [1] and Semenza et al. [2, 3]. Further research on this issue will elucidate the regularities of oxygen spatial dynamics within a cell, including preferred "entry gates", "utilization funnels", and possible O_2 "depot sites".

Abbreviations: BK_{Ca} channels, large conductance calcium-activated potassium channels; CSE, cystathionine gamma-lyase; FIH, factor inhibiting HIF; HIF, hypoxia-inducible factor; HO-2, heme oxygenase-2; PHD protein, prolyl hydroxylase domain protein; ROS, reactive oxygen species.

MITOCHONDRIA

Molecular oxygen is the terminal electron acceptor in the processes of ATP generation in the mitochondria [4-6]. These cellular organelles consume up to 90% oxygen received by the cells via diffusion and convective mass transfer [5, 7, 8]. For many years, these observations have made mitochondria the main subjects in the search for the intracellular oxygen sensors [6, 9, 10].

Most authors looking for the initial point in the intracellular oxygen homeostasis pathway were by default searching for the hypoxic O_2 sensor. The scientist hoped to identify either molecular machinery or process that would change its state upon decrease in the oxygen concentration, and such change should have a significant impact on cell functioning and its fate.

At the first glance, the mechanism of oxygen sensing was expected to be found at the terminal points of oxygen supply to the cells. It was suggested that this mechanism would be based on sensing the ATP content. If the amount of stored ATP reaches the minimal allowed level, this switches on the "alarm" signal, and the cell initiates the hypoxic response. This hypothesis was inspired by the theoretical model by Atkinson [11] and his equation for calculating the cell energy charge. However, so far, no expected mechanism for maintaining the normal oxygen status has been found; moreover, in the recent review, Waypa et al. [6] dubbed such mechanism a "poor engineering solution".

Here are the arguments against the ATP-dependent mechanism of oxygen sensing. Firstly, ATP synthesis requires multiple organic substrates in addition to oxygen. Hence, the amount of ATP does not reflect the oxygen supply only. Secondly, it remains unclear how an individual mitochondrion and the entire mitochondrial pool in the cell could affect the diffusional mass transfer of oxygen in the extracellular space and the cytosol? Thirdly, direct measurements of oxygen content in the cells demonstrated that the ATP production is maintained up to the anoxic threshold (2 torr, or 2 mm Hg) [12, 13]. Hence, measuring ATP content will not ensure detection of the oxygen supply within the physiological hypoxia range [6].

The absence of answers to these questions, as well as fruitless 40-year-long search for the ATP-dependent mechanism of intracellular oxygen sensing have led to the conclusion that mitochondria, if they are involved in the control of oxygen homeostasis in the cells, achieve it via some other mechanisms.

In this connection, thiol oxidation with hydrogen peroxide has attracted considerable interest of researchers. However, the authors of reviews on this topic, who are very cautious in their conclusions, suggest that the oxygen sensitivity of the stages of H_2O_2 interaction with thiol-containing molecules (glutathione, glutaredoxins, thioredoxins, and peroxiredoxins) is the evidence of optimization of the delicate balance in this alliance rather than indicates the intracellular oxygen sensory transduction [14-16].

Another possibility of the involvement of mitochondria in the formation of cell response to hypoxia could be based on the signaling functions of reactive oxygen species (ROS) produced by the electron transport chain (ETC) enzymes [4, 5, 17-19]. It has been known for a long time that ROS generation increases with the increased oxygen supply to the cells [20, 21]. A decrease in the production of prooxidants under hypoxic conditions has been mentioned in the earlier studies [22, 23]. However, other authors have reported an enhanced production of hydrogen peroxide by the mitochondria in cardiomyocytes and pulmonary artery smooth muscle cells under hypoxic conditions [24-26]. Their conclusions were based on the data from different laboratories that investigated the intracellular distribution of ROS at low oxygen concentration using different techniques [27-29].

It was experimentally proven that in addition to the peroxidation activity, ROS exhibit regulatory functions in numerous cellular processes, including proliferation, differentiation, cell aging, regulation of transcription factors, and inflammatory response [19, 30, 31]. It was suggested that peroxidants, such as H_2O_2, could be involved in the regulation of intracellular oxygen homeostasis [32, 33]. This hypothesis was corroborated by direct measurements of the ROS concentration dynamics using the thiol redox sensor roGFP in the pulmonary artery smooth muscle cells [34]. Under normoxic conditions hydrogen peroxide activated roGFP by 20% in the in the cytosol, 45% in mitochondrial intermembrane space, and 70% in the mitochondrial matrix. Under hypoxic conditions, roGFP activation increased by 35 and 65% in the cytosol and the intermembrane space, respectively, but significantly decreased in the mitochondrial matrix. Similar changes were observed in the isolated arterial smooth muscle cells [32]. These data provided a direct proof that the H_2O_2 concentration in the cytosol increases under hypoxic conditions. The results produced by Waypa et al. [6] were later confirmed by other authors [8, 35-37].

Recently, it was demonstrated that the main source of ROS playing a pivotal role in the cell oxygen homeostasis is complex III of the ETC [4, 38, 39]. The mechanisms of mitochondrial ROS involvement in the regulation of cellular response to hypoxia are shown in Fig. 1. Under hypoxic conditions, complex III releases some of superoxide anion radicals into the intermembrane space [10, 40], where they are converted into H_2O_2 by superoxide dismutase [41, 42]. Next, hydrogen peroxide is released into the cytosol, where it modulates the activity of prolyl hydroxylase 2 (PHD2) [4, 9, 10].

