Environmental Oxygen is a Key Modulator of Development and Evolution: From Molecules to Ecology

Oxygen-sensitive pathways pattern the developing organism, linking genetic and environmental components during the evolution of new traits

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Oxygen is a key regulator of both development and homeostasis and a promising candidate to bridge the influence of the environment and the evolution of new traits. To clarify the various ways in which oxygen may modulate embryogenesis, its effects are reviewed at distinct organizational levels. First, the role of pathways that sense dioxygen levels and reactive oxygen species are reviewed. Then, the effects of microenvironmental oxygen on metabolism, stemness, and differentiation throughout embryogenesis are discussed. Last, the interplay between ecology and development are reexamined with a focus on the evolution of tetrapods, including during the emergence of a novel mechanism that shapes amniote limbs—interdigital cell death. Both genetic and environmental components work together during the formation of organisms, highlighting the importance of a multidisciplinary approach for understanding the evolution of new traits.

1. Introduction

Oxygen is far more than just a requirement for energy generation; it is also a regulator of homeostasis at the cell, tissue, and organism levels. Life on Earth emerged \( \approx 3.7 \) billion years ago under a strongly reducing, anoxic environment. While very low levels of oxygen started to appear in the oceans and alongside the first appearance of aerobic life during the Archean age, it was the Great Oxygenation Event that would forever change the redox environment of the planet, cementing oxygen as the main electron donor during photosynthesis and later supporting the radiation of aerobic, mitochondria-containing eukaryotes. \(^2\) Since then, the ability to respond to surrounding oxygen levels has been a critical adaptive feature of both prokaryotes and eukaryotes \(^3\) both to avoid oxidative stress and as a component of several metabolic networks. \(^4\) Given that it is such an ancient feature, it is perhaps unsurprising that oxygen signaling may have acquired new functions over the course of evolution, including during development.

This topic can be approached from many angles, a feature that may pose a challenge when trying to obtain new insights for answering multidisciplinary questions, such as the role of ecological niches in the development of different species. Thus, it is useful to consider the various levels at which oxygen may affect organisms in a stepwise manner (Figure 1). At the single-cell level, multiple pathways are able to sense both the dioxygen \((\text{O}_2)\) concentration and the redox potential of the cell. Dioxygen levels are sensed directly by hypoxia-sensitive pathways, most notably hypoxia-inducible factors \((\text{HIFs})\), although other mechanisms exist in metazoans, namely, the oxygen-sensitive histone lysine demethylase \((\text{KDM})\) and the cysteamine dioxygenase/N-degron pathways \(^7\) (Figure 1a). In addition, the production and degradation of reactive oxygen species \((\text{ROS})\) are critical for maintenance of the redox balance of the cell. \(^8\) Several proteins undergo changes in structure and/or activity according to their redox state, leading to the activation or repression of cell signaling pathways and transcription factors \(^9\) (Figure 1a).

Dioxygen- and redox-sensitive pathways detect the dynamic distribution of oxygen across tissues and may lead to holistic changes in the cellular state (Figure 1b). The evolution of aerobic metabolism supported the appearance of novel metabolic pathways and improved energy generation. \(^2,3\) As organisms increase in complexity, microenvironmental oxygen levels could also regulate cell potency and the timely differentiation of each tissue during embryogenesis. \(^10,11\) The formation of the cardiovascular and hematopoietic systems drives global changes in oxygen availability, \(^12\) restricting certain developmental events to specific timeframes.

Finally, the environmental oxygen available to different species is highly dependent on their ecology (Figure 1c). The oxygen concentration is dependent on whether an animal is aquatic or terrestrial during each stage of its life cycle, as well as on intrinsic differences in the respiratory, cardiovascular, and hematopoietic systems among animals. \(^13\) These variations may potentially...
Oxygen can influence development at multiple levels. a) At the single-cell level, signaling pathways can sense both dioxygen (O$_2$ gas) and the redox potential of the cell. Among the dioxygen sensors, HIF transcription factors are stabilized only under hypoxia, while some histone lysine demethylases (KDM) and the N-degron pathway are repressed under hypoxic conditions.[7] Additionally, the balance between the production and degradation of ROS is critical for the redox balance of the cell. Many signaling pathways are potentially regulated by ROS-mediated oxidation, including mitogen-activated protein kinases (MAPKs), the multifunctional protein Ref-1, iron-regulated proteins (IRP1/2), and the key antioxidant system Nrf2/Keap1.[9] b) The oxygen level in the tissue, or microenvironmental oxygen, is a key modulator of the cellular state. Integration between oxygen- and nutrient-sensitive pathways regulates metabolism, which in turn can modulate the cell cycle and epigenetics.[8] Notably, maintenance of stemness is associated with low aerobic metabolism, while differentiation is linked to high oxidative phosphorylation (OXPHOS) levels and energy production.[11] c) The available oxygen is ultimately dependent on the environment surrounding each individual, which may even change throughout development. Aquatic animals are often subject to environmental hypoxia. In contrast, atmospheric oxygen is highly concentrated and is a constantly available to terrestrial species.[13]

