REVIEW SUMMARY

OXYGEN SENSING

Oxygen-sensing mechanisms across eukaryotic kingdoms and their roles in complex multicellularity

Emma U. Hammarlund*†, Emily Flashman, Sofie Mohlin, Francesco Licausi*†

BACKGROUND: Animals and land plants are the most diverse complex multicellular life-forms on Earth, and their success intricately links a capacity for adhering cells to perform different tasks at different times. The performance of cell tasks, however, can be both dependent on and challenged by oxygen. Oxygen acts as the final electron acceptor for aerobic respiration but also participates in reactions to generate metabolites and structural macromolecules; recently, oxygen also has come to the fore for its signaling role in developmental programs in animals and plants. Today, the relative oxygen concentration within multicellular organisms integrates information about cell position, metabolic state, and environmental conditions. For the rise of complex life, the capacity to link oxygen perception to transcriptional responses would have allowed organisms to attune cell fates to fluctuations in oxygen availability and metabolic needs in a spatiotemporal manner.

ADVANCES: Recent discoveries of oxygen-sensing mechanisms in different eukaryotic kingdoms allow us to compare molecular strategies dedicated to this task and the outputs that these produce. Remarkably, higher plants and animals converged, from a functional perspective, to recruit dioxygenase enzymes to posttranslationally modify transcriptional regulators for proteasomal degradation at the relatively "normoxic" conditions. In this way, transcriptional responses can be repressed at higher oxygen levels (which is context dependent) but are specifically elicited under hypoxia. The mitigation of the effects of prolonged hypoxia is also similar in animals and plants: reduction of metabolic rate, avoidance of toxicity of anaerobic by-products, and prevention of cell injury upon reoxygenation. Recent geological and phylogenetic investigations allow us to reconstruct the origin of such molecular switches in the eukaryotic clade and compare it with the development of organ-grade multicellularity. The results support the perspective that oxygen-consuming enzymes evolved sensory functions depending on the contingent requirements imposed by the environment and developmental programs. Considering that these sensing machineries evolved at a time (in the Neoproterozoic and early Paleozoic eras) when atmospheric oxygen concentrations were substantially lower than today, and in marine settings where redox is prone to vary, they may have played a major role in guiding development and homeostasis in response to endogenous oxygen dynamics. The broad scope of oxygen sensing and response machineries for multicellular success is further highlighted when hijacked during tumorigenesis to support uncontrolled growth in a variety of conditions and stresses.

OUTLOOK: The broad role of oxygen-sensing systems in the survival and evolution of complex multicellular life requires further exploration, including into the commonality and conservation of the oxygen-sensing machineries. That higher plants and animals adopted alternative solutions to direct their primary hypoxia responses, despite their ancestors likely being equipped with the same enzymatic repertoire, may describe differences in their respective environmental, cellular, and organismal features and histories. Broadly, by shifting focus from exploring oxygen-sensing mechanisms as primarily a response to oxygen shortage for aerobic respiration, we can potentially reveal previously unidentified ways in which these systems can be manipulated for clinical and agricultural benefit. By such an approach, we will gain further insight to their broad scope and the challenges that multicellular life is exposed to, today as in geologic history. ■

The list of author affiliations is available in the full article online.
*These authors contributed equally to this work.
†Corresponding author. Email: emma.hammarlund@med.lu.se (E.U.H.); francesco.licausi@plants.ox.ac.uk (F.L.)
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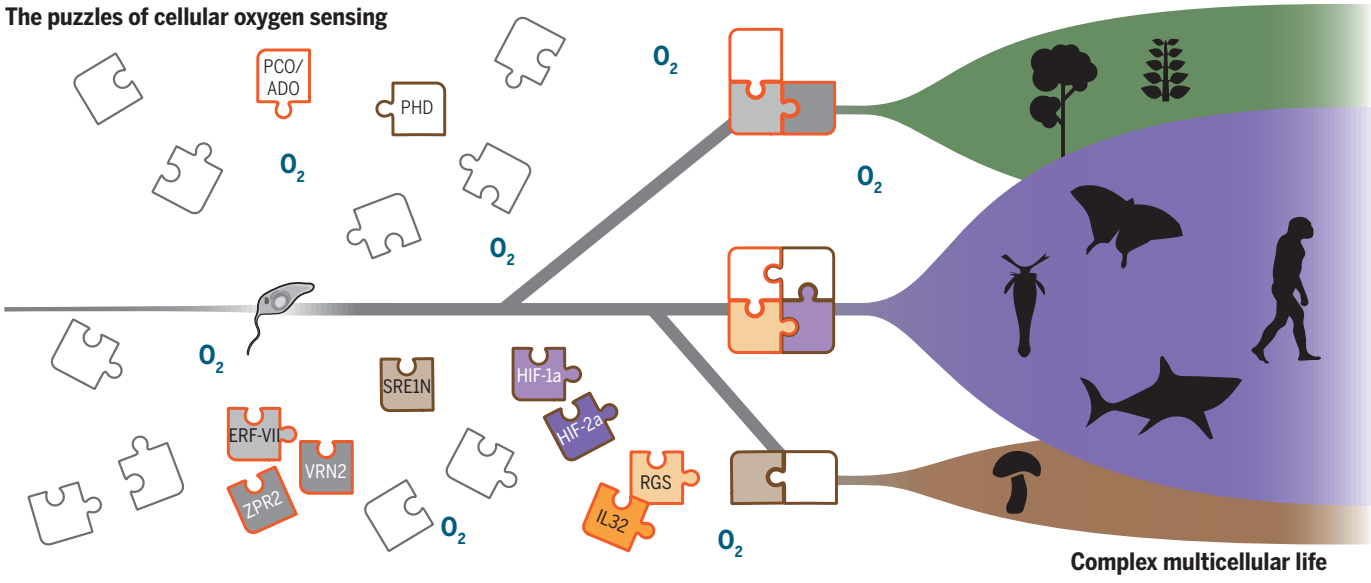

Eukaryotic kingdoms convergently recruited dioxygenases to sense fluctuations in ambient oxygen and to respond under hypoxia. Oxygen sensing allows cells to attune their metabolism and fate to spatiotemporal requirements, a critical component in complex multicellularity. The basal oxygen-sensing mechanisms use alternative targets in plants, fungi, and animals—kingdoms that alone demonstrate the capacity to form tissues of different complexities.

REVIEW

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Oxygen-sensing mechanisms across eukaryotic kingdoms and their roles in complex multicellularity

Emma U. Hammarlund[1,2,3]*†, Emily Flashman[4], Sofie Mohlin[1,5], Francesco Licausi[6,7,8]*†

Oxygen-sensing mechanisms of eukaryotic multicellular organisms coordinate hypoxic cellular responses in a spatiotemporal manner. Although this capacity partly allows animals and plants to acutely adapt to oxygen deprivation, its functional and historical roots in hypoxia emphasize a broader evolutionary role. For multicellular life-forms that persist in settings with variable oxygen concentrations, the capacity to perceive and modulate responses in and between cells is pivotal. Animals and higher plants represent the most complex life-forms that ever diversified on Earth, and their oxygen-sensing mechanisms demonstrate convergent evolution from a functional perspective. Exploring oxygen-sensing mechanisms across eukaryotic kingdoms can inform us on biological innovations to harness ever-changing oxygen availability at the dawn of complex life and its utilization for their organismal development.

The rise of Earth's most complex and sizable life-forms—animals and land plants—remains enigmatic. Out of all of life's diversity, only animals and land plants have multiple organs such as a brain and lungs or roots and leaves. Animals and plants therefore represent two distinctly successful versions of complex multicellularity, but the inferred causes for their success are opposing. Although the rise of animals is commonly explained as a result of environmental change (increased oxygen) that unleashed the full potential of biological innovations (*1*, *2*), the rise of plants is explained with biological innovations unleashing a capacity to live with environmental change (for example, in aquatic or terrestrial environments) (*3*). However, recent and transdisciplinary insights demonstrate that animals and land plants share a particularly versatile capacity to perceive and respond to fluctuating oxygen conditions (*4*, *5*). Here, we propose that the acquisition of the capacity to perceive and respond to the variable presence of oxygen must have been central to the rise of complex life. To evaluate this hypothesis, we consider two worlds in parallel, and bridge their information: the modern world, in which an oxygen-sensing capacity provides key functions to animals and plants, and the historic one, in which neither oxygen-sensing mechanisms nor complex multicellular organisms were yet fully in place.