Enzymes of the prolyl hydroxylase domain (PHD) protein family require molecular oxygen as a substrate for the degradation of hypoxia-induced factor (HIF) and its HIF-α isoform in particular, which occurs under normoxic conditions. During hypoxia (i.e., at a lower oxygen concentration in the cytosolic environment), the PHD2 activity decreases, which prevents HIF degradation. HIF translocates to the nucleus and induces transcription of "hypoxic" genes, thus providing the cell response to hypoxia [43].

The question arises of how the increase in the cytosolic ROS concentration in hypoxia could result in the PHD2 inhibition and HIF stabilization. According to Waypa et al. [6] and other researchers [44, 45], ROS partially denature enzyme molecules, resulting in the PHD activity decrease.

Until recently, mitochondria had been considered as the most likely candidates for the location of oxygen sensors in eukaryotic cells [4, 6, 10]. There is enough accumulated evidence indicating that the byproducts of the bioenergetics activity of mitochondria (ROS) modulate cytosolic processes involved in the cell response to hypoxia [6, 8, 39]. In this context, prooxidants can be considered not as O_2 sensors, but rather as O_2 markers. In modern terms, mitochondrial ROS participate in the signaling pathways ensuring the maintenance of oxygen homeostasis in hypoxic cells [4, 10]. One might expect more than one input to this signaling pathway.

OXYGEN-SENSITIVE PLASMA MEMBRANE ION CHANNELS

Cell plasma membrane is a first barrier on the path of molecular oxygen to the sites of its intracellular utiliza-

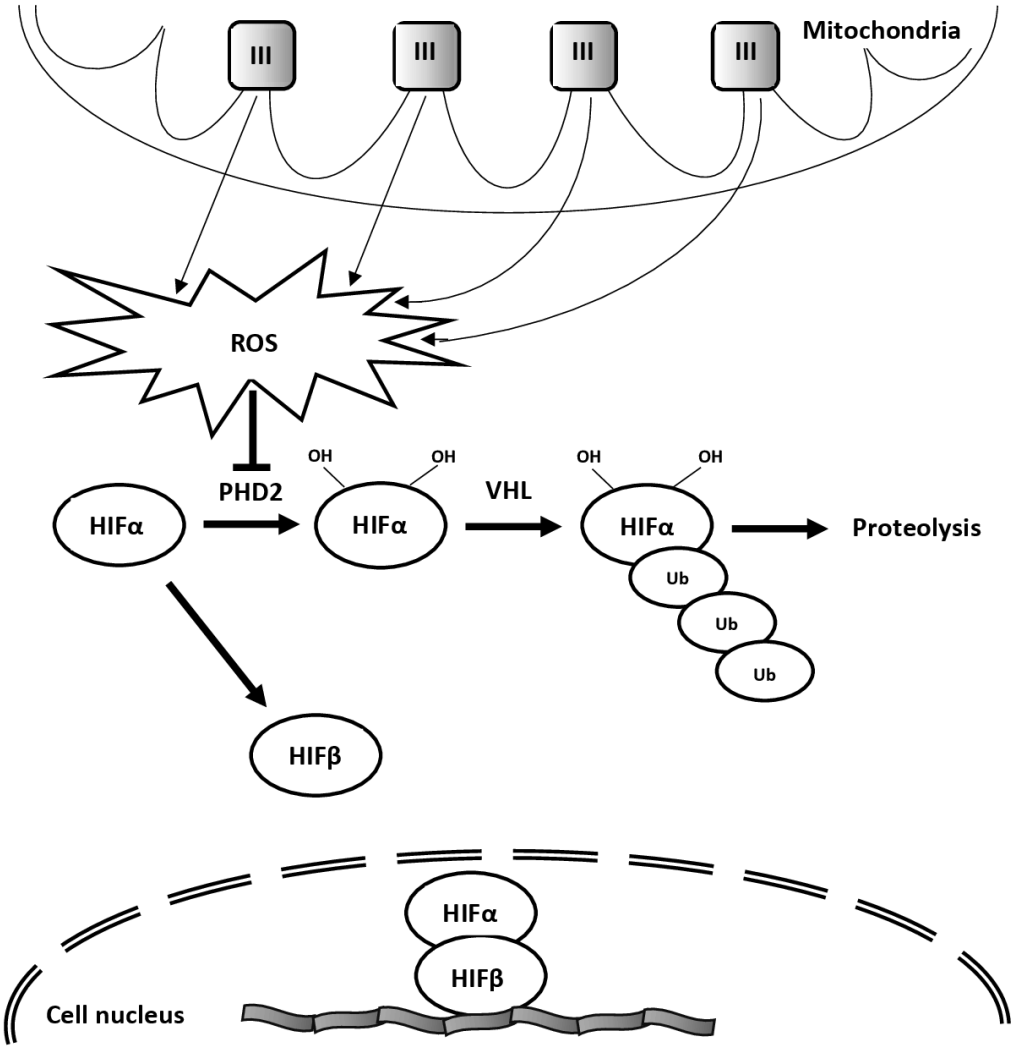

Fig. 1. Inhibition of HIF-α subunit metabolism by the mitochondrial ROS produced under hypoxic conditions: III, complex III of the ETC; PHD, prolyl hydroxylase; VHL, von Hippel–Lindau factor; HIF, hypoxia-induced factor; Ub, ubiquitin.

tion. According to Benjamin Lewin [46], very small and neutral oxygen molecules easily penetrate the lipid bilayer of the cell membrane. A common property of all living cells is the existence of regulated ion concentration gradients across the plasma membrane. The dynamics of changes in the ion gradients is manifested through the membrane potential, which is generated by multiple classes of ion channels incorporated into the lipid bilayer. In animal cells, the membrane potential is formed mainly by K^+, Na^+, and Cl^- ions. The channels transporting these ions are regulated by multiple effectors (electric field, concentration of Ca^{2+} and H^+ ions, ATP molecules, etc.) [47].