Figure 1. Oxygen can influence development at multiple levels. a) At the single-cell level, signaling pathways can sense both dioxygen (O$_2$ gas) and the redox potential of the cell. Among the dioxygen sensors, HIF transcription factors are stabilized only under hypoxia, while some histone lysine demethylases (KDM) and the N-degron pathway are repressed under hypoxic conditions.[7] Additionally, the balance between the production and degradation of ROS is critical for the redox balance of the cell. Many signaling pathways are potentially regulated by ROS-mediated oxidation, including mitogen-activated protein kinases (MAPKs), the multifunctional protein Ref-1, iron-regulated proteins (IRP1/2), and the key antioxidant system Nrf2/Keap1.[9] b) The oxygen level in the tissue, or microenvironmental oxygen, is a key modulator of the cellular state. Integration between oxygen- and nutrient-sensitive pathways regulates metabolism, which in turn can modulate the cell cycle and epigenetics.[8] Notably, maintenance of stemness is associated with low aerobic metabolism, while differentiation is linked to high oxidative phosphorylation (OXPHOS) levels and energy production.[11] c) The available oxygen is ultimately dependent on the environment surrounding each individual, which may even change throughout development. Aquatic animals are often subject to environmental hypoxia. In contrast, atmospheric oxygen is highly concentrated and is a constantly available to terrestrial species.[13]

Thus, we propose that all of the aforementioned levels should be considered in an integrated manner to understand the roles of oxygen during evolution. This review will present an integrated discussion of how oxygen orchestrates several developmental steps, with a focus on the implications for the evolution of species.

2. HIF Pathways Regulate Multiple Steps of Metazoan Development

2.1. Evolution of the HIF-PHD Pathway

Pathways that are able to sense variations in dioxygen levels, especially HIFs, are the core components of hypoxia sensing in metazoans. HIF transcription factors are heterodimers composed of an oxygen-regulated HIFα subunit and a stable HIFβ subunit (also known as ARNT) that can bind to hypoxia response elements (HREs) in the genome.[6] Under well-oxygenated conditions, HIFα is hydroxylated by members of the oxygen-dependent prolyl hydroxylase domain family (PHD or EGLN).[6] In turn, the von Hippel–Lindau tumor suppressor protein (pVHL) ubiquitinates HIFα, marking it for proteasomal degradation and thus inhibiting the transcriptional activation mediated by HIFs.[6] Additionally, the oxygen- and α-ketoglutarate-dependent factor inhibiting HIF-1α (FIH1) can disrupt the interaction between the HIF complex and its transcriptional coactivators.[4,17] Multiple feedback pathways fine-tune the activity of the HIF-PHD pathway and integrate the oxygen response to nutrient availability and metabolic control of the cell.[6,17,18]

Proteins of the HIF pathway are conserved within several metazoan groups, including cnidarians[19] and placozoans;[20] only FIH is absent in placozoans.[20] In contrast, key components of this pathway are lacking from sea sponges and cnemophores.[22] Interestingly, both groups inhabit low-oxygen environments and can withstand periods of anoxia.[21] Consistent with the hypothesis that early animals had low oxygen requirements,[22] other oxygen-sensitive prolyl hydroxylases are found in other clades, such as bacteria[22] and fission yeast.[22] Additional oxygen-sensitive pathways are also found in metazoans, as will be discussed later in this review.

While HIF-1α is found throughout metazoans, HIF-2α (or EPAS1) is a vertebrate innovation.[24] This duplication has enabled the appearance of nonredundant transcriptional targets, expression across distinct cell types, and differential regulation by PHD and VHL proteins.[17,25] HIF1-α is ubiquitously expressed, and its target genes have been traditionally associated with mitigating acute hypoxia (e.g., promoting glycolysis and inhibiting oxidative phosphorylation) and improving oxygen availability (e.g., proangiogenic activity).[12,26] In contrast, HIF-2α exhibits more restricted expression, and its targets have been linked to regulation of the cell cycle, erythropoiesis, and chronic adaptive...
2.2. Roles of HIFs during Embryogenesis