Free oxygen profoundly affects eukaryotic cells. On the one hand, molecular oxygen acts as a terminal electron acceptor that yields unprecedented energy during aerobic respiration and builds metabolites. On the other hand, reactive chemical species that contain oxygen can change the configuration and function of nucleic acids, sugars, lipids, proteins, and metabolites. The paramount impact that fluctuating oxygen availability has for cell function and constitution makes the capacity to perceive oxygen vitally important for any eukaryotic organism, especially when the organism is multicellular. Complex multicellular organisms are defined by their persistent three-dimensional organization, in which adhering cells can perform different tasks of labor at different times (*6*–*8*). Cell clustering per se represents a state that buffers environmental chemical fluctuations and stabilizes internal gradients. However, internal oxygen gradients also change dynamically as a function of cell respiration. Cells will therefore experience different oxygen availability depending on both their spatial and temporal (spatiotemporal) position. These fluctuations in dynamic internal oxygen gradients combined with oxygen's power to affect cell functions therefore make the capacity to perceive oxygen an adaptation with considerable but yet underappreciated scope. If the capacity to sense oxygen is combined with specific responses to different oxygen concentrations, it would also facilitate spatiotemporal induction of different cellular functions.

Oxygen sensing is the ability by which modern organisms detect changes in the amount of oxygen within and between cells, coupled to a context-dependent response. As of today, oxygen sensing is commonly described as the acute response to oxygen concentrations below the respiratory requirements (hypoxia) of the host. This allows tissue homeostasis when, for example, a muscle experiences oxygen depletion during a fast run or when a root's access to oxygen is blocked by waterlogging. However, although the necessity of an acute response to hypoxia makes sense to us humans, as obligate aerobes, the normalcy of hypoxia offers another perspective: Oxygen levels below the ambient concentration can be argued to be normal for certain tissues in plants (*9*) and most tissues in animals (*10*–*12*). Hypoxia also prevailed globally at the time in Earth history when oxygen sensing evolved, with atmospheric oxygen concentrations presumably below ~5% (*13*, *14*). The hypoxia-response machineries reach beyond coping with hypoxia to coordinate different cell fates (future identities and tasks) in accordance with—and despite—oxygen availability and fluctuations.

Here, we present a broad look at oxygen-sensing mechanisms across eukaryotic kingdoms and time, to place their role within the context of evolving complex multicellularity (Box 1). We describe the rarity of complex multicellularity over the history of life, the prevalence of fluctuating environmental oxygen conditions, and the necessity to perceive these fluctuations. We then review the different known oxygen-sensing mechanisms and their roles for modern forms of multicellularity, discussing the conceptual gaps that present opportunities to explore the hierarchical order, evolution, and impacts of cellular oxygen sensing.

The historic arena: Hypoxic, variable, and largely devoid of multicellularity

Complex multicellular organisms are rare in the long history of life when compared with the diversity of unicellular organisms. The diversity of unicellular prokaryotes (Archaea and Bacteria) and eukaryotes (protists in the broad sense, including Protozoa, Chromista, and Archezoa) is estimated to supersede the collective phylogenetic diversity of animals, plants, and fungi by at least an order of magnitude (*15*, *16*). The degree of organismal complexity can also be compared by the diversity of cell types that make up tissues. With that view, vascular plants and particularly animals are by far more complex than all other known organisms (*17*–*19*). Simple multicellularity is an aggregation of cells where spatiotemporal coordination of labor is lacking, and this has evolved independently multiple times (*20*). Complex multicellularity, however, has diversified only six times across geologic history: three within the plant kingdom (red algae, brown algae, and land plants), twice in the kingdom of fungi, and once as the animal kingdom (*7*). Out of these events, only animals and land plants (Embryophyta) form organ

[1]Translational Cancer Research, Department of Laboratory Medicine, Lund University, Scheelevägen 8, 223 81 Lund, Sweden. [2]Nordic Center for Earth Evolution, University of Southern Denmark, Campusvej 55, 5230 Odense M, Denmark. [3]Department of Geology, Lund University, Sölvegatan 12, 223 62 Lund, Sweden. [4]Chemistry Research Laboratory, University of Oxford, Mansfield Road, Oxford OX1 3TA, UK. [5]Division of Pediatrics, Department of Clinical Sciences, Lund University, 221 00 Lund, Sweden. [6]Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RB, UK. [7]PlantLab, Institute of Life Sciences, Scuola Superiore, Sant'Anna, 56124 Pisa, Italy. [8]Department of Biology, University of Pisa, Pisa, Italy.
*These authors contributed equally to this work.
†Corresponding author. Email: emma.hammarlund@med.lu.se (E.U.H.); francesco.licausi@plants.ox.ac.uk (F.L.)

Box 1. Glossary.

Cell fate: The future identity of a cell (or its daughter cells) and the accompanying phenotype or task to perform within its tissue and context.
Enzymatic proteolysis: Breakdown of proteins into peptides by the action of proteases, often organized in complexes, such as the proteasome. In cells, proteolysis is often directed by cascades of posttranslational modifications, including ubiquitination, that label a protein for degradation.
Enzymes: Proteins that can catalyze a chemical reaction (biocatalyst) and thus offer a kinetic potential to chemical reactions. Oxygen-dependent enzymes discussed in this review include 2-OG–dependent dioxygenases, thiol dioxygenases, PCOs, and ADO.
Fe(II)/2-oxoglutarate (2-OG)–dependent dioxygenase: Oxidoreductase enzymes that catalyze incorporation of oxygen atoms into a variety of substrates. 2-oxoglutarate is concomitantly converted to succinate and CO_2.
Thiol dioxygenases: Fe(II)-dependent enzymes that catalyze the oxygen-dependent oxidation of free thiols (-SH) to sulfinic acid ($-SO_2H$).
Plant cysteine oxidases (PCOs): A group of thiol dioxygenases that catalyze dioxygenation of cysteinyl (Cys) residues at the N termini of substrate proteins, such as the ERF-VII TFs.
2-Aminoethanethiol dioxygenase (ADO): A thiol dioxygenase that regulates stability of N-terminal Cys-initiating proteins IL32 and RGS4 and -5 in humans through Cys dioxygenation and the N-degron pathway.
Eukaryotic kingdoms: Protista, Plantae, Animalia (Metazoa), and Fungi.
Eumetazoa: A basal animal clade and sister group to Porifera (sponges). Eumetazoans have either radial (for example, cnidaria) or bilateral symmetry (invertebrates or vertebrates).
Hypoxia-response machinery: Cellular system that consists of one component that perceives a decrease in oxygen availability (such as an enzyme) and one that induces a response (such as a TF) to trigger cellular adaptation.
Oxygen sensing: The ability to detect changes in the amount of oxygen and mount an adaptive response.
Redox: Chemical reactions in which the oxidation states of atoms change.
Stemness: Cell ability of self-renewal through division and differentiation into specialized cell types.
Spatiotemporal division of cell fate: When cells in an organ perform different functions at the same time in a coordinated manner.
Transcription factor (TF): A protein that controls the rate of transcription of genetic information from DNA to mRNA. They bind to DNA in a sequence-specific manner. The main TFs discussed in this Review are HIFs and ERF-VIIs.
Hypoxia inducible factors (HIFs): Members of the basic helix-loop-helix (bHLH) family, consisting of an α subunit and a β unit (ARNT). Generally, the HIFs are constitutively expressed, but their α subunit is degraded via Fe(II)/2-OG–dependent oxygenases in the presence of oxygen.
Group VII ethylene response factors (ERF-VIIs): Cys-initiating members of the ERF/APETALA2 (ERF/AP2) family. Some ERF-VIIs are constitutively expressed but degraded through the activity of PCOs in the presence of oxygen.
Viridiplantae: Green plants, consisting of the clades Chlorophyta and Streptophyta, under which land plants (Embryophyta) and vascular plants (Tracheophyta) are subdivisions. A subdivision of vascular plants is flowering plants (angiosperms).

systems. Although the ages of the first or last common eukaryotic ancestor as well as when eukaryotic kingdoms diverged are debated, the diversification of eukaryotes is considered late by most. Records of molecular clock estimates and microfossils suggest that it took at least a billion years before the diversification of the animal and plant (Viridiplantae and Streptophyta) kingdoms began some 0.8 billion years (Ga) ago in the Cryogenian Period (*21*, *22*). Thereafter, animal diversity "exploded" in the Cambrian and Ordovician periods (0.54 to 0.44 Ma ago), which was also when land plants (Embryophyta) and vascular plants (Tracheophyta) originated (Fig. 1) (*3*, *21*). Thus, organ-grade complex multicellularity evolved only twice, both relatively late in Earth history. The rarity of successful transitions from unicellular eukaryotes to complex multicellular life suggests that cellular and environmental components necessary to facilitate persistent and complex multicellularity are difficult to align. One environmental component, and proposed cause for the rise of animal diversity in particular, is how environments would have become permissive through the inferred increase in free oxygen (*1*, *2*, *23*). However, the increased ability to sense fluctuations of free oxygen can also be inferred as a biological component and cause of the rise of diversity of animals as well as in plants.

