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Oxygen, reactive oxygen species and developmental redox networks: Evo-Devo Evil-Devils?

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ABSTRACT Molecular oxygen (O_2), reactive oxygen species (ROS), and associated redox networks are cornerstones of aerobic life. These molecules and networks have gained recognition as fundamental players in mechanisms that regulate the development of multicellular organisms. First, we present a brief review in which we provide a historical description of some relevant discoveries that led to this recognition. We also discuss the fact that, despite its abundance in nature, oxygen is a limiting factor, and its high availability variation impacted the evolution of adaptive mechanisms to guarantee the proper development of diverse species under such extreme environments. Finally, some examples of when oxygen and ROS were identified as relevant for the control of developmental processes are discussed. We take into account not only the current knowledge on animal redox developmental biology, but also briefly discuss potential scenarios on the origin and evolution of redox developmental mechanisms and the importance of the ever-changing environment.

KEY WORDS: *oxygen, reactive oxygen species, metazoan, development, Eco-Evo-Devo, NADPH oxidase*

Introduction: oxygen and reactive oxygen species

Oxygen is the third most abundant element in the universe (Trimble, 1997), the most abundant element on earth's crust (Sosa Torres *et al*., 2015) and in living organisms (Campbell *et al*., 1999). Evidence for the existence of oxygen has a long history. Ibn al-Nafis in 1250 described the pulmonary circulation and how the air mingles with blood through invisible pores in the lungs to form the "vital spirit" (arterial blood). Pulmonary circulation was then rediscovered three hundred years later by Michael Servetus (Severinghaus, 2016). However, oxygen's, or "fire air", discovery is attributed to Carl Wilhelm Scheele and Joseph Priestley circa 1770. Their work was followed by Antoine Lavoisier, he actually coined the term oxygen and was the scientist who established the basis to start understanding oxygen chemistry (Sosa Torres *et al*., 2015). However, one hundred years before, Joseph Mayow obtained in 1674 the first experimental evidence supporting the biological relevance of "particula igneo-aerea" or "spiritit nitro-aereo" (later shown to be oxygen), when he observed that a mouse died when placed in a closed glass vessel along with a burning candle (Mayow, 1674). This discovery was most commonly attributed to Joseph Priestley, a self-considered "aerial philosopher". Still Priestley's contributions are no less significant, as he demonstrated that plants "restore" what he called "putrid air" to make it breathable: "I prefently had the moft indifputable proof of the reftoration of putrid air by vegetation"…"as this putrid air was thus eafily reftored to a confiderable degree of fitnefs for refpiration, by plants growing in it". Priestley's exceptional insights lead him to also propose, apparently for the first time, the oxygen cycle through photosynthesis and respiration. As described in his own words: "the injury which is continually done to the atmofphere by the refpiration of fuch a number of animals, and the putrefaction of fuch maffes of both vegetable and animal matter, is, in part at leaft, repaired by the vegetable creation. And, notwithftanding the prodigious mafs of air that is corrupted daily by the abovementioned caufes; yet, if confider the immenfe profufion of vegetables upon the face of the earth, growing in places fuited to their nature, and confequently at full liberty to exert all their powers, both inhaling and exhaling, it can hardly be thought, but that it may be a fufficient counterbalance to it, and that the remedy is adequate to the evil" (Priestley, 1772). Hence the conceptual foundations for the understanding of the relevance of living organisms' interactions with the environment at a global scale started long time ago, thanks to the insight of

Abbreviations used in this paper: Cys, cysteine; H_2O_2, hydrogen peroxide; Nox, NADPH oxidase; O_2, molecular oxygen; ROS, reactive oxygen species.

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these natural philosophers. Since their time the current landscape of oxygen's importance in biological sciences has broadened.

For example, now we know that under standard conditions of temperature and pressure, oxygen is in the form of dioxygen (O_2), a transparent and odorless gas that represents about 20.95% (volume/volume) of the earth's atmosphere.

While dioxygen is very stable, under the effect of ultraviolet radiation coming from sunlight it can form ozone at the upper atmosphere (Sosa Torres *et al.*, 2015). In 1840 Christian Friedrich Schönbein was the first scientist to generate ozone in the lab (Schönbein, 1840) and by 1881 Hartley found that ozone was capable of absorbing ultraviolet light, which in addition to its presence in the atmosphere led him to consider "whether the limitation of the solar spectrum may not be due to ozone" (Hartley, 1881), an effect demonstrated 30 years later (Fabry and Buisson, 1913). It is currently accepted that ozone is fundamental for life as we currently know it, by virtue of its capacity to strongly absorb harmful UV rays in the range of 200 to 300 nm (Canuto *et al.*, 1982). Oxygen is also central as a structural constituent of living matter since in combination with other light elements like hydrogen, carbon, and nitrogen it forms the building blocks of major organic macromolecules i.e. proteins, nucleic acids, polysaccharides, and lipids, which together with water represent most of an organism's total organismal weight matter (Lehninger, 1984).