The HIF-PHD pathway regulates several steps during the early development of metazoans, beginning as early as the zygote. One example of the complex regulatory effects of HIF-PHD is found during the dorsoventral patterning of sea urchin embryos (Figure 2a). The egg’s mitochondria are maternally concentrated on the prospective ventral side of the egg, creating a gradient of redox potential. In addition, the hifα mRNA, initially available throughout the egg, is preferentially degraded on the ventral side of the early blastula. Together, these mechanisms lead to the stabilization of Hifα exclusively on the dorsal side of the blastula, expression of dorsal genes in an HRE-dependent manner, and subsequent inhibition of the expression of ventral genes such as nodal. An asymmetric mitochondrial distribution has been described in the oocytes of many other species, including arthropods, ascidians, and vertebrates, and has been linked to hydrogen peroxide production during the polarization of Caenorhabditis elegans zygotes. It is unknown whether intrinsic redox gradients or differential activation of HIF regulates the early axial patterning of other animals as well.

During early vertebrate embryogenesis, the HIF-PHD pathway is critical for the formation of the major oxygen delivery systems of the body: the cardiovascular and hematopoietic systems. Complete knockout of several components of the HIF-PHD pathway in mice is lethal due to severe cardiovascular defects. The hypoxic environment within the early embryo is required for the differentiation of hemangioblasts, early precursors of both endothelial and blood cells, in a HIF-dependent manner. Primitive hematopoiesis provides embryos with a few cell types, including red blood cells, while blood vessels form in both embryonic and extraembryonic regions. Even before circulation has started, HIF-regulated heart morphogenesis occurs. This synchronization allows the timely development of the cardiovascular and hematopoietic systems, as well as the extraembryonic membranes, within the hypoxic microenvironment of the vertebrate embryo in a conserved pattern.

The hypoxic milieu of early embryos regulates the development of other structures, such as the neural crest (Figure 2b). In both zebrafish and Xenopus laevis, HIF-1α activity in neural crest cells is required for epithelial-to-mesenchymal transition (EMT) and chemotaxis through regulation of Twist and Cxcr4 expression, respectively. HIF-1α is also necessary for the production of cephalic neural crest cells in chicken embryos prior to the establishment of circulation in the head. Interestingly, increased hypoxia led to hypermotility or excessive EMT of chicken neural crest cells, revealing that neural crest development is tightly regulated at both the genetic and environmental levels. HIF-2α has also been proposed to play a role in the development of the trunk neural crest, although in a different manner, promoting both the multipotency and proliferation of the neural crest. Notably, although cells expressing conserved regulatory pathways with neural crest cells exist in chordates, the neural crest is considered a morphological innovation of vertebrates that acquired its multipotency throughout vertebrate evolution. It is fascinating to speculate whether HIF-2α played a role in the evolution of this tissue.

2.3. Roles of HIFs during Organogenesis

As the organism grows, simple diffusion becomes insufficient to provide the necessary oxygen concentration to the increasingly complex tissues. During organogenesis, the availability of oxygen within tissues is a central driver of angiogenesis for establishment of a mature vascular network. Gradients of hypoxia function as morphogens guiding angiogenesis through expression of vegfa, a classic HIF-1α target gene, as well as through autonomous effects in the formation of vascular organs such as the placenta and the heart. HIF-2α is also...
required for vascular remodeling at postvasculogenesis stages in mice.\textsuperscript{44} This system is not exclusive to vertebrates; insects have a tracheal system of tubes that are formed in a manner akin to angiogenesis.\textsuperscript{45} Tracheal remodeling is induced by hypoxic tissues in both Rodhnius and Drosophila;\textsuperscript{45} mutation of components of the HIF-PHD pathway abrogates this process in the latter species.\textsuperscript{46} Thus, HIF provides a conserved strategy to direct vessel formation toward oxygen-deficient tissues according to an oxygen gradient from organogenesis to adult life.\textsuperscript{12,42,45}

The HIF-PHD pathway also has several tissue-specific roles, including in skeletogenesis, adipogenesis, and immunity.\textsuperscript{27} The formation of vertebrates’ endochondral bones is one of the most interesting examples of a developmental process regulated by oxygen in both space and time. The first step of this process is the formation of avascular mesenchymal condensations, in which HIF-1α is required for chondrocyte identity, survival, and proliferation.\textsuperscript{47-49} The cartilage matures in a highly organized fashion and eventually undergoes apoptosis, which leads to the invasion of both blood vessels and osteoblasts.\textsuperscript{47,50} Increasing HIF signaling in mouse osteoblasts through VHL knockout increased angiogenesis and a marked increase in bone volume.\textsuperscript{51,52} Endochondral bones have been found in all extant osteichthyan groups investigated so far, albeit with slight histological differences.\textsuperscript{53} In contrast, chondrichthyan bones have no bona fide bones but display both calcified and noncalcified cartilage.\textsuperscript{54} The diversification of skeletal cell types has been proposed as a key factor during the evolution of endochondral ossification in vertebrates.\textsuperscript{55} We further propose that this increase in the complexity of chondrogenic and osteogenic genetic programs could also have allowed the differential regulation of angiogenesis and/or oxygen availability in each of the developmental steps of the skeleton. Thus, oxygen sensing by the HIF-PHD pathway is critical for multiple steps of development, from the patterning of the hypoxic early embryo to the formation of specific tissues.