Since 1988, molecular oxygen has been included in the list of factors affecting the activity of membrane K^+ ion channels. The data published by Lopez-Barneo et al. [1] were unexpected and, therefore, have been carefully verified in the following years. The first review on the effect of oxygen deficiency on the large conductance calcium-activated potassium (BK_{Ca}) channels (BK_{Ca}) was published nine years later, which cited 73 publications on this topic [48]. The authors of this review reported that inactivation of BK_{Ca} channels with the decrease in the oxygen partial pressure in the incubation medium to >20 torr caused no doubts. This same effect was found in various cytomorphic models (arterial smooth muscle cells; neurons of the cortex, hippocampus, and substantia nigra; carotid body type I cells; neuroepithelial bodies at the bronchial bifurcation). It is important to mention that BK_{Ca} channels respond to hypoxia both in the composition of undamaged membranes of intact cells and during recording in the patch clamp mode. The authors of the review suggested that other types of ion channels might be modulated by oxygen as well.

At the next stage of investigations on the oxygen sensitivity of K^+ channels, researches have become interest-

ed in the structure of these molecular aggregates and search for domains directly responsible for the oxygen concentration-dependent sensory signaling in the channel vicinity during closing of selective K^+ channels [49]. At least three families of oxygen-sensitive potassium channels including more than 14 types of channels have been identified [50, 51]. However, no oxygen-sensitive domains have been found in the α- and β-subunits of various types of potassium channels [49, 51].

At the third stage, the principles of the *membrane hypothesis* have been postulated [47, 49, 52]. This hypothesis was formed in an attempt to answer the questions formulated by the beginning of the XXI century. Why are only potassium channels sensitive to the changes in O_2 concentration? How can they function for a long time without support of mitochondria during patch clamping? Do BK_{Ca} channels act as oxygen sensors or do they represent only an effector component in the cell response to hypoxia? Why does the response of potassium channels to the decrease in the oxygen concentration occur very fast?

Several groups of researchers have proposed the existence of unknown protein ensembles associated directly or indirectly with the oxygen-sensitive potassium ion channels [47, 49]. Glutathione-activated protein kinase, heme oxigenase-2 (HO-2), NADP(H) oxidase, and other proteins were considered as plausible components of these ensembles [47, 53, 54].

Close association between BK_{Ca} channels and HO-2 was established in the experiments conducted in the rat carotid body type I cells using mass spectrometry and co-immunoprecipitation [55]. By catabolizing heme molecules, HO-2 produces carbon monoxide, biliverdin, and Fe^{2+}. Under normoxic conditions, NADP(H) and molecular oxygen participate in this reaction as cofactors. It was shown that carbon monoxide can activate BK_{Ca} channels [56]. Under hypoxic conditions, when oxygen becomes deficient, the stimulating effect of carbon monoxide on BK_{Ca} channels declines and potassium ion conductance decreases. In this case, HO-2 molecules serve as oxygen sensors for BK_{Ca} channels. Another group of authors demonstrated the inhibitory effect of H_2S on BK_{Ca} channels in the carotid body type I cells from mice, rats, and humans under hypoxic conditions [57, 58]. Due to these observations, CO and H_2S (intracellular gaseous transmitters) were included into the model of oxygen sensitivity of potassium channels [52].

Later, the membrane hypothesis has been expanded and eventually finalized [51, 59]. Plasma membrane ion channels play an important role in the different modalities of sensory signaling processes, either as primary sensory elements or as downstream components of the effector network [60]. Potassium channels in the vertebrate type I carotid cells are an example of effector-specific channels. Among them are Kv3, Kv4, BK_{Ca}, and TASK families [50, 52]. It was demonstrated that conductance of these potassium channels depends on the concentration of molecular oxygen on the plasma membrane surface [1, 51]. However, the question still remains on what part of the ion channel molecular structure or what channel-associated satellite molecule channel can act as the oxygen sensor.

The authors of the membrane hypothesis Chris Peers and Nanduri Prabhakar believe that potassium channels belong to the effector part of the signaling cascade in the oxygen-sensitive cytosystems [49, 51, 52], while the functioning of molecules associated with potassium channels should be considered as oxygen-susceptible [47, 53, 54]. The list of these molecules includes HO-2, NADP(H), cystathionine gamma-lyase (CSE), guanylate cyclase (GC), cyclic guanosine monophosphate (cGMP), and protein kinase G (PKG).

The interactions of the membrane potassium ion channels from different families with the associated proteins are shown in Fig. 2. HO-2 catabolizes heme and converts it into biliverdin, iron (II) ion, and carbon monoxide. NADP(H) and molecular oxygen serve as cofactors during heme degradation under normoxia [61]. These above-mentioned processes occur, for example, in glomus cells of the carotid bodies [56, 62].

CO molecules activate BK_{Ca} channels [57, 63, 64]. Hypoxia decreases production of carbon monoxide in the carotid bodies. The same effect was observed for the HO-2 inhibitor zinc protoporphyrin-9 [62]. In both cases, CO deficit leads to the closing of BK_{Ca} channels [51].

Hence, a decrease in the molecular oxygen concentration in the cytoplasmic environment of constitutive HO-2 associated with the BK_{Ca} channels decreases its catalytic activity. This results in the reduction of CO synthesis. The CO deficit during hypoxia development leads to the closing of potassium channels.

This disrupts the dynamics of membrane potential formation in the glomus cells of carotid bodies and some other oxygen-sensitive cells.