Free oxygen began to build up in the atmosphere about halfway through Earth's 4.6-Ga history, instigated by the cyanobacterial capacity for oxidative photosynthesis. The signs of free atmospheric oxygen are visible in the rock record at ~2.45 Ga ago, through indirect geochemical evidence (*24*). Before this, however, free oxygen was produced and seemingly present in marine shallow (shelf) settings (*25*) at the trace concentrations that allow biosynthesis of steroids (*26*). After this, and for more than the following 2 Ga, atmospheric oxygen concentrations were likely predominantly as low as what oceanographers call severely hypoxic (<2%) or hypoxic [<5 to 7%, albeit this is context dependent (*27*)] (*14*). Over the Cambrian and Ordovician periods, when animals diversified and vascular plants originated, geochemical reconstructions estimate that the atmosphere had 2 to 5% or, at most, 10% oxygen (*13*, *14*, *28*, *29*). Not until ~150 Ma after the rise of animals and plants, in the Silurian or Devonian periods, did global oxygenation approach modern levels (Fig. 1) (*30*). Thus, conditions at the time when animals and plants and their oxygen-sensing mechanisms originated and diversified can be considered hypoxic by today's standards.

Variability of oxygen concentrations is a physical imperative on Earth's surface. Even today, when the atmosphere is richly oxygenated (21% O_2), oxygen levels vary dramatically both in soil and within the ocean. Respiration of biomass may consume oxygen faster than it is replenished, whether in soil or water (in water, gas diffusion is four orders of magnitude slower than in air). Animals and green plants evolved in the ocean, where production and respiration of biomass together with physical mixing, such as from winds and waves, result in constantly variable environmental oxygen conditions. The long history of oxygen fluctuations in shallow marine niches would have posed a ceaseless challenge to nascent multicellular organisms with limited capacity to perceive and respond to these variations. Thus, both the challenges and opportunities for eukaryotic life to intermittently encounter free oxygen attributes an evolutionary importance to the cellular mechanisms that perceive it. Before the development of cellular mechanisms to perceive and to orchestrate organismal responses to changes in oxygen conditions, complex multicellular life would have struggled to sustain in niches with fluctuating conditions on Earth's surface (*31*).

The past and present of oxygen sensing

Oxygen sensing acts as a transducer of hypoxic signaling, which is best illustrated by the primary hypoxia-response machineries in plants and animals. These machineries function through the action of oxygen-dependent enzymes that repress the operation of transcription factors (TFs). In an oxygen-dependent reaction, these enzymes catalyze a posttranslational modification of the TF that reduces its stability (*4*, *5*). Oxic conditions therefore lead to degradation

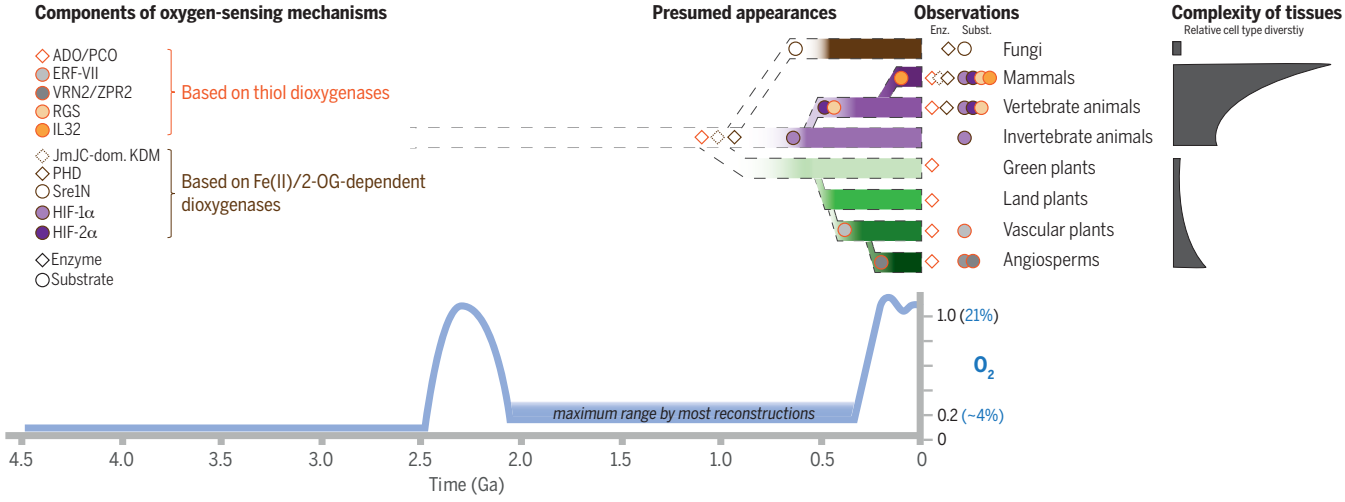

Fig. 1. Increasing complexity of oxygen-sensing mechanisms and the extent of complexity within multicellular organisms over Earth's history of 4.6 Ga. Enzymes (diamonds) and substrates (circles) form components of oxygen-sensing mechanisms, based on thiol dioxygenases (orange outlines) and Fe(II)/2-OG–dependent dioxygenases (brown outlines). We depict the presumed appearance of the oxygen-sensing components during the divergence of the eukaryotic animal, plant, and fungi kingdoms (dashed lineages). We depict the onset of the respective diversifications of fungi (Basidiomycota and Ascomycota) with differentiated tissues (brown) (*138*); invertebrate animals, vertebrates, and mammals (purple) (*23*); and green, land, and vascular plants (green) (*3*). Observations of enzymes (Enz.) and substrates (Subst.) for each group of organisms include when found in sequences, when determined to have an oxygen-sensing role, or both. Complexity of tissues for each group of organisms is represented by their maximum number of different cell types (diversity) (*6*, *17*, *19*). Reconstructions of atmospheric oxygen levels in the past, which constrain ranges of min-max oxygenation, agree upon a maximum oxygenation of ~0.2 of modern levels (<~4% oxygen) for the Mesoproterozoic Era (1.6 to 1.0 Ga ago) and Neoproterozoic Era (1.0 to 0.6 Ga ago) (thick blue field) (*14*, *139*). The maximum oxygenation of ~4% is presumed for the time interval when eukaryotic kingdoms diversified (0.8 to 0.5 Ga ago) (*21*, *22*), meaning that the evolution of oxygen-sensing mechanisms is rooted in hypoxic conditions. Geochemical indications and modeling efforts indicate that high atmospheric oxygen concentrations, as today, persisted at 2.5 to 2.0 Ga ago and then from 0.4 Ga ago (the Devonian Period) onward (*13*, *14*, *24*, *28*, *30*, *139*).

of these TFs and, hence, inactivity of the hypoxic responses. In hypoxic conditions, however, reduced activity of the oxygen-sensing enzymes allows stabilization of the TFs, which direct the response to hypoxia by up-regulating a suite of genes that trigger adaptation. Enzymes are particularly suited to act as sensors because their rate of activity is proportional to the amount of substrate available—they can elicit a graded response to oxygen.