3. The KDM and ADO/N-Degron Pathways Also Sense Dioxigen Levels in Metazoans

While HIFs are essential for oxygen sensing in metazoans, this pathway is absent from or has unrelated functions in other phyla.\textsuperscript{55} However, several other oxygen-dependent dioxygenases exist in the human genome, and some could potentially serve as hypoxia sensors in cells.\textsuperscript{18} Two additional pathways conserved between metazoans and plants have been identified: KDMs and N-terminal cysteine dioxygenases (ADOs).

Multiple studies have revealed that the epigenetic landscape can be modulated by hypoxia at the chromatin level; it has been proposed that JmjC-domain-containing histone lysine demethylases could act in an oxygen-sensitive manner.\textsuperscript{55} This hypothesis has been confirmed in human cells: the enzymatic activity of both KDM5A\textsuperscript{56} and KDM6A\textsuperscript{57} was inactivated under hypoxia in an HIF-independent manner, leading to increased genomic histone methylation. Additionally, KDM6A promoted hypoxia-induced inhibition of myoblast differentiation in vitro.\textsuperscript{57} Myogenesis is also inhibited by environmental hypoxia during development in an HIF-dependent manner,\textsuperscript{58} revealing that oxygen can regulate the same process through multiple mechanisms. Oxygen-sensitive KDMs have important implications for the development of preimplantation mammalian embryos, as embryonic stem cells (ESCs) generally have open chromatin, and their pluripotency is positively modulated by hypoxia.\textsuperscript{59}

The other dioxygen-sensing mechanism involves N-terminal cysteine dioxygenases, or cysteamine (2-aminoethanethiol) dioxygenase (ADO) in humans.\textsuperscript{60} This pathway was originally identified in plants, in which ERFVII transcription factors are destabilized in an oxygen-dependent manner, inhibiting the hypoxic response.\textsuperscript{61} Under normoxia, these dioxygenases oxidize the N-terminal cysteine of their substrates, targeting them for proteasomal destruction by the N-degron pathway. Potential targets in mammals have been implicated in oxygen regulation during cardiovascular development.\textsuperscript{60} Thus, a dioxygen sensor that operates by posttranslational modification exists in metazoans, potentially transducing hypoxia responses more rapidly than the transcription-dependent HIF pathway.\textsuperscript{60} Thus, the HIF-independent dioxygen sensors in metazoans indicate additional possibilities for the regulation of cell potency by hypoxia during development.

4. ROS and Redox-Modulated Pathways

In addition to dioxygen-sensing pathways, metabolic and signaling pathways sensitive to ROS are also critical adaptations to the oxic realm. Maintenance of physiological ROS levels is required for the stability of the genome and macromolecules,\textsuperscript{8} including in the developing organism, as seen in diabetic embryopathies or upon exposure to teratogenic drugs.\textsuperscript{62} Sources of ROS exist throughout several cellular compartments, including the mitochondrial electron transport chain, peroxisome, and endoplasmic reticulum, and ROS can be directly synthesized by NADPH oxidases.\textsuperscript{8,10} Several proteins contain cysteine residues that can be oxidized by ROS, inducing structural and/or functional changes that modulate cell signaling.\textsuperscript{9} This section is by no means a comprehensive review of all ROS-modulated pathways but will briefly review some of the pathways with developmental or evolutionary implications.

The earliest organisms that employed oxygenic photosynthesis appeared hundreds of millions of years before the Great Oxigenation Event.\textsuperscript{2} Molecular phylogenetic analyses suggested that the first antioxidant systems preceded this event as well and included the enzymes superoxide dismutase, catalase, and peroxiredoxin.\textsuperscript{5} Antioxidant systems are regulated by several oxidant sensors in extant organisms. For instance, the redox-sensitive transcription factors OxyR and SoxR are at the core of the antioxidant response in\textit{Escherichia coli}, rapidly inducing the expression of the thioredoxin and glutathione systems, iron scavengers, metabolic enzymes, and DNA repair proteins in addition to the previously mentioned enzymatic antioxidants.\textsuperscript{63} Genes activated by the cell’s antioxidant systems are widely conserved.\textsuperscript{5} The phenotype of mutant mice for core antioxidant/prooxidant genes has been reviewed in detail,\textsuperscript{10,12} ranging from early embryonic lethality with abundant cell death to an increased sensitivity to oxidative stress in adult animals.