Oxygen also plays fundamental roles in cell biochemistry and physiology. It is required in eukaryotic cell respiration for ATP production, where mitochondria are responsible for about 80% of the total ATP production in aerobic cells through the electron transfer chain (Halliwell and Gutteridge, 2015). The principles of electron transfer through the respiratory chain up to the final acceptor, oxygen were discovered by David Keilin (Keilin and Hardy, 1925, Keilin *et al.*, 1939, Slater, 2003). However, this pathway was found not to be completely efficient, since some electrons "leak" and partially reduce oxygen generating reactive oxygen species (ROS) like superoxide, hydrogen peroxide and hydroxyl radicals. Eukaryotic cells process about 10^{12} O_2 molecules per day and release 0.1% to 2% of them as partially reduced oxygen in the form of different ROS (Ames *et al.*, 1993, Hansford *et al.*, 1997). It is important to emphasize that ROS is a collective term that refers to free radical and non-radical oxygen species. Free radicals are atoms or molecules "capable of independent existence that contains one or more unpaired electrons" (Halliwell and Gutteridge, 2015). For example, dioxygen, superoxide and hydroxyl are free radicals, in contrast to hydrogen peroxide, which is not a free radical. Many different reactive species exist like nitrogen species, sulfur species, carbonyl species, and selenium species, all-important in living systems (Halliwell and Gutteridge, 2015, Sies *et al.*, 2017) however, the present review focuses only on oxygen and oxygen species.

Early studies suggested that toxic free radicals are formed during normal oxidative metabolisms, and their negative effects can be exacerbated when O_2 is supplied at higher-than-atmospheric concentrations or during the exposure of biological systems to intense ionizing radiation (Gerschman *et al.*, 1954). Later, Irwin Fridovich developed the hypothesis that *in vivo*, the high reactivity of superoxide, hydrogen peroxide and hydroxyl radicals are responsible for the toxic effects of oxygen (Fridovich, 1978). Further evidence supporting ROS toxicity was obtained by the discovery of the respiratory burst in phagocytes, a process that protects organisms from infections. During the respiratory burst phagocytes increase dramatically their oxygen consumption (Baldridge and Gerard, 1932) and produce high amounts of superoxide and hydrogen peroxide, both which possess bactericidal effects (Rossi and Zatti, 1964). This increased ROS production was found to be dependent on NADPH oxidase activity (Babior, 1984, Babior *et al.*, 1973, Rossi and Zatti, 1964). Unfortunately, ROS generated during respiratory burst cause adjacent tissue damage and are potent inducers of the inflammatory response (Hardy *et al.*, 1994, Mundi *et al.*, 1991). To counteract the negative effects of ROS different antioxidant activities, enzymatic, and non-enzymatic mechanisms, evolved in living organisms. The antioxidant enzymatic mechanisms involve the action of different enzymes that metabolize specific ROS, for example superoxide dismutase metabolizes superoxide into hydrogen peroxide and catalase, glutathione peroxidases and peroxiredoxins, which then metabolize hydrogen peroxide. The non-enzymatic mechanisms involve antioxidant molecules such as glutathione and thioredoxin (Halliwell and Gutteridge, 2015).

Then the concept of oxidative stress came into stage. The original idea referred to "an imbalance between oxidants and antioxidants in favor of the oxidants, potentially leading to damage" (Sies *et al.*, 2017). The interest on the characterization of the damage inflicted by oxidative stress to different macromolecules such as nucleic acids, proteins, and lipids, linked ROS to the etiology of a variety of diseases like ischemia reperfusion damage, rheumatoid arthritis, cancer, age-related diseases, and aging itself (Halliwell and Gutteridge, 1984). However, the notion of "oxidative stress" has been refined through the years. More recently it was proposed that when oxidative damage is caused by an excessive load of ROS then it should be referred as "oxidative distress", and it should be distinguished from "oxidative eustress", a state in which ROS are formed in amounts that support physiological functions (Niki, 2016, Sies, 2017). This change in paradigm responded to the abundant evidence showing that ROS are part of "normal" aerobic life and participates in redox signaling in the control of cell responses. Almost seventy years ago Barry Commoner and his collaborators (Commoner *et al.*, 1954) detected for the first time the formation of free radicals in biological samples obtained from plants and animals, in which free radicals were notably abundant in frog's unfertilized eggs. The identified free radicals were not oxygen based, however this particular paper experimentally demonstrated that living organisms generate important quantities of protein free radicals in normal tissues and proposed that they were most likely intermediaries involved in different oxidation-reduction processes. Some years later ROS were found in healthy normal tissues and organs like the kidney, the thyroid, in fibroblasts, osteoclasts, in other non-phagocytic cells and even in mouse embryonic tissues (Burdon, 1995, Cross and Jones, 1991). In addition, ROS were shown to stimulate cell proliferation in different cell types in tissue culture (Burdon, 1995). Currently it is known that ROS participate as signaling molecules in the control of cell proliferation, death, differentiation, and migration, all indispensable processes for animal embryonic development. ROS are produced in different cell compartments by different enzymatic activities like fatty acyl-CoA oxidase, xanthine oxidase, cytochrome p450 systems, cycloxygenases and lipoxygenases, NADPH oxidases (Nox), and the respiratory chain of mitochondria (Covarrubias *et al.*, 2008, Sies *et al.*, 2017). But we have focused our attention on two of these main sources: mitochondria and Nox. Nox are membrane-associated enzymes that catalyze the reduction of molecular oxygen to form superoxide