Sensing through Fe(II)/2-OG–dependent oxygenases

The first identified and most characterized hypoxia-response machinery is the system of hypoxia-inducible factors (HIFs) in animals, the discovery of which was recognized with the 2019 Nobel Prize in Physiology or Medicine (*32*). HIFs are heterodimers that consist of α subunits and the aryl hydrocarbon receptor nuclear translocator (ARNT), or β subunit. HIF-α subunits are stabilized at hypoxia, leading to the transcription of hundreds of genes that promote adaptive responses. In physiologically oxic conditions, however, the oxygen-dependent prolyl hydroxylase (PHD) enzymes catalyze hydroxylation of specific prolyl residues in HIF-α proteins that enable their recognition by ubiquitin ligase complexes [commonly the von Hippel Lindau (VHL) protein] and subsequent degradation through the proteasome (*33*). A second hydroxylase enzyme [factor-inhibiting HIF (FIH)] catalyzes hydroxylation of an asparagine (Asn) residue in HIF-α to reduce the transactivation capacity of HIFs. The PHDs and FIH are Fe(II), 2-oxoglutarate (2-OG), and oxygen-dependent enzymes, whose rate of activity is sensitive to oxygen availability, particularly the PHDs (*34*–*36*). This means that even a small decrease in oxygen availability can potentially result in a reduction in HIF hydroxylation to enable HIF stabilization and activation of its transcriptional response (Fig. 2). Although PHD-like enzymes are conserved even in bacteria (*37*) and fill an oxygen-dependent regulatory role in yeast (*38*), their oxygen-sensing function appears refined with the involvement of HIF and the ubiquitin-proteasome system (*39*). All eumetazoans (animals with bilateral and radial symmetry) except the ctenophores possess the HIF-1α subunit, whereas only vertebrate animals possess the HIF-2α subunit (*40*, *41*).

Eukaryotes and prokaryotes involve Fe(II)/2-OG–dependent dioxygenases in a number of important biological functions (*42*). Although the catalytic rate of all these enzymes depends on oxygen availability, whether or not these enzymes can act as oxygen sensors in the hypoxia-response machinery depends on two factors: (i) whether the impact of their activity is transduced through their substrates to induce a response and (ii) whether their rate of activity is limited by the range of oxygen concentrations present in the cell. Despite overall conservation of enzyme structure and catalytic mechanism, different Fe(II)/2-OG–dependent dioxygenases are rate-limited by oxygen at different concentrations. The PHDs are rate-limited at relatively high oxygen concentrations. FIH activity can, however, tolerate mild hypoxia for HIF Asn-hydroxylation and even more severe hypoxia for non-HIF substrates (*43*). Other members of this enzyme family are restricted only at very low oxygen concentrations to initiate an adaptive response at severe hypoxia. For example, ten-eleven translocation (TET) DNA demethylases only lose their activity in severely hypoxic tumors, leading to DNA hypermethylation (*44*).

A subset of Fe(II)/2-OG–dependent oxygenase enzymes, the Jumonji C (JmJC) domain–containing histone lysine demethylases (KDMs), has been reported to demonstrate an oxygen-sensing role across a broader range of oxygen concentrations. The status of histone methylation can affect chromatin packing and transcriptional responses by regulating access of TFs to promoter regions. Recent studies have connected the oxygen sensitivity of some KDMs with

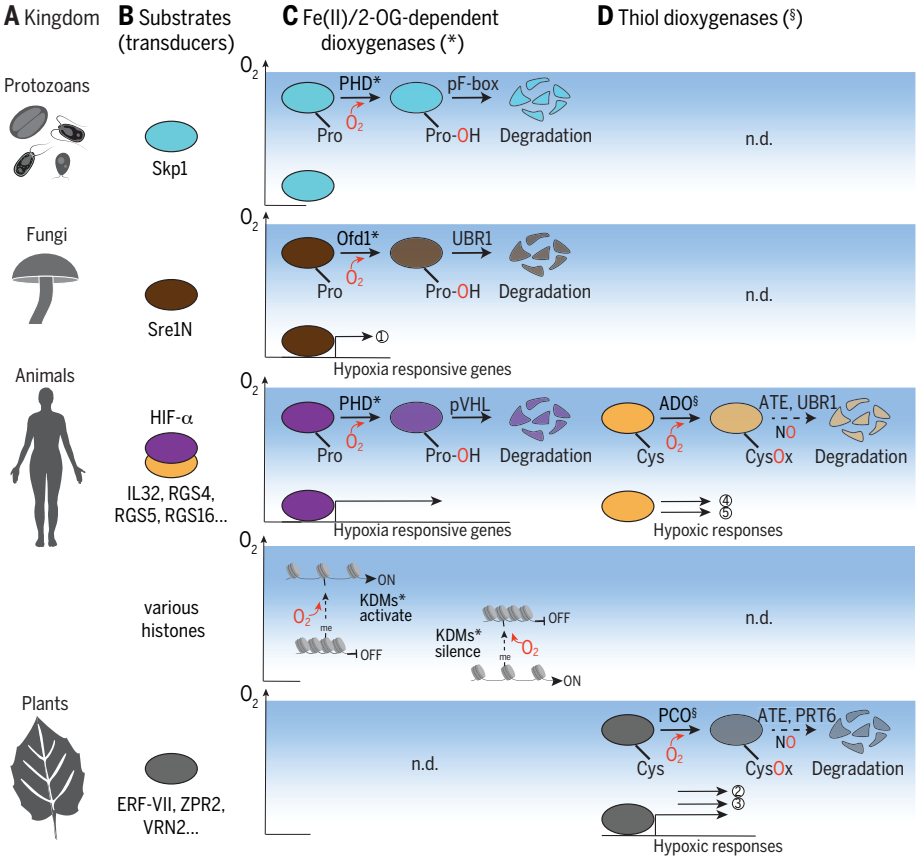

Fig. 2. Direct mechanisms for oxygen sensing and hypoxic signaling. (**A**) Complex multicellular organisms within the eukaryotic kingdoms. Shown are protozoa, fungi, vascular plants, and animals. (**B**) The transducers of hypoxia-response machineries. Shown are the Pro-containing proteins that may be hydroxylated and degraded in the presence of oxygen (blue field) or stabilized by hypoxia (white fields): Skp1; sterol regulatory element-binding protein TF (Sre1N) and HIF; or the Cys-initiating proteins ERF-VII, ZPR2, VRN2, IL32, and RGS4 and -5. Histone (de)methylation can be also modulated in an oxygen-dependent manner in eukaryotes. (**C**) The sensory components based on Fe(II)/2-OG–dependent dioxygenases (*) are PHDs or Ofd1, or JmJC-domain–containing KDMs. (**D**) The sensory components based on thiol dioxygenases (§) are PCO and ADO. By contrast, proteolysis (involving also proteins such as pF-box, UBR1, ATE, PRT6, and pVHL proteins and NO) and demethylation occur at relatively high oxygen concentrations (blue shading). Cellular responses at hypoxic conditions (hypoxic responses) are context and substrate dependent: (1) When the stabilized protein is a TF (Sre1N, ERF-VII, and HIF-α), hypoxia-responsive genes are induced, and the hypoxia responses are of different scopes (length of black arrows). Also, (2) of the PCO substrates in plants VRN2 regulates chromatin condensation, whereas (3) ZPR2 controls activity of TFs. Of the ADO substrates in animals, (4) RGS4 and -5 control G protein signaling, and (5) IL32 controls inflammation by interacting with an unknown receptor. In animals, demethylation by the JmJC-domain–containing KDMs can both activate and silence gene expression in an oxygen-dependent manner.

altered histone methylation status in hypoxia (*45*–*47*). Of these, KDM5A and KDM6A play a role in cell differentiation and fate restriction and have implications for tumorigenesis (*48*).