Therefore, HO-2 from the vertebrate type I cells of carotid bodies is the intracellular component that senses molecular oxygen concentration and transmits the signal to the effectors (BK_{Ca} channels). The authors believed that this validated their version of membrane hypothesis [51]. Its heuristic power was corroborated in the studies conducted at the system level. Thus, young and healthy volunteers showed reduction of hypoxic ventilatory response following short-term inhalation of carbon monoxide [65].

In addition to the CO-dependent mechanism, the activity of potassium channels in the vertebrate glomus cells of carotid bodies can be regulated by another signaling pathway, whose central component is CSE [66, 67]. This enzyme synthesizes intracellular gaseous mediator H_2S [51, 57, 68]. Under normoxic conditions, the synthesis of hydrogen sulfide is at its minimum; however, H_2S generation increases proportionally to the decrease in the oxygen concentration under hypoxic conditions [57, 69].

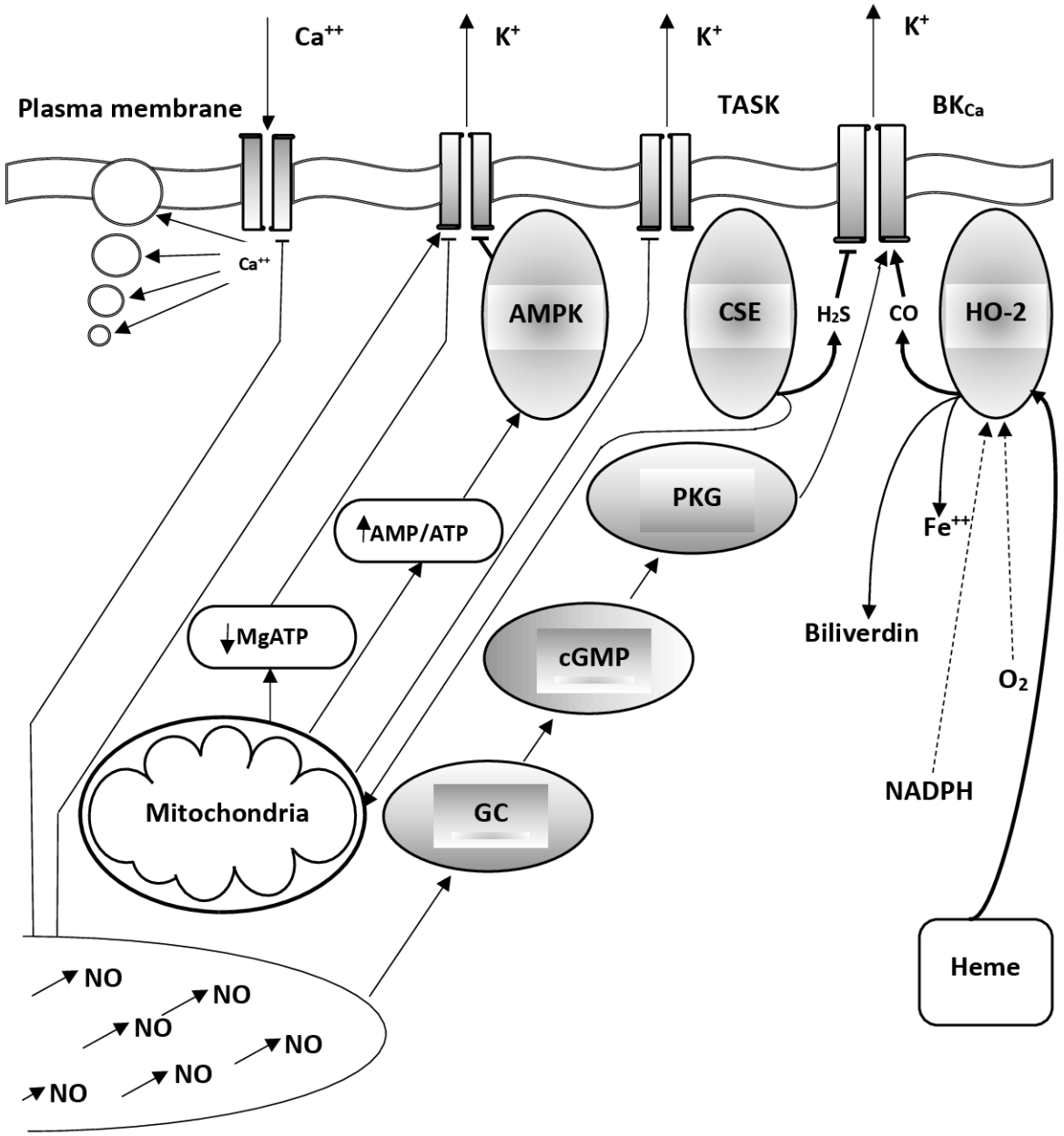

Fig. 2. Interaction of various families of membrane potassium channels with associated proteins; gray ovals, proteins associated with channels; →, activating effect; ⊣, blocking effect; ◎, mediator-containing vesicles produced by glomus cells of carotid bodies; AMPK, AMP-activated protein kinase; CSE, cystathionine gamma-lyase; HO-2, hemoxygenase-2; PKG, protein kinase G; MgATPase, magnesium-activated ATPase; cGMP, cyclic guanylate monophosphate; GC, guanylate cyclase.

Activation of H_2S synthesis as a response to hypoxia is absent in the CSE-knockout mice. The CSE inhibitor propargyl-L-glycine also impedes production of intracellular hydrogen sulfide [59]. It is generally accepted nowadays that CSE is the major producer of intracellular H_2S in hypoxic cells [51]. Artificial increase in the content of hydrogen sulfide in the cells of carotid bodies activates this organ proportionally to the degree of exposure [57, 70]. Accumulated experimental data indicate that the product of CSE activity (H_2S) is an obligatory participant of the formation of response of carotid body cells to hypoxia.