Redox-sensitive transcription factors also regulate the antioxidant response in metazoans, including Nrf2. Members of the cap “n” collar transcription factor family play diverse roles in hematopoiesis, heme metabolism and antioxidant re-
sponse across several species.[64] Under stress conditions, Nrf2 is transllocated to the nucleus and induces the transcription of several antioxidant defense genes.[9,64] Although Nrf2 is found in the yeast genome, its protective role was acquired only during the evolution of metazoans.[65] Additionally, this pathway increased in complexity throughout evolution concomitant with the rise in atmospheric oxygen levels, including the recruitment of Keap1 in vertebrates.[65] An even more extreme example of gain of function of an ancient pathway is iron metabolism. Free iron catalyzes the production of hydroxyl radicals,[79] thus, the iron balance must be carefully regulated to avoid oxidative stress.[9,66] Due to the redox-sensitive properties of iron, several proteins have iron-containing heme moieties with a variety of functions in the cell.[67] One of these proteins is hemoglobin, which may protect tissues against oxidative stress induced by hypoxia and HIF, as in Drosophila.[68] The expression of hemoglobin in red blood cells, a safe and efficient oxygen-transporting system, is a key component in the evolution of the vertebrate circulatory system.

Several adaptive signaling pathways have been described as playing a role during development. In mammals, the multifunctional protein Ref-1/APE is responsible for the redox activation of a large number of stress-inducible transcription factors, including AP-1 transcription factors and HIF-1α.[69] Ref-1 is required for mouse embryonic survival due to its central role in both DNA repair and redox regulation and the regulation of many developmentally active pathways in a TRX-dependent manner.[10,61] Interestingly, the redox function is exclusive to this clade,[70] revealing that new functions may appear during evolution and in turn modulate a great number of pre-existing pathways. The activation of transcription factors such as AP-1 by oxidants is still detected in other clades, such as in the yeast factor Yap1, where it also occurs in a TRX-dependent fashion.[71] The mitogen-activated protein kinases p38 and JNK, also referred to as stress-activated protein kinases, can also be positively regulated by ROS[9] and have been linked to osteoblast differentiation in mice[72] or apoptosis-induced proliferation in Drosophila.[73]

Finally, controlled production of ROS may also drive specific developmental events. As ROS regulate the balance between cell survival and death,[74] high production of these radicals can lead to programmed cell death during development,[75,76] elimination of cells or even whole structures are also an important step during the development of many organs. A notable example is the removal of the interdigital membranes between the fingers,[77] which requires the production of ROS[78] and activation of DNA damage pathways.[79] Thus, pathways of adaptive response to ROS may be ancient features but have acquired new roles throughout evolution.

5. Microenvironmental Oxygen Levels and Metabolism Regulate Cell Potency and Differentiation During Development

5.1. Aerobic Metabolism and Cell Potency Have Been Closely Linked Throughout Evolution

The regulation of signaling pathways by oxygen is not a one-way street—cells respond and adapt to local oxygen tension, especially in a dynamic setting such as embryogenesis. This connection can be traced to the evolution of aerobic metabolism, which involves the rewiring of several pre-existing anaerobic metabolic networks and the appearance of novel reactions and metabolites.[80] These new factors include not only antioxidants and more efficient energy-generating pathways but also oxygen-dependent chromatin modulators and the synthesis of sterols and polyunsaturated fatty acids, which are critical for cellular compartmentalization in eukaryotes.[1] The redox and nutritional states remain closely related in metazoans,[8] as well as metabolism and the cell cycle.[81] epigenetics,[82] and stemness.[83] Thus, taking a holistic approach to understanding how aerobic metabolism can regulate the cell state can provide insights into the role of oxygen during evolution and development. Many unicellular organisms optimize their metabolism for energy generation during starvation but proliferate when nutrients and oxygen are abundant.[81] As uncontrolled proliferation is damaging to multicellular organisms, this pattern is still found in a different context: differentiated cells primarily employ oxidative phosphorylation to generate energy though aerobic metabolism even when resources are available.[81] In contrast, proliferating cells have a high glycolytic rate, generating many intermediate metabolites that support macromolecular synthesis during cell division instead of being completely oxidized for ATP generation, which has been called the Warburg effect.[81]