or hydrogen peroxide (Lambeth, 2004). As mentioned above, Nox were initially identified in the respiratory burst that occurs in different phagocytic cells, however by the end of the last century Nox1 was found to be expressed in non-phagocytic normal tissues like the colon, prostate, uterus, and vascular smooth muscle (Suh *et al.*, 1999). Soon after, other Nox genes, Nox3, 4, and 5, were found expressed in embryonic tissues suggesting that Nox expression in different normal tissues account, at least in part, for the reported ROS presence in non-phagocytic cells (Cheng *et al.*, 2001). It was found that Nox mediate a variety of responses like cell proliferation (Suh *et al.*, 1999), cell death, migration and differentiation and at a molecular level they participate in signalling mediated by different hormones and growth factors (Lambeth, 2004).

So far we have only discussed part of the prooxidant and antioxidant players of the truly complex redox network systems involved in the regulation of fundamental biological processes, however more detailed discussions on these topics exist (Halliwell and Gutteridge, 2015, Sies *et al.*, 2017). In what follows of the present review we will focus on aspects that we consider of particular relevance to the current knowledge on animal redox developmental biology and we will discuss some potential scenarios about the origin and evolution of redox developmental mechanisms and the importance of the ever-changing environment. We illustrate the main points with selected examples. Since classical and current literature on redox biology is truly overwhelming, we apologize to authors of many excellent works that are not cited mostly due to the limitation of space.

Oxygen availability

Despite oxygen being currently abundant and ubiquitous on earth's biosphere, through most of the history of life that was not the case (Fig. 1). Even today for organisms in nature, oxygen can represent a limiting factor, as it is not uniformly distributed either in air or water. This fact is commonly overlooked not only when considering the environmental variations in which the development of different organisms occurs, but also in labs, even though we attempt to tightly control experimental conditions. In general, it is taken for granted that O_2 is unlimited and it is assumed that the concentration is under what is commonly call normoxia, unless it is specified that samples are exposed to hypoxia or hyperoxia.

Although it seems counterintuitive, hypoxia is known to be required for the proper development of different tissues and organs (as we will discuss in more detail briefly), however hypoxia can have deleterious effects on the development in different organisms, depending on the severity of oxygen restriction, the duration and the developmental stage of the hypoxic event (Ritchie *et al.*, 2017). Severe hypoxia or even complete absence of oxygen or anoxia can cause developmental delay and fetal retardation in fish (Kajimura *et al.*, 2005), can induce developmental defects or can provoke embryo death in different animal species (Ritchie *et al.*, 2017), or even induce more specific effects on particular molecular pathways like interfering with nodal signaling that affect oral-aboral axis specification in sea urchin embryos (Coffman *et al.*, 2014).