In 2010, Li et al. [58] were the first to elucidate the effect of excessive intracellular hydrogen sulfide on ion channels of the glomus cells of carotid bodies. It was established later that H_2S targets BK_{Ca} channels, but its effect is different from the one exhibited by carbon monoxide [59]. Intracellular hydrogen sulfide promotes the influx of calcium ions into the glomus cells [70]. It also inhibits potassium channels from the TASK family via suppressing oxidative phosphorylation in the glomus cell mitochondria [70, 71]. However, the main target of hydrogen sulfide in the type I cells of carotid bodies is BK_{Ca} channels [59, 72].

Under normoxic conditions, CSE displays low activity. Its activity and, therefore, H_2S generation, increase with the development of intracellular hypoxia proportionally to the degree of oxygen deficit. H_2S inhibits BK_{Ca} channels and indirectly (via mitochondria) decreases the conductance of potassium channels from other families (e.g., TASK). Hence, CSE from the vertebrate type I cells of carotid bodies represents another intracellular sensor that converts the sensory information on the intracellular oxygen concentration into signals to the effectors (BK_{Ca} channels). Therefore, vertebrate type I cells of carotid bodies have that more than one mechanism for transition of sensory information on the intracellular oxygen concentration for the regulation of potassium channel activity. CO and H_2S influence in the opposite directions the effector part of the oxygen-sensitive signaling pathway during hypoxia development. When the CO concentration decreases, BK_{Ca} channels lose the support of this mediator, while increase in the H_2S concentration leads to the closing of potassium channels. Hence, under hypoxic conditions, the cells remain under protection of two different mechanisms.

The authors of the latest version of the membrane theory believe that the third intracellular gaseous transmitter – NO – also participates in the regulation of intracellular oxygen homeostasis. It has been known for a long time that hypoxia decreases the activity of NO synthase (NOS), including its neuronal form nNOS in the glomus cells of carotid bodies [73]. nNOS is not expressed in the type I cells and, according to the authors, is delivered into these cells from the surrounding efferent fibers of the glossopharyngeal neurons [51, 73, 74]. It was found that artificial increase in the NO content in the cells of carotid bodies activates BK_{Ca} channels [75]. However, these results were not confirmed under different experimental conditions [76]. It was established that the stimulating effect of NO on BK_{Ca} channels is mediated by guanylate cyclase (GC), cyclic guanosine monophosphate (cGMP), and protein kinase G (PKG) [77, 78].

It has been long known that glomus cells of carotid bodies are characterized by the fastest cellular response to acute hypoxia. There are no doubts nowadays that membrane potassium channels from different families take part in this response. BK_{Ca} channels are involved in several signaling pathways of hypoxic cellular metabolism as effector elements. HO-2 serves as an oxygen sensor affiliated with BK_{Ca} channels. The molecular mechanisms of cytoplasmatic response to chronic hypoxia are also well known.

CYTOPLASMIC MECHANISMS OF OXYGEN SENSITIVITY

Carotid bodies are an example of systemic oxygen sensors [79, 80]. HO-2, in turn, represents an oxygen sensor associated with the plasma membrane of the type I cells of carotid bodies [51]. Furthermore, all Metazoa cells have a cytoplasmic signaling pool, the central element of which is HIF [81]. This pool contains should contain one more cellular oxygen sensor.

The history of HIF discovery and studies is briefly presented in the work by Szewczak [82]. The studies on the regulatory role of erythropoietin (Epo) have resulted in the discovery of the gene encoding this protein [83], followed by the identification of cytoplasmic HIF that induced transcription of the Epo gene [2]. It was then found that HIF induces transcriptional cascade of genes in response to the reduced oxygen supply in mammalian cells [3, 84].

The structure and functions of HIFs have been discussed in numerous reviews [85-87] (Fig. 3).

Oxygen partial pressure in the cell cytoplasm under normoxia is 7 to 20 torr (Fig. 3a). Under these conditions, HIF molecules undergo proteasomal degradation. As the first step, HIF interacts with FIH (factor inhibiting HIF), which results in the cleavage of asparagine fragment from the HIF molecule. The reaction proceeds in the presence of sufficient amounts of oxygen, 2-oxoglutarate, iron (II) ions, and ascorbic acid [87].

In parallel with this, prolyl hydroxylase hydroxylates two proline residues in the HIF α-subunit with the release of carbon dioxide and succinic acid [88]. Next, HIF-α interacts with the von Hippel–Lindau tumor suppressor protein and undergoes ubiquitination and final degradation by the proteasome [80, 84].

The mechanism of HIF degradation during the initial stages of mild hypoxia accompanied by a moderate decrease in the partial oxygen pressure in the cytoplasm is different (Fig. 3b). HIF is not hydroxylated by prolyl hydroxylase and the intermediate products of HIF-α and HIF-β degradation do not undergo proteasomal degradation [84, 87].

HIF metabolism under severe hypoxia is shown in Fig. 3c. Under these conditions, the initial steps of HIF proteolysis involving FIH and PHD become impossible, since severe hypoxia inhibits asparagine hydroxylation in HIF. In this case, HIF-α and HIF-β in cooperation with the transcription co-activator P300/CBP induce transcription of target genes, whose function is to overcome the oxygen deficit [80, 84, 85].