As multicellular tissues increase in complexity, precise control of differentiation capacity (potency), and proliferation with no exhaustion of potential (self-renewal)—the core stem cell properties—is required during the development and homeostasis of vertebrates and invertebrates.[84,85] Many adult stem cells have low mitochondrial metabolism and immature mitochondria, which is linked to the maintenance of their quiescence while avoiding overactivation and stem cell exhaustion.[83,85] For instance, in hematopoietic stem cells, increases in oxidative phosphorylation, fatty acid oxidation, and ROS generation are linked to a loss of stemness and subsequent differentiation.[83,86] These cells reside in a low-oxygen niche in the bone marrow[87] and may lose their stemness if exposed to normoxia.[88] Planarian stem cells or neoblasts also have few mitochondria[89] and share pluripotency markers with mammalian ESCs or germ cells.[90] Thus, modulation of the hypoxic response and ROS signaling have been linked to the evolution of stemness.[11,24] As will be discussed below, the metabolic profiles regulate cell potency and differentiation during embryogenesis as well.

5.2. Metabolic Patterns Control Vertebrate Development

Oxygen availability changes throughout development, a phenomenon that leads to global changes in the metabolic profile of the embryo. For instance, mice employ predominantly glycolytic metabolism before the placental circulation is established, as simple oxygen diffusion is only sufficient at very early embryonic stages, during which an increase in oxidative metabolism is found.[91] In chicken eggs, a drop in the concentration of oxygen in the egg chamber, indicating increased uptake by the embryo, is concurrent with the development of the chorioallantoic circulation.[11] An increase in the complexity and number of mitochondrial cristae is also detected as aerobic metabolism becomes more prevalent in older murine and avian embryos.[39]
Zebrafish embryos also do not require a complete cardiovascular system as an oxygen source during the first days of life and display a shift in the abundance of glycolytic metabolites toward oxidative intermediates during embryogenesis. As an important consequence, these metabolic shifts restrict certain developmental events to specific time windows. ESCs require high glycolytic metabolism to maintain their pluripotency and ability to self-renew and to regulate their epigenetic machinery. A gradient of glycolytic metabolism exists in the primitive streak of mice and chicken embryos, which is required for the posterior extension of the body axis, maintenance of this highly proliferating tissue and correct differentiation of the paraxial mesoderm and neural tube. Additionally, the emergence of the neural crest takes place prior to the perfusion of the neural tube (Figure 2b). This process depends on hypoxia; conversely, dysregulation of hypoxia signaling caused by gain-of-function mutations in HIF-2α leads to tumorigenesis in some neural crest-derived tissues. 

Regulation of mitochondrial metabolism also takes place in specific tissues during organogenesis. For instance, mouse skeletal muscle development is modulated in a redox-sensitive manner. Akin to what is observed in adult stem cells, a hypoxic microenvironment and signaling are required for the self-renewal of myoblasts, while increased oxygenation leads to differentiation and fusion of the myofibers. In the same way, the HIF-1α-induced glycolytic signature present during early heart development allows increased mitochondrial oxidative metabolism as the differentiation of the heart trabecula progresses. In mammals, defective oxygen delivery, as seen in placental mammals, may also lead to specific defects in oxygen-sensitive tissues, such as the kidney medulla. Thus, microenvironmental oxygen levels regulate cell potency and metabolism, as well as timely differentiation. These highly conserved processes were important for the evolution of multicellularity and for the regulation of each developmental step of extant metazoans.

6. Environmental Oxygen Can Have Adaptive and Nonadaptive Consequences During Development

6.1. Environmental Oxygen Availability throughout Development and Life History

The classic approach to studying the evolution of new traits, including during development, is to investigate how changes in the genetic program affect the phenotype. However, the environment also plays an instructive role during embryogenesis. Developmental plasticity has been described for several traits, such as sex determination based on temperature (e.g., fishes, amphibians, and reptiles) or morphological changes based on the presence of predators (e.g., planktonic crustaceans). The interplay among ecology, evolution, and development (“eco-eco-devo”) has been recently considered when investigating other aspects, such as the role of symbionts during development. Here, the role of the environment in oxygen availability throughout development will be briefly discussed, focusing on tetrapods—amniotes and amphibians—as examples.

One of the most notable features of amniotes is the presence of extraembryonic membranes, highly vascularized structures that provide a constant source of oxygen to the growing organism. The extraembryonic membranes may exchange oxygen with the air, as seen in egg-laying amniotes, or with the mother’s blood, as seen in placental mammals. This high oxygen availability has been linked to high metabolic rates in amniotes especially in endothermic mammals and birds. Although extant animals hardly experience an increase in the atmospheric oxygen concentration, such an event has been suggested to play a role in the evolution and radiation of large placental mammals; experimental hyperoxia also has a positive effect on the developmental rate and body size of amniotes. In contrast, avian embryos that develop under hypoxia present severe vascular defects and oxygenation defects caused by placental deficiency lead to defects in developing mammals, including growth restriction.