In yeast and protozoa, certain Fe(II)/2-OG dioxygenases also play oxygen-sensing roles. In fission yeast, a sterol regulatory element-binding protein TF (Sre1) promotes adaptation to hypoxic conditions, and the activity of Sre1 is controlled by an oxygen-sensitive Fe(II)/2-OG–dependent dioxygenase, Ofd1 (*49*). This mechanism controls cholesterol synthesis and uptake in yeast (as does its homolog in animals) (*50*). In protozoa, oxygen-sensing PHDs catalyze hydroxylation of S-phase kinase-associated protein 1 (Skp1), an essential subunit of a ubiquitin-ligase complex. This hydroxylation transduces the oxygen-dependent regulation of different developmental stages in the protozoan life cycle. At these stages, certain oxygen concentrations work as environmental triggers and correspond to concentrations at which the enzymes are rate limited (*51*, *52*). This rate limitation suggests that the oxygen sensitivity of these reactions has been fine-tuned and advantageous during evolution. The function of this oxygen sensitivity is clear from slime molds (Mycetozoa, normally solitary amoebae), for which it facilitates regulation of cells of different differentiation states during the formation of their multicellular fruiting bodies (*53*).

Prokaryotes also have a variety of Fe(II)/2-OG–dependent oxygenases with a wide range of roles, including in protein translation (*37*, *54*). Broadly, none of these has yet been reported as being highly sensitive to oxygen (except for a thermophilic ribosomal oxygenase under high-temperature conditions) (*55*). Bacterial oxygen sensing is instead achieved with different mechanisms that involve either conformational change of a DNA-binding protein upon oxygen binding or phosphorylation cascades that result in transcriptional upregulation in hypoxia. Bacterial oxygen sensing is described in detail in several reviews [for example, (*56*)].

Sensing through thiol dioxygenases and the Arg branch of the N-degron pathway

Vascular plants exploit a different hypoxia-response machinery, albeit with features in common with the HIF system of eumetazoans. With these, constitutively expressed TFs belonging to the group VII of the ethylene response factor family (ERF-VIIs) are stabilized in hypoxia to enable transcription of a suite of genes that promote adaptive responses (*57*, *58*). In physiologically oxic settings, the ERF-VIIs are degraded via the Arg/N-degron pathway, a process of degradation signaling in which the identity at the N terminus of a protein dictates its stability (*59*, *60*). Plant cysteine oxidases (PCOs) catalyze dioxygenation of cysteinyl (Cys) residues at N termini (Nt) of the ERF-VII TFs (*61*, *62*), which are subsequently arginylated by arginyl-transferases (ATEs) and then presumably recognized by the ubiquitin ligase proteolysis 6 (PRT6) (*63*). Basal nitric oxide (NO) levels are also required for this process (*64*). This recognition leads to the degradation of the ERF-VIIs. So, in plants, PCOs act as sensors, whereas the ERF-VIIs transduce the hypoxic signal into a response.

PCOs are Fe(II)-dependent thiol dioxygenases whose rate of activity with respect to ERF-VII oxidation is sensitive to oxygen availability (*65*), similar to the metazoan PHDs involved in HIF regulation. Apparently, these two hypoxia-response mechanisms have evolved separately but fulfill similar roles. Unlike the HIF hydroxylases, for which activity toward non-HIF substrates is uncertain (*66*), the PCOs appear to have multiple substrates, including little zipper protein 2 (ZPR2) and vernalization 2 (VRN2) (*57*, *67*). There are therefore several "response" components controlled by the oxygen-sensing PCOs. This means that a hypoxic response can be transduced through several pathways, depending on the cellular context. Although the degree of oxygen sensitivity of the PCOs toward these and other alternative

substrates is yet unreported, the hypoxia dependence of this function is clear. ERF-VIIs first appeared in vascular plants, and ZPR2 and VRN2 became Cys-initiating proteins in flowering plants (angiosperms) (*68*, *69*). On the other hand, the enzymatic asset of the Arg/N-degron pathway and PCOs required to operate oxygen-dependent degradation of Nt-Cys-degrons can be traced back to unicellular eukaryotic ancestors of plants and animals (*69*). Thus, it can be hypothesized that other Cys-initiating proteins control the hypoxia response in lower plants. In this perspective, the identification and characterization of Cys-initiating TFs is of particular interest.

Intriguingly from an evolutionary perspective, a human homolog of the PCOs, the enzyme 2-aminoethanethiol dioxygenase (ADO), was recently identified as regulating the stability of certain N-terminal Cys-initiating proteins in humans [interleukin-32 (IL32) and regulator of G protein signaling 4 and 5 (RGS4/5)] through the Arg/N-degron pathway (*70*). ADO acts as a separate human oxygen sensor by means of an equivalent mechanism to that of the PCOs in plants. ADO activity toward RGS4/5 is particularly oxygen sensitive, with a rate dependence close to that of the PHDs (*36*). So far, no Nt-Cys-degron TF has been identified as an ADO substrate; thus, the response component of this machinery does not amplify the transduction of the hypoxic signal similarly to HIF or ERF-VIIs. Nevertheless, the commonality in oxygen-dependent proteolysis (degradation) mediated by thiol-dioxygenases in plants and animals is striking and may suggest the existence of an ancestral mechanism in early eukaryotes.

These observations hint at both convergence and divergence of oxygen-sensing machineries in complex multicellular eukaryotes. On the one hand, both metazoans and vascular plants converged to the aerobic degradation of constitutively expressed transcriptional regulators. The key sensory dioxygenases and protein substrates differ, but the proteostatic logic that enables the activation of adaptive responses is very similar. On the other hand, the presence of both enzymes in the plant and animal kingdoms indicate a preference toward either system, possibly to accommodate specific developmental, physiologic, or metabolic requirements. In the evolutionary perspective of oxygen perception, it is remarkable that plant and animal species share few conserved mechanisms when compared with the high diversity displayed in bacteria, archaea, and fungi (*56*, *71*).

The power of hypoxia-response machineries

The roles of oxygen-sensing mechanisms have been explored at the cellular, individual, and evolutionary levels, often under the assumption that hypoxia is a "stress." Here, however, we evaluate whether the adaptations provided by oxygen sensing allow cells and individuals to cope with fluctuations and internal gradients in oxygen availability on both temporal and evolutionary time scales. We also consider their capacity for spatiotemporal coordination of cell labor and fates.

Guarding against oxygen fluctuations—homeostasis

Oxygen concentrations perpetually fluctuate in Earth's surface environments as a function of consumption, diffusion, and resupply. Similarly, oxygen concentrations fluctuate within and outside of organisms, tissue, and cells. When oxygen concentrations are temporarily lower than the organism's respiratory requirements, responses act to maintain homeostasis through reversal or mitigation. Homeostatic responses to hypoxia typically involve mRNA reprogramming, which represses energetically expensive pathways and up-regulates those associated with adaptation or avoidance (*72*–*74*).

Temporal oxygen deficit for metabolic reactions requires activation of alternative pathways that minimize oxygen consumption (*75*, *76*) but also may induce the activity of essential enzymes that use oxygen as a substrate (*77*, *78*). When severe hypoxia shifts sugar metabolism toward substrate-level phosphorylation at the expense of the oxidative pathway, this is achieved by facilitating carbon entry into the glycolytic pathway (*74*), putting on the brakes to pyruvate channeling into the tricarboxylic acid (TCA) cycle and redirecting it to fermentative reduction. Albeit different in eukaryotic kingdoms, these ancillary reactions sustain the carbon flux through glycolysis by the regeneration of oxidized nicotinamide adenine dinucleotide (NAD^+) and, concomitantly, prevent the inhibition of glycolysis by its own products. The majority of animals as well as some fungi reduce pyruvate to lactate by means of hypoxia-inducible lactate dehydrogenase (LDH) (*79*), whereas yeasts rely exclusively on alcohol fermentation by means of a two-reaction pathway that decarboxylates pyruvate and reduces the resulting acetaldehyde (*80*). In Viridiplantae, both metabolic strategies of reducing pyruvate, either from LDH or through alcohol fermentation, are activated under hypoxia, with additional contribution of formate, hydrogen, acetate, and alanine synthetic pathways (*81*, *82*). Higher plants evolved toward a preference for ethanol fermentation because lactic acid deprotonation contributes to cytosolic acidosis and thus jeopardizes cellular functioning and integrity. Removal of fermentative products is also facilitated in the animal and plant kingdoms—for example, with the up-regulation of lactate exporters (*83*, *84*). In contrast to lower species in which fermentation appears controlled by substrate availability or posttranslational regulation, genes coding for enzymes and transporters involved in this metabolic adaptation to hypoxia are found incorporated in the main hypoxia response in metazoans and higher plants (*74*, *76*). From this perspective, transcriptional regulation of the genes coding for this metabolic adaptation is a recent acquisition, concomitant to the increase in developmental complexity in both kingdoms. Thus, convergence toward this regulation seems to offer an ecological advantage for complex multicellular systems to cope with the temporal offset between metabolic requirements and oxygen availability (Fig. 3).