According to the latest estimates, the transcriptional cascade initiated by HIF could involve up to 1500 genes [84, 89, 90]. Cell response to hypoxia involving metabolic HIF pool is slow: the earliest consequences of genome activation in response to the intracellular oxygen deficit become noticeable after an hour [91]. The peak of gene expression under chronic intracellular hypoxia is reached after 24 h [80]. Mice deficient by HIF die on the 10th day of embryonic development, which indicates that this mechanism of cellular oxygen homeostasis regulation is essential already at the prenatal stage [92].

Analysis of metabolism changes in Metazoan cells in response to the reduced oxygen concentration reveals the

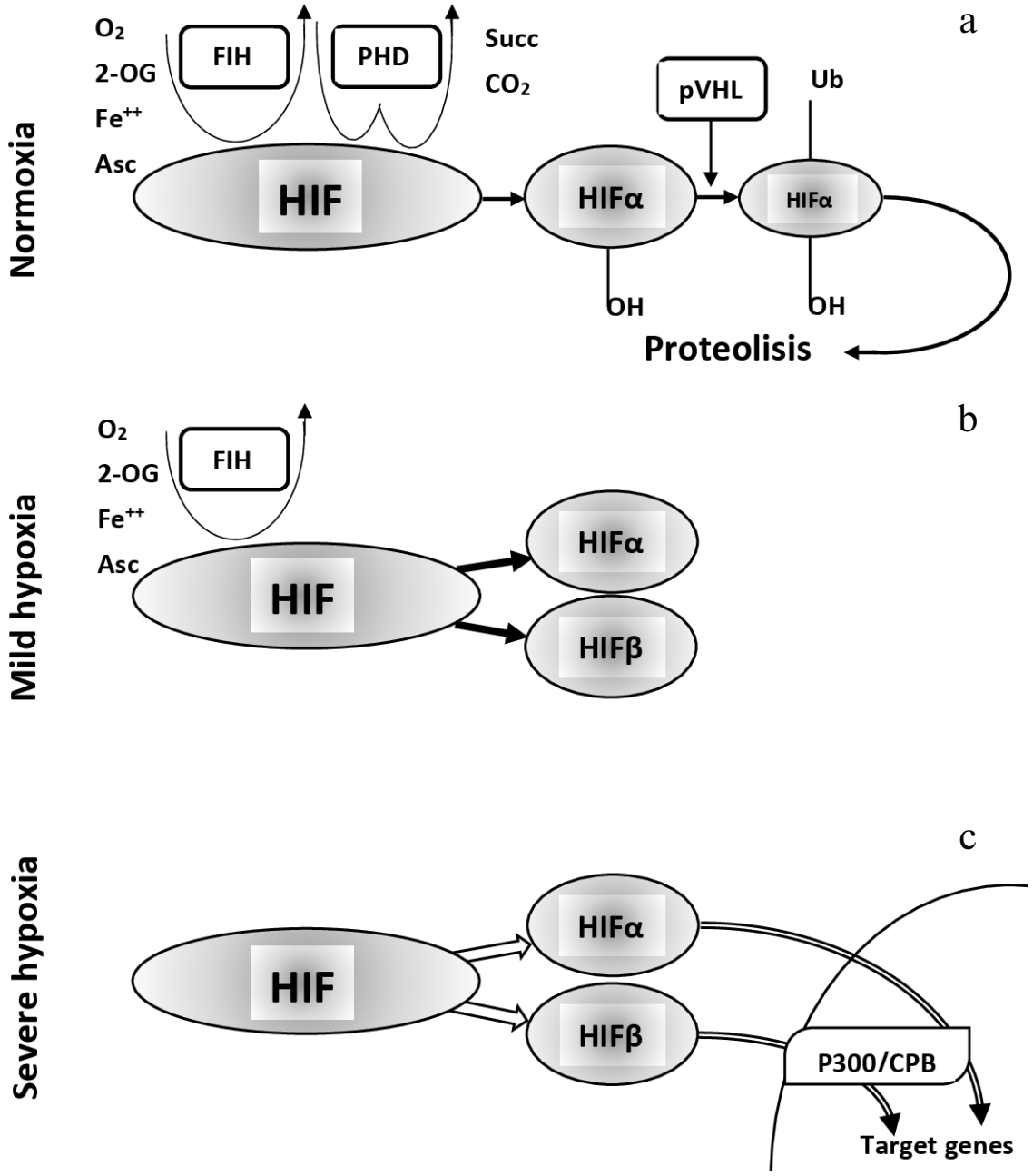

Fig. 3. Oxygen-sensitive pool of cytoplasmic metabolites involving HIF: a) normoxia; b) mild hypoxia; c) severe hypoxia. FIH, factor inhibiting HIF; 2-OG, 2-hydroxyglutarate; Asc, ascorbic acid; PHD, prolyl hydroxylase domain protein; Succ, succinic acid; pVHL, von Hippel–Lindau tumor suppression protein; Ub, ubiquitin; P300/CBP, transcription coactivator.

existence of a cluster of two hundred oxygen-sensitive genes that induce cascades of post-translational protein modifications. Hydroxylases are essential components of this cluster; among them, special attention of researchers has been drawn to prolyl hydroxylases [81, 93].

Under normoxic conditions, PHD enzymes associated with the translation of HIF molecules in the cytoplasm continuously hydroxylate proline in the α-subunit of the HIF heterodimeric molecule. The first hydroxylation occurs at position P564; the second – at P402, which results in the oxygen-dependent degradation of HIF-1α [91, 94]. Hence, the PHD enzymes are the closest (proximal) regulators of HIF activity [9]. This implies that HIF hydroxylases (including PHD enzymes) should be considered as cytoplasmic oxygen sensors, rather than HIF itself [93, 95].