On the other hand, amphibians have no extraembryonic membranes, relying on the development of respiratory structures for oxygenation even prior to hatching. Additionally, they have a complex life cycle that is subject to variations in environmental oxygen according to each species’ habitats in embryonic, larval, and adult phases. A major factor for oxygen availability in amphibians is whether the habitat is aquatic or terrestrial. The oxygen concentration and availability are much higher in air than in water; to the extent that water is not common as an exclusive source of oxygen for adult amphibians. In addition, bodies of water are subjected to variations in oxygen concentration according to temperature, movement, crowding, and seasonality, making resistance to hypoxia an important adaptation of aquatic amphibians. Thus, aquatic eggs are smaller and less metabolically active than terrestrial eggs, and aquatic larvae have adaptations in their cardiovascular, hematopoietic, and respiratory systems to improve oxygen uptake. These adaptations may also be responsive to variations in environmental oxygen throughout the life cycle: the terrestrial eggs of several species hatch after flooding—as well as after experimental hypoxia—due to poor oxygenation. In the same way, an aquatic or terrestrial environment regulates gill regression and hatching competence accordingly. A frog that can lay eggs in both environments in an oxygen-dependent manner. Finally, some amphibians do not present a free-living larval stage at all; these directly developing animals hatch only after completing metamorphosis.

Some of the directly developing species lay their eggs on land and are exposed to high atmospheric oxygen concentrations akin to amniote eggs. However, adaptive traits are susceptible to modulation by life history strategies. Other systems that are potentially regulated by oxygen can also be modulated by these changes, even in nonadaptive ways. For instance, the myocardia of zebrafish and aquatic newts have a remarkable regenerative capacity. In contrast, mice only display cardiac regeneration until 7 days of age, when this process becomes very limited. Interestingly, this loss in potential was correlated with an increase in mitochondrial complexity and ROS production in the heart, leading to cell cycle arrest in cardiomyocytes. Thus, the oxygen-rich environment of the mouse heart leads to loss of the heart’s regenerative potential in comparison with that of the fetal heart or that in aquatic species. We propose that differences in life history strategy and oxygen availability across species might affect developmental mechanisms dependent on oxygen and ROS in both adaptive and nonadaptive ways.
Figure 3. Differences in life history strategies modulate the development of amniotes and amphibians. a) There exists a major difference between amphibians and amniotes during development. Amniotes have many extraembryonic membranes rich in blood vessels that allow them to efficiently exchange gases and nutrients. These membranes are connected to the eggshell and egg yolk in reptiles, birds, and egg-laying mammals (“In the egg”) or to the mother in placental mammals (“In the uterus”). b) Some amphibians hatch from their eggs early and live freely as tadpoles or larvae. Then, they undergo metamorphosis and change their body shape to that of an adult individual (“Biphasic development”). Other amphibians complete their metamorphosis inside the egg, and an immature adult animal emerges by the end of the embryonic period, without a tadpole stage (“Direct development”). c) The high oxygen levels available to adult mice are associated with loss of the regenerative potential of the heart. [15] Neonatal mice and adult zebrafish are exposed to relatively low oxygen levels, a condition that is correlated with the maintenance of cycling cardiomyocytes, low ROS production, and heart regeneration capacity. [15] d) While aquatic tetrapods exhibit no interdigital cell death during development, the presence of terrestrial eggs in amniotes or directly developing amphibians is correlated with the induction of cell death [16]. The cooption of limb patterning pathways such as interdigital BMP and an increase in ROS production under high oxygen levels led to the appearance of cell death as a novel limb-shaping mechanism. [16]

6.2. Environmental Oxygen and the Appearance of Interdigital Cell Death During Tetrapod Evolution

As discussed above, changes in environmental oxygen may lead to unexpected consequences during evolution: nonadaptive phenotypes may eventually lead to morphological innovations and become integrated into developmental processes. [108] Our group recently proposed that the increased amount of oxygen available to terrestrial eggs leads to the production of ROS between digits during development and the induction of cell death. [11] The removal of the interdigital webbings by cell death is unique to amniotes: amphibians employ a different mechanism to achieve the same goal (Box 1). Cell death has allowed the appearance of novel limb shapes, such as the singular morphology of the feet of aquatic birds such as the moorhen, [109] the split hands and feet of chameleons, [110] and finger loss in some mammalian clades, such as horses and camels [111]. Understanding how this system appeared in amniotes would provide valuable insights into the evolution of limb morphology. In this last section, a hypothesis regarding an ecological shift leading to the appearance of a novel step during limb development—interdigital cell death—will be addressed (Figure 3d).