Counteracting hypoxia through reoxygenation is also activated in animal and plant tissues. In most vertebrates, for example, this is attained through the local generation of new blood vessels (angiogenesis) and of synthesis of erythrocytes that carry oxygenated hemoglobin (*85*). Plants, instead, lack a dedicated oxygen distribution system, and thus certain species that are adapted to flooding acquired the ability to form hollow paths along stems and roots (aerenchyma) for unrestricted gas diffusion from above-water organs to submerged tissues (*86*). When the whole plant is underwater, rapid growth of stems and leaves can be deployed for emergence and ensure oxygen acquisition (*87*). Neither aerenchyma formation nor the elongation of organs are controlled by oxygen availability directly but rather through the gas hormone ethylene, whose synthesis is enhanced by submergence (*88*, *89*).

Fluctuations in oxygen are concomitant with fluctuations in reactive oxygen species (ROS) and reactive nitrogen species (RNS). A burst of hydrogen peroxide (H_2O_2) and NO has been reported to occur in animal and plant cells when these are challenged by severe oxygen deficiency (*90*–*93*). Additional and more severe ROS accumulation is also expected as normoxic conditions are restored. Genes regulated by HIFs and ERF-VII in animals and plants, respectively, code for scavengers of both ROS and NO, as well as enzymes involved in redox homeostasis, such as glutathione peroxidase, superoxide dismutase, glutathione *S*-transferases, and thioredoxins (*94*, *95*). In turn, ROS and NO contribute to HIF and ERF-VII regulation at the transcriptional and posttranslational level in animal and plant cells, respectively (*64*, *96*).

Although strategies to reverse the effects of short-term hypoxia are kingdom specific (reoxygenation), mitigation of the effects of prolonged hypoxia can be related to similar strategies in animals and plants: reduction of the metabolic rate, avoiding toxicity of anaerobic by-products, and preventing cell injuries upon reoxygenation. Oxygen-sensing machineries assist animals and plants in these strategies to cope with the imbalances that fluctuating oxygen availability causes to the cellular environment. Collectively, these systems contribute to the ability of multicellular organisms to maintain homeostasis.

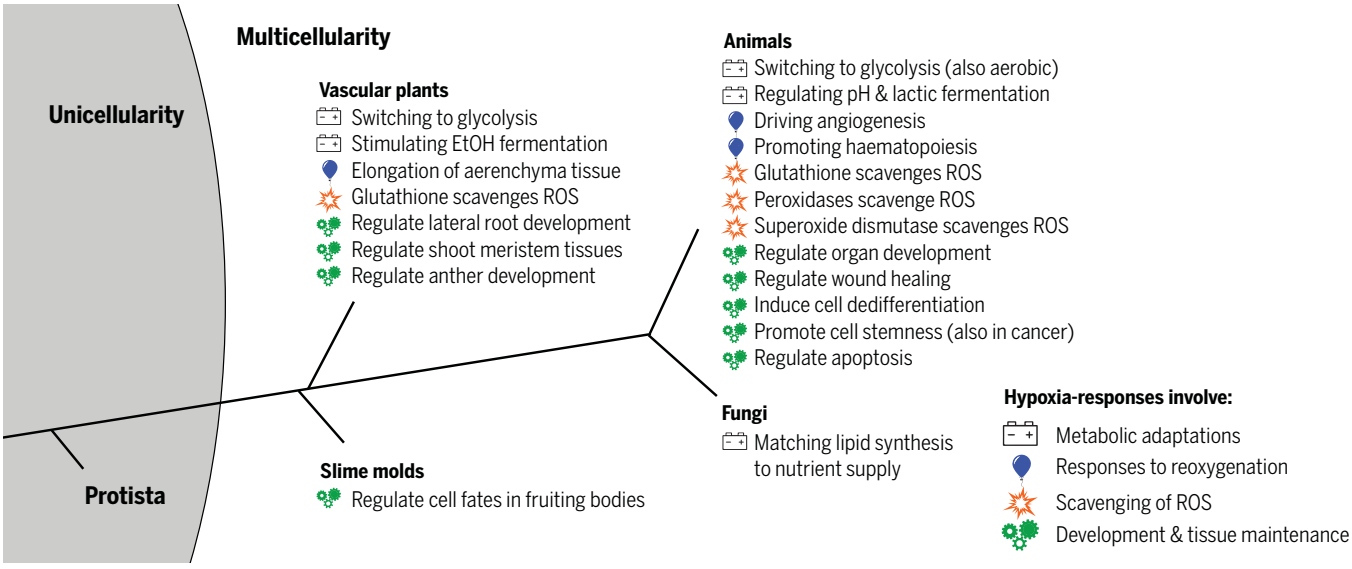

Fig. 3. Roles that hypoxia-response machineries appear to be involved in across eukaryotic kingdoms. Also shown are representative roles listed as related to mediation of metabolism (battery), oxygen resupply (balloon), scavenging of ROS (explosion), or development and tissue maintenance (cogs).

A capacity to modulate cell fate

The importance of oxygen availability and gradients for cells to multiply and differentiate has been recognized for a long time, and so has the cellular capacity to sense these. A wide range of oxygen concentrations have been measured across plant and animal organs and observed to vary throughout developmental stages (*68*, *97*). Mammalian embryogenesis occurs mainly at low oxygen concentration, and several types of stem and progenitor cells are embedded in hypoxic niches, where oxygen gradients drive their differentiation (*98*). Similarly in plants, the proliferative tissues responsible for producing new organs, the meristems, have also been shown to be embedded in hypoxic niches (*67*). Furthermore, detrimental cell growth hijacks the oxygen-sensing machinery and may induce cellular responses contrary to what oxygen gradients would dictate. In solid tumors, the uncontrolled proliferation of cells and their active metabolism often exceed the delivery capacity of the surrounding blood vessels, limiting oxygen availability (*99*). It is now 30 years since the discovery that the HIF system enables and supports tumorigenesis (*100*, *101*). Similarly in higher plants, tumor-like tissues, such as calli or galls, experience oxygen limitations, and ERF-VIIs support the metabolism and proliferation of highly dividing and undifferentiated cells (*102*, *103*).

Clues from cancer: Uncoordinated formation of multicellularity

The cancer field has put more emphasis on HIFs than has any other field, owing to the contribution of these factors to the success of tumor multicellularity. HIF-α is produced and degraded in the cytoplasm and, when stabilized for long enough, translocates to the nucleus. Localization and functions for the HIF-α subunits, however, appear to differ (*104*). In the case of HIF-1α, nondegraded protein is more or less exclusively present in the nucleus. Its activity is rather uniform by how it induces target gene expression in response to hypoxia in virtually any cell. That HIF-1α plays a reliable role in the immediate cellular response to hypoxia argues for its seminal role as an adaptation to acute oxygen fluctuations. HIF-2α, however, displays cytoplasmic in addition to nuclear localization (*105*–*107*). Insight from tumor multicellularity demonstrates that functions of HIF-α subunits do not always overlap. Whereas the HIF-1α subunit, which is specific to all eumetazoans except ctenophores, can be regarded as a fast response to metabolic alterations, the vertebrate-specific HIF-2α subunit is demonstrated to contribute to the success of tumors by modulating cell fate. Albeit that HIF-2α also plays a modest role in metabolic regulation, its main functions are regulation of cell fate, cell immaturity (stemness), and metastasis and to establish a hypoxia-mimicking phenotype in oxygenated milieus (pseudohypoxic niches) (*108*–*111*).