The family of hypoxia-induced transcription factors (HIFs) is the central element of the oxygen concentration signaling pool. HIF is a heterodimeric protein consisting of the oxygen-sensitive HIF-α subunit and constitutive

HIF-β subunit [4, 96]. Higher multicellular organisms contain three types of HIF-α subunits: HIF-1α, HIF-2α, and HIF-3α. The first one is expressed in most, if not all, mammalian cells; HIF-2α and HIF-3α were found in some endothelial and connective tissues [80, 85, 97]. In addition to oxygen, the pool of HIF metabolites is affected by ROS, nitrogen oxide, HSP90, and other molecules with signaling properties [6, 10]. Below, we will discuss these regulatory processes in detail.

RELATIONSHIP BETWEEN MAIN MECHANISMS OF CELL OXYGEN SENSITIVITY

Mitochondrial, membrane, and cytoplasmic mechanisms of the oxygen homeostasis maintenance are component of cellular metabolism, and, hence, cannot avoid their mutual influence on each other [49, 54]. By 2008, Ward [54] has identified three plausible mechanisms explaining the impact of oxygen-sensitive processes in the mitochondria on the cell plasticity and energy metabolism. In the last years, an opinion has been formed that the main effect of mitochondria on the cytoplasmic and membrane mechanisms of oxygen sensitivity is realized mainly through ROS [4, 6, 8, 39].

The metabolic pool of cytoplasmic processes, in which HIF plays the role of master regulator, is shown in Figs. 1 and 3. ROS (H_2O_2) leave mitochondria (complex III), diffuse through the cytosol, and suppress the activity of prolyl hydroxylase-2 [41, 42]. This process intensifies with the hypoxia development. As a result, HIF degradation stops and HIF variants translocate to the nucleus, where they induce transcription of hypoxia-associated genes, resulting in the formation of the cellular response to hypoxia [43]. This mechanism cannot be fast; hence, the response to hypoxia involving HIF pool is effective only in the case of chronic oxygen deficiency.

In parallel to this, HIF influences oxidative phosphorylation in the mitochondria [80, 98], e.g., by affecting transcription of the pyruvate dehydrogenase kinase gene. This kinase phosphorylates the E1α subunit of pyruvate dehydrogenase, thus inactivating this enzymatic complex that converts pyruvate into acetyl coenzyme A. HIF can affect this process via either glucose deprivation or limited use of fatty acids. In the latter case, HIF-1 induces transcription of microRNA miR210, which, in turn, downregulates expression of the iron-sulfur cluster assembly scaffold proteins ISCU1/2. This results in the inhibition of tricarboxylic acid cycle, suppression of oxidative phosphorylation, and reduction of oxygen consumption by the mitochondria [99, 100]. Hence, during hypoxia development, HIF regulates ROS production by the feedback mechanism.

The HIF pool also affect the antioxidant potential of the cells. Under hypoxic conditions, HIF-1 and HIF-2 activate transcription of the respective genes, leading to the initiation of additional mitochondrial synthesis of glutathione, which is the main component of the antioxidant systems [101].

Hence, the HIF pool and mitochondrial metabolism are coupled via a feedback mechanism that ensures to a certain degree the maintenance of oxygen homeostasis in the cell. Two feedback loops connecting mitochondrial and HIF pools in the cell (prooxidant and antioxidant) counteract the action of each other (Fig. 4).

The interactions between the mitochondrial metabolic pool and membrane-associated mechanisms of oxygen homeostasis have been investigated to a lesser degree [4, 52, 102]. During rapidly developing hypoxia, complex I of the ETC increases production of ROS ($O_2^{\cdot-}$) [4, 103] that, in turn, inhibit conductance of calcium channels located in the cell plasma membrane [4]. An increase in the ROS concentration in the vicinity of the plasma membrane inner surface blocks the flow of potassium ions through the TASK channels [54]. BK_{Ca} channels close for the same reason [6]. Hence, it can be considered proven that under conditions of rapidly developing hypoxia, ROS inhibit the conductance of calcium and potassium channels located in the vicinity of mitochondria associated with these channels [102, 103]. Hence, the byproducts of mitochondrial metabolism facilitate cell depolarization by inhibiting several families of potassium channels under hypoxic conditions (Fig. 4).

Fine changes in the membrane-associated microdomains formed by ion channels and mitochondria are intensively studied. Interactions of the oxygen-sensitive membrane mechanisms and HIF pool in the cell cytoplasm also attract a lot of interest from the researchers.

There are only a few experimental studies on the signal exchange between the oxygen-sensitive plasma membrane channels and HIF pool. We failed to find any review on this topic. In 2006, Tajima et al. [104] reported the effect of HIF pool in human melanoma cells subjected to long-term hypoxia on the increase in the conductance of calcium-dependent potassium channels (K_{Ca}), which was a result of HIF-1α overexpression in response to the oxygen deficit in the culture medium. Later, similar effect was observed for the voltage-gated potassium channels (K_v) in rat pulmonary artery smooth muscle cells [105] and for TASK-2 potassium channels in mouse WEHI-231B cells [106].

It was also found that during prolonged hypoxia, HIF-2α represses genes responsible for the synthesis of the BK_{Ca} β_1 subunit [107].

Recently, direct effect of prolyl hydroxylases from the HIF pool on the cation channels of the TRP family (TRPA1 and TRPV3) has been reported. Under normoxic conditions, prolyl hydroxylases not only direct HIF to the proteasomal degradation but also inhibit the activity of TRPA1. This inhibition diminishes with the decrease in the oxygen partial pressure in the cytoplasm and ceases under hypoxic conditions [108, 109].