Although issue regression through cell death is an important morphogenetic mechanism, the exact molecular pathway
promoting cell death in the interdigits is still unknown. Interdigital Bmp signaling integrates multiple pathways in the limbs and induces interdigital cell death in a precise timeframe, as extensively reported.\(^{77,112,113}\) The integrity of other cellular components are also required for interdigital tissue regression, including of both apoptotic and non-apoptotic pathways, cell cycle inhibitors, and the extracellular matrix remodeling machinery.\(^{27,114}\) Moreover, an additional factor is required: the production of ROS and concomitant downregulation of antioxidant defenses.\(^{78,115}\) Modulation of atmospheric oxygen levels changed the number of dying cells in both chickens and mice in a ROS-dependent manner,\(^{16,116}\) indicating the possibility that environmental oxygen might regulate limb development directly.

Classic experiments revealed that cell death did not occur in the interdigital membranes of amphibians.\(^{117,118}\) However, all of the investigated species exhibited biphasic development with aquatic larvae or tadpoles.\(^{117,118}\) Later studies described interdigital cell death in two species: seepe salmonander (Desmognathus aeneus)\(^{119}\) and the coqui frog (Eleutherodactylus coqui).\(^{16}\) Unlike previous reports,\(^{117,118}\) these two animals have terrestrial directly developing eggs (Figure 3b). To address the possibility that the increased oxygen availability was responsible for this discrepancy, the concentration of atmospheric oxygen was increased around an amphibian that typically lacks interdigital cell death, the African clawed frog (X. laevis). Surprisingly, this treatment was sufficient to induce increased ROS production and cell death specifically in the interdigital regions.\(^{16}\) The oxygen tension itself was distinct between the interdigital regions of chicken and frog, and the oxygen tension itself could be modulated by atmospheric oxygen in the limbs of both species.\(^{16}\) Hence, a new possibility arose: environmental oxygen could regulate the production of ROS within the interdigital membranes, leading to cell death.

However, why is the interdigital region permissive to induction of cell death in the first place? The answer may lie in other limb patterning mechanisms that are also important for interdigital cell death. Bmps are expressed in the interdigital regions of amphibians,\(^{120,121}\) where they are required for digit patterning\(^{122}\) a shared feature between amphibians and amniotes (Box 1). Similarly, the maturation of hypoxic digit cartilage\(^{123}\) promotes remodeling of the interdigital vasculature, which is required for cell death in mice.\(^{116}\) This vascular remodeling took place at the same stage at which cell death starts in chickens or can be induced in amphibians;\(^{16}\) additionally, transgenic X. laevis tadpoles with an increased density of blood vessels in the limbs exhibited ectopic interdigital cell death.\(^{16}\) Thus, active Bmp signaling and abundant vascular perfusion are features present in the interdigital region of tetrapods that may make this region permissive to the induction of cell death when environmental oxygen levels are sufficiently high (Figure 3d).

We propose that terrestrial direct development is important for increasing oxygen availability and ROS production within the limbs. However, it is not clear whether induced cell death initially played an adaptive role in limb morphogenesis. It is possible that this environmentally induced process emerged only as a byproduct of increased ROS levels in limbs of tetrapods with terrestrial direct development. Subsequently, modulation of oxygen- and redox-sensitive pathways integrated this new step into the limb development of amniotes coopting other digit-patterning mechanisms, in an example of genetic assimilation (Figure 3d).\(^{108}\) Future research will be essential to uncover how this environmentally induced, novel phenotype became an adaptive trait of amniotes.

7. Conclusions and Outlook

The sensing of hypoxia and the redox state of the cell started as a mechanism for survival in an oxygenated world but became integrated into multiple steps of the development of metazoans. These environmental signals are integrated with other signaling pathways, metabolism, cell cycle regulation, and epigenetic machinery, regulating both early embryonic patterning and later morphogenesis. Developmental processes regulated by oxygen or ROS include dorsoventral patterning of the zygote, neural crest migration, formation of the cardiovascular system, and endochondral ossification. In addition, the ecology and life history of each species directly influences oxygen availability, which may
lead to both adaptive and unexpected effects during their development, such as the appearance of interdigital cell death in amniontes.

The link between environment and development has been increasingly examined in the last years, but there is still a lot to investigate in this exciting field. Exploring how oxygen availability modulates the formation of diverse tissues will generate invaluable insights about the link between ecology and phenotype. Finally, further investigating oxygen-sensing pathways at a molecular level across multiple species can elucidate how these adaptive systems acquired novel developmental roles throughout evolution.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

developmental plasticity, ecology, evolution, hypoxia-inducible factor, interdigital cell death, metabolism, microenvironmental oxygen levels

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