The pseudohypoxic phenotype is a consequence of HIF-2α accumulation in normoxic tumor cells, including such with the capacity to self-renew (a stem cell trait typically associated with hypoxia). Some of these HIF-2α–expressing cells are located in perivascular niches, despite their access to oxygen in these areas (*107*, *112*). This phenomenon is particularly well studied in the cancer forms glioblastoma and neuroblastoma (*107*, *112*), for which there are no correlations between HIF-1α expression and outcome, whereas expression of HIF-2α predicts poor prognosis and distal metastasis. It is not the collected expression itself that falls out as a predictor but rather the presence of this small fraction of HIF-2α–positive perivascular tumor cells. These constitute a rare cell type within the tumor that coexpresses several stem cell markers, strengthening HIF-2α as a promoter of stemness. The link between the pseudohypoxic phenotype, stemness, and the formation of tumor multicellularity is also supported by how mutations in *EPAS1* (encoding HIF-2α) directly induce tumor formation (*113*). As an example of the complexity of this protein, a HIF-2–specific inhibitor, PT2385 (*114*), which prevents ARNT binding and transactivation capacity, does not affect downstream transcription, cell proliferation, or in vivo tumor growth in neuroblastoma (*105*, *115*, *116*). In glioma, HIF-2α localizes to extranuclear polysomes to promote translation of a large but distinct set of proteins (*117*). These and other data suggest that the HIF-2α protein displays additional, although as yet mainly unknown, functions. We thus know that HIF-2α is expressed in the cytoplasm in addition to the nucleus. HIF-2α has ARNT-independent functions, and it is plausible that it forms complexes with proteins other than ARNT to initiate transcription. In addition, HIF-2α might form protein complexes in the cytoplasm to promote translation, stabilization, and secretion of proteins important for stemness, pseudohypoxic phenotypes, and tumor cell metastasis. In essence, HIF-2α appears to mediate a hypoxic or nonhypoxic cellular response—that associates with cell stemness [for example, (*118*)]—independently of surrounding oxygen concentrations.

Clues from coordinated multicellular development

Developmental pathways are affected by the hypoxic transcriptional regulators in both animals

and plants. These regulators act as switches or "pacemakers" of stem cell proliferation and differentiation.

During animal development, HIF-1α and its binding partner ARNT are ubiquitously expressed, whereas HIF-2α expression is more tissue- and time-restricted (*119*, *120*). Among invertebrate animals, hypoxia and subsequent HIF-1α accumulation are associated with growth and stem cell proliferation during development in fruit flies and mosquitos (*121*, *122*). In vertebrates, HIF-1α is ubiquitously expressed throughout development, and homozygous deletion is lethal (*123*, *124*). HIF-1α and HIF-2α are demonstrated to promote the generation of new blood vessels and branching of existing ones. However, *EPAS1* appears to have evolved before the downstream *erythropoietin* (EPO) gene, a promoter of increased systemic oxygen carrying capacity, and now, HIF-2α directly promotes erythrocyte differentiation from bone marrow progenitors (*125*). During development, expression of HIF-2α is temporally and spatially restricted. Endothelial cells display high expression of HIF-2α continuously, whereas cells of the developing sympathetic nervous system (SNS) express HIF-2α mRNA and protein during discrete periods of mammalian development (*120*, *126*, *127*). The central roles by which the animal-specific HIFs regulate hypoxic cell functions is suggested to have facilitated primitive animals to cope, and fully access, oxic niches during evolution (*41*).

In higher plants, developmental processes are intertwined with the activity of at least three classes of transcriptional regulators characterized by an exposed N-terminal cysteine, which is regulated by the oxygen-dependent branch of the N-degron pathway. First, ERF-VIIs have been shown to repress opening of the protective hypocotyl hook and cotyledon greening as well as to antagonize hormone signaling in the root for timely shaping of its architecture (*128*, *129*). Second, ZPR2 binds and inactivates homeodomain III–type TFs, regulators of meristem maintenance and new organ formation (*67*). Third, VRN2 is assembled into the polycomb repressive complex 2 (PRC2) that represses gene expression through histone methylation (*57*). One of the best characterized targets of this complex is the *FLOWERING LOCUS C* gene, a repressor of the transition toward reproductive development in *Arabidopsis*.

Epigenetic control of developmental pathways through histone methylation in both kingdoms is also controlled in part by oxygen-dependent KDMs. Although recent reports confirmed the hypoxia sensitivity of members of this protein family in human cells, with consequences for differentiation in several cell line model systems (*45*, *46*, *48*, *130*), this has not yet been explored in plants. However, several JmjC-domain–containing KDM proteins have been shown to control aspects of plant development in *Arabidopsis*, including germination, flowering, and callus formation (*131*).

Oxygen-sensing machineries as adaptations for nascent multicellularity

In all kingdoms of life, enzymes that detoxify ROS are ubiquitously present, which indicates that their evolution predated photosynthetic oxygenation (*132*). ROS can be locally produced by abiotic photolysis on a largely anoxic planet, according to geochemical and geophysical studies of both Earth and Mars (*133*). Continuous scavenging of ROS through enzymatic or metabolic assets, however, is likely to be costly for cells. Instead, the acquisition of mechanisms for perception and response would be of higher gain. This perception and response would engage detoxification only when oxygen and ROS levels exceeded a danger threshold and thus be metabolically cheaper. The multitude of oxygen-sensing strategies in prokaryotes and eukaryotes probably reflects adaptation to environmental niches distinguished by oxygen dynamics, in addition to the regulation of specific metabolic needs (*56*). The acquisition of mechanisms for ROS perception and scavenging would have pioneered the utilization of free oxygen as a resource. With a control of ROS detoxification, oxygen-sensing machineries could also be co-opted to express oxygen-requiring genes when this substrate is available and conversely suppress them in favor of anaerobic strategies in case of hypoxia.

The convergent evolution in complex eukaryotes toward selective proteolysis of transcriptional regulators that control hypoxia responses suggests the superiority of this strategy over others that use free oxygen. In all cases considered, the recruitment of specific transcriptional regulators by way of oxygen-dependent regulatory pathways occurred long after the "invention" of oxygen-consuming enzymes that operate on amino acid residues (*40*). These enzymes could have evolved from their original metabolic function toward a role as sensors. In turn, the transcriptional regulators acquired the specific residues that are substrates of the sensor enzyme in strategic positions to subdue the kinetics of the reaction to physiologically relevant oxygen levels. To constitutively synthesize transcriptional regulators is also costly, but the cost can be balanced by the advantage of rapid activation once degradation is inhibited. By contrast, bacteria exploit posttranslational regulation that is faster and less energy demanding, such as phosphorylation or dimerization (*56*). This type of regulation, however, is not as effective or can possibly not guarantee a sufficient level of specificity. The oxygen-sensing systems in plants and animals are fine-tuned through several layers of modification, although they all seem to be subordinated to the proteolytic system. For example, whereas most translated HIF-α proteins are proteasomally degraded in oxic conditions through PHD- or VHL-mediated ubiquitination, a second layer of HIF regulation is driven by FIH, which catalyzes oxygen-dependent asparagine hydroxylation of HIF-α. This second layer prevents the interaction between HIF-α and transcriptional activators. That FIH hydroxylates the asparagine motif less efficiently on HIF-2α than on HIF-1α could be one molecular explanation as to why HIF-2α is stabilized at higher oxygen concentrations (*43*, *134*). Essentially, although the road to proteolysis can be retarded or distinctly coupled to interval oxygen concentrations, the oxygen-sensing machineries across eukaryotic kingdoms master the route along which proteins are degraded by oxygen.

The commonality between organisms that exploited selective proteolysis as a solution to oxygen sensing resides in their complex multicellularity. Remarkably, HIF-α and ERF-VII and the regulatory proteins involved in their oxygen-dependent regulation are, at the outset, expressed and subsequently degraded. This hints at the need for a unified system to perceive and interpret oxygen gradients that are unavoidably generated throughout growth and development. As compared with diffusible signal molecules produced endogenously, such as hormones, molecular oxygen provides a faster and more direct connection to the metabolic needs of aerobic cells. Within a persistent three-dimensional organization in which respiration and transport regulate the mass balance of oxygen, cells are continuously exposed to variable oxygen concentrations, depending on their position in space and time. The multiple layers of oxygen perception and response demonstrate a capacity by which this variability is recorded and translated during growth, development, and activities of daily living in the eukaryotic kingdoms with complex multicellularity. This capacity appears more restricted in protozoa and fungi that also demonstrate both lower phenotypic and cell type diversity than those of animals or vascular plants. In all cases, however, the oxygen-sensing mechanisms contribute to the spatiotemporal induction of cellular functions, which is one of the defining features of complex multicellular life (Fig. 4) (*6*–*8*).