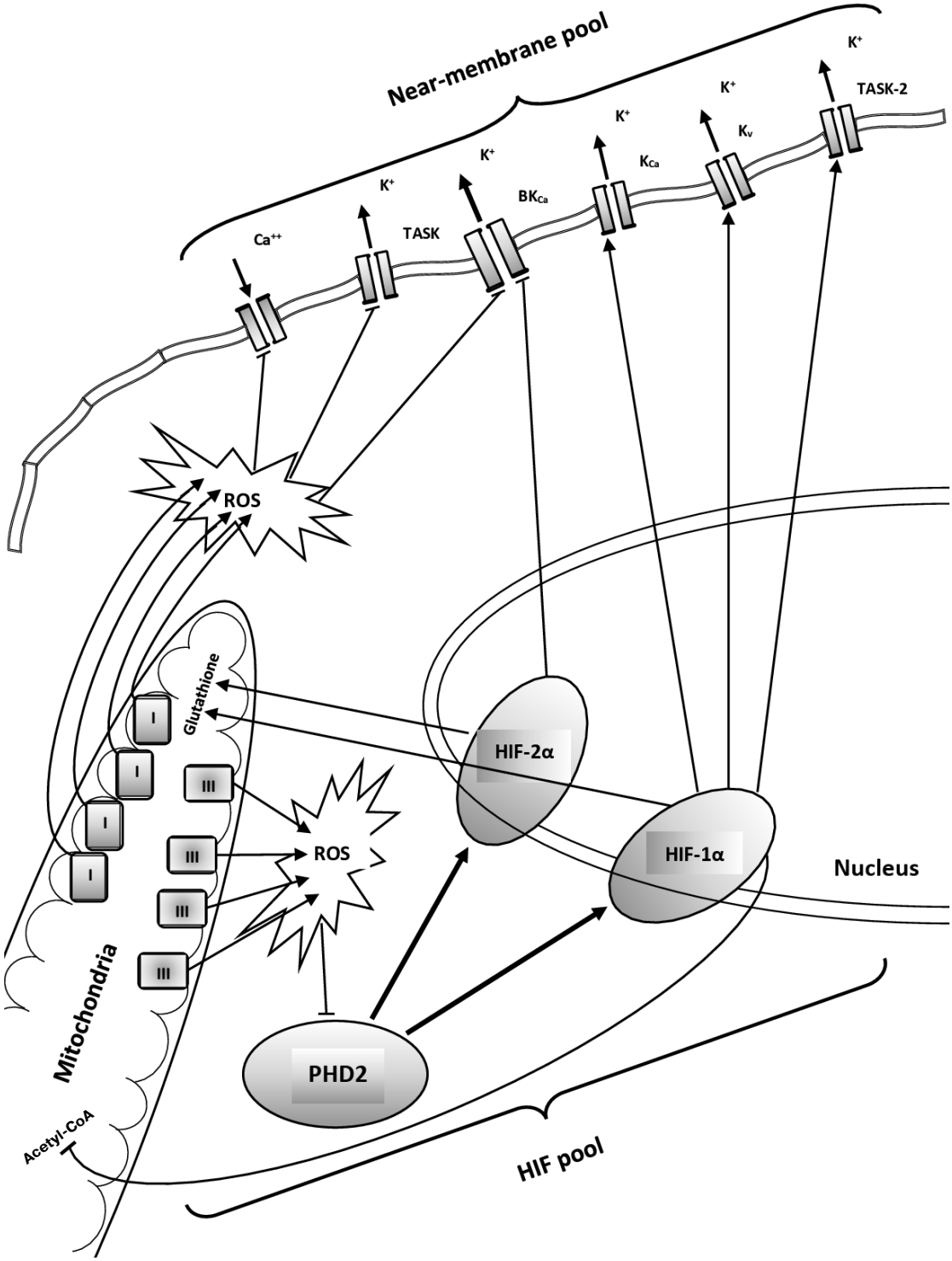

Fig. 4. Relationship between the mitochondrial metabolic pool, cytoplasmic HIF pool, and the near-membrane mechanisms of oxygen sensitivity. I and III, ETC complexes I and III, respectively; PHD2, prolyl hydroxylase domain protein 2.

The obtained data indicate the opposite effects (activating and inhibitory) of the cell HIF pool on the membrane-associated mechanisms of cell oxygen homeostasis regulation. Further studies are required to clarify the priorities of this mechanism [110].

No evidence of the reciprocal effect of the plasma membrane oxygen-sensitive potassium channels on the HIF pool has been found yet. The lack of such data could be explained by an enormous difference in the rate of response of ion channels (several seconds) and HIF pool

metabolism (tens of minutes) to the hypoxic stimuli. In this case, slower processes could affect the faster ones, but not *vice versa*.

Hence, the main mechanisms of cell oxygen homeostasis regulation (cytoplasmic and membrane) and the modulating effect of mitochondrial metabolism are closely interconnected and supplement each other during exposure to hypoxic stimuli of various modality.

Based on the data presented in the review, we can conclude that the cells of Metazoa have several mechanisms for sensing the intracellular oxygen content. In the case of rapidly developing hypoxia, oxygen shortage is detected by the near-membrane pool of potassium channels and associated proteins. In the case of chronic hypoxia, metabolic pool with HIF as a master regulator participates in the cell response to the oxygen concentration decrease. The influence of mitochondrial metabolisms on these two different mechanisms of oxygen sensing is limited to the modulating effects mediated by ROS. It must be emphasized that the discussed intracellular mechanisms are quite successful in responding to the oxygen deficit in the cell. Excessive (relative to the norm) concentrations of oxidants could be also found in cells during organism exposure to hyperoxia; however, their effects are realized via yet poorly understood mechanisms [111-113].

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