Conceptual gaps

The role of oxygen sensing for complex multicellular life and evolution appears crucial, but conceptual gaps remain on several layers. On the biomolecular and cellular level, for example, the presence of Nt-Cys-degron TFs that are ADO substrates could inform us about the most ancestral of oxygen-sensing strategies. Also, the existence of parallel oxygen-sensing strategies based on 2-OG–dependent oxygenases, such as KDMs, remain to be investigated

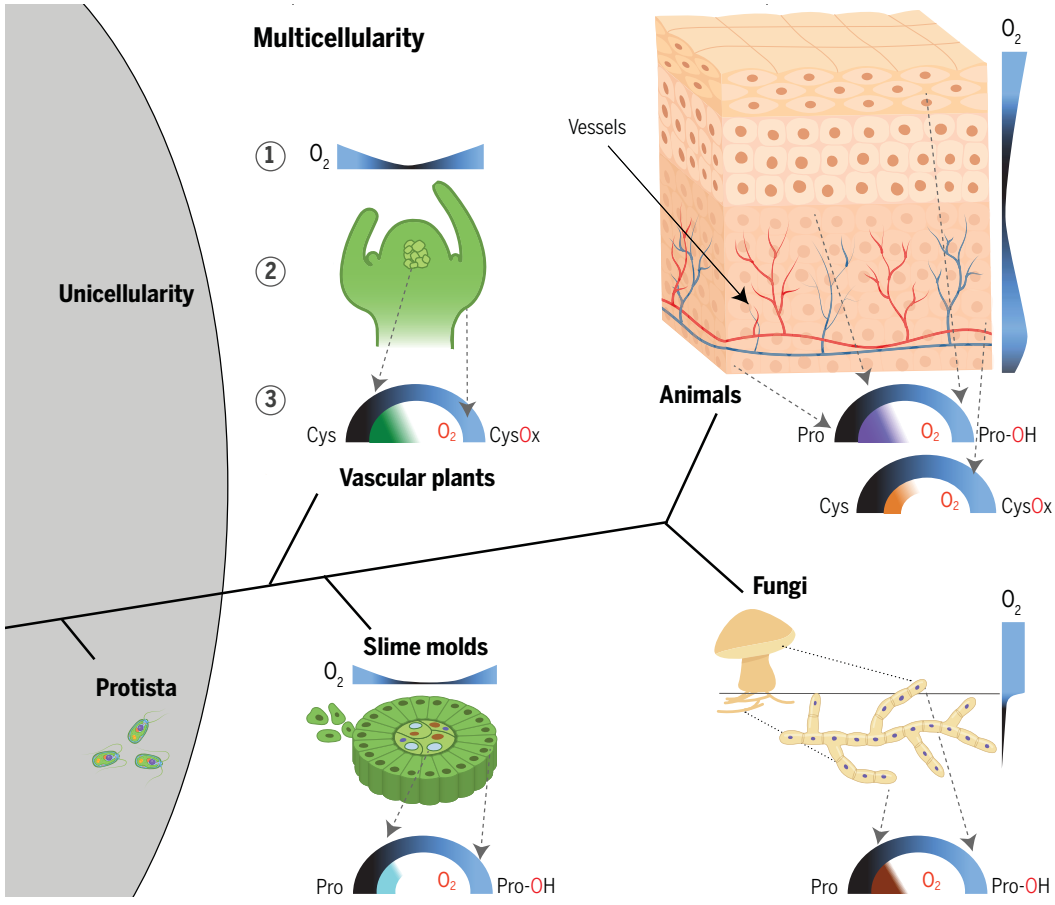

Fig. 4. Oxygen-sensing mechanisms across the eukaryotic kingdoms. Complex multicellularity converged to the recruitment of enzymatic control of the stability of regulatory proteins at specific endogenous oxygen concentrations (depicted for animals). (1) Endogenous oxygen gradients and (2) the spatial position of perceiving cells are stylized, with (3) a dial depicting the sensing (outer half circle) and response (inner half circle). In the dial, proteins are labeled as oxidized (Pro-OH/CysOx) at oxic conditions (blue) or nonoxidized (Cys/Pro) at hypoxia (black). The inner half circle of the dial represents the stabilization of a protein (half halo) or as a TF (pie chart; color coding and substrates are as in Fig. 2). Thiol-dioxygenases stabilize TFs in green plants (Viridiplantae), and 2-OG–dependent dioxygenases stabilize TFs in animals (Eumetazoa and Cnidaria) and Fungi. Protein stabilization (not TFs) is also regulated by thiol-dioxygenases in mammals and by 2-OG-oxygenases in slime molds.

in plants and fungi (*64*, *135*). The involvement of NO in the hypoxic signaling also deserves special attention. In animal cells, NO and RNS affect HIF expression, stability, and activity in a complex manner, depending on local concentrations and cellular milieu (*96*). Moreover, the Arg/N-degron pathway has been shown to act as a sensing mechanism to detect low NO levels both in animals and plants, although the response elicited by such conditions only marginally overlaps with those to hypoxia (*136*). The proteolytic element(s) positively affected by NO remains to be identified but seems to lie elsewhere than thiol-dioxygenase activity (*136*). Future efforts will likely shed light on defining the contribution of this signaling molecule to oxygen sensing in plants, animals, and their ancestors.

On the evolutionary level, the commonality in oxygen-sensing mechanisms between the plant and animal kingdoms is striking, but land plants and animals adopted alternative solutions to direct their primary hypoxia responses, even though their ancestors were likely equipped with the same repertoire of dioxygenases. The acquisition of oxygen-sensing function may be associated with a reduction in oxygen affinity that matches variations in oxygen concentration occurring in the cells and tissues. These variations are in turn determined by environmental, ecological, and organismal features, such as metabolic rates and anatomy. Thus, fundamental differences in internal and external characteristics could have driven the distinct selection of biochemical pathways to direct selective proteolysis in these two systems—but what are these defining characteristics, and what trade-offs followed with the different systems? Even for most modern multicellular organisms, we lack information on endogenous oxygen gradients and their fluctuations. However, many enzymes use oxygen as a cofactor but appear not to contribute to oxygen-sensing machineries. This suggests that either their oxygen-sensing potential is unexplored or their function is to catalyze other specific reactions, irrespective of the environment. Nevertheless, constraining the details and hierarchical order of oxygen-sensing systems will allow investigations of whether components were shared by a common ancestor or shared after the divergence of the respective kingdoms.

Last, expanding our understanding of the evolution of oxygen sensing, by learning from geological history, will allow identification of opportunities for further beneficial manipulation of these systems for both clinical and agricultural purposes. Already, synthetic biology approaches that involve interchange of regulatory modules between organisms from different kingdoms constitute an innovative strategy to adapt oxygen sensitivity and the magnitude of response machineries (*137*). For example, transfer of oxygen-sensing components from plant to yeast has contributed to defining features of these systems and could readily help to develop drugs that interfere with their functioning (*136*). We advocate a

shift in focus, away from exploring oxygen sensing as primarily a response to oxygen shortage for aerobic respiration and toward considering it as a mechanism that enables multicellularity to cope with and even use fluctuations in oxygen concentrations. We predict that this will reward us with new perspectives on the broad scope of oxygen-sensing mechanisms and the challenges that multicellular life is exposed to, today as in geologic history.

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Oxygen-sensing mechanisms across eukaryotic kingdoms and their roles in complex multicellularity

Emma U. Hammarlund, Emily Flashman, Sofie Mohlin and Francesco Licausi

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Origins and evolution of hypoxia response

In our current oxygen-rich atmosphere, the ability of eukaryotic cells to sense variation in oxygen concentrations is essential for adapting to low-oxygen conditions. However, Earth's atmosphere has not always contained such high oxygen concentrations. Hammarlund *et al.* discuss oxygen-sensing systems across both plants and animals and argue that the systems are functionally convergent and that their emergence in an initially hypoxic environment shaped how they operate today.

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