But what exactly are normoxia, hypoxia and hyperoxia? Are

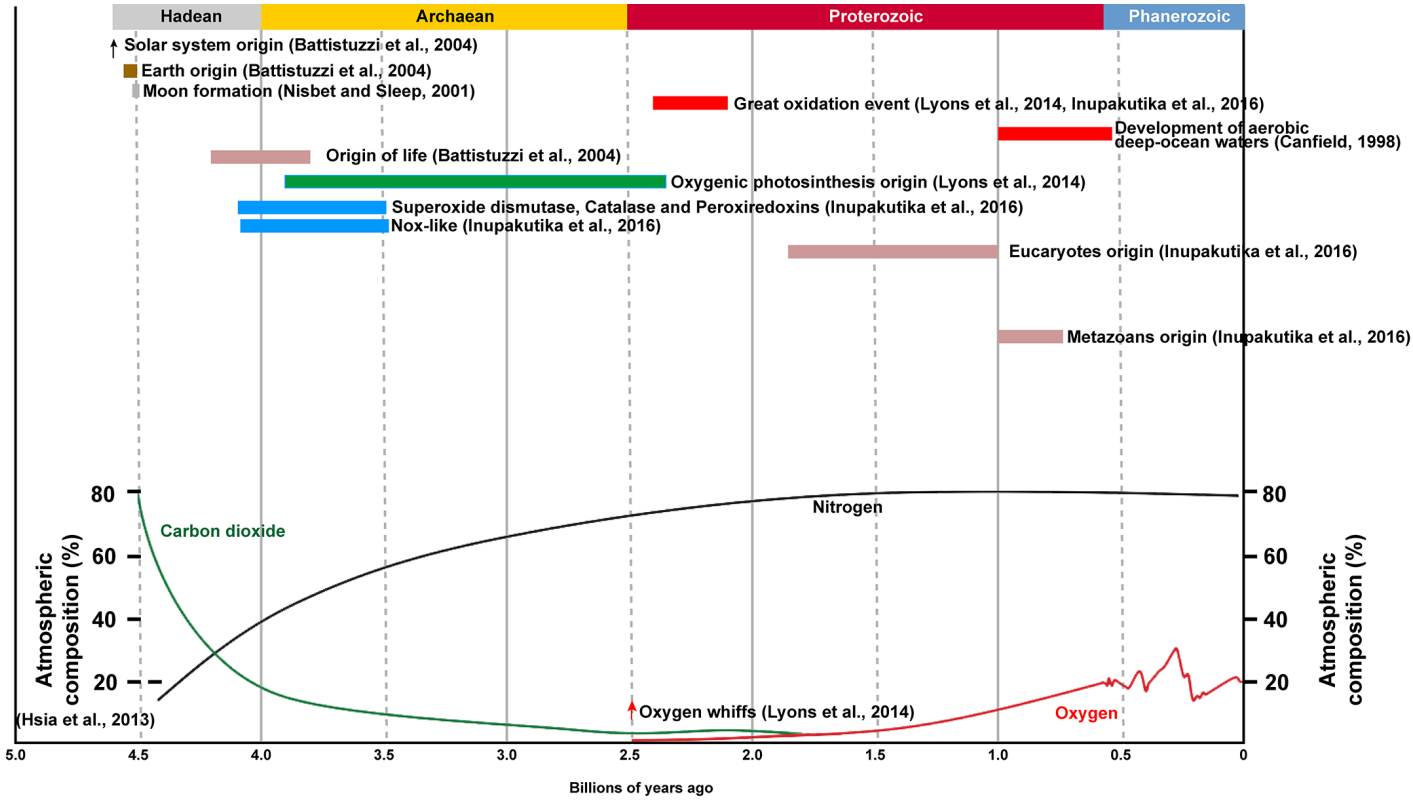

Fig. 1. Timeline of some main solar system, earth and biological events. *At the top, colored bars indicate Earth history eons. At the bottom, the red arrow indicates the approximate date for the generation of proposed O_2 whiffs (Lyons* et al.*, 2014). This model of the evolution of atmospheric gas composition is modified from data of Hsia* et al.*, 2013 and Lyons* et al.*, 2014.*

these absolute or relative conditions of O_2 availability?

In normoxia the atmospheric dry air is a mixture of different gases: 20.95% oxygen, 78.09% nitrogen, 0.93% argon and 0.03% carbon dioxide. The concentration of the different gases in air can be considered constant but it is important to note that this applies when ambient air is dry, at 0°C (standard temperature), at sea level, and under constant atmospheric pressure and constant gravity. In these conditions one atmosphere exerts an equivalent pressure of a mercury column of 760 millimeters (mmHg) of height in a manometer. Other units, like the Pascal (Pa = N/m^2) where N is a measure of force, the Newton (N = kg × m/s^2) (BIPM, 2019), are used to express the pressure of gases; however, the most commonly used units of measurement in medicine and life sciences are the mmHg and percentage (%). Each of the gases present in air exert a specific partial pressure, and the partial pressure of oxygen (PO_2) at sea level is 159 mmHg equivalent to the 20.95% (Hainsworth, 1981, Wenger *et al.*, 2015). However, the air pressure decreases exponentially as the altitude increase, and it can be calculated with the following formula: $P_{air} = P_{Atm} \times e^{-(0.127 \times alt)}$), were P_{Atm} is the atmospheric pressure at the sea level and (alt) is the altitude in kilometers (Wenger *et al.*, 2015). Therefore, the PO_2 decline imposes rising hypoxic conditions when the altitude increases, potentially affecting the development of different organisms. This is more evident in newcomers to high altitude or in animal experimental models when exposed to hypoxia. For example, different insects (*Manduca sexta*, *Tenebrio molitor* or *Drosophila melanogaster*) exposed to chronic low but ecologically realistic PO_2 present developmental delay and increased mortality rates (Dillon *et al.*, 2006). Around the world different human groups live at high altitude, 2,500 meters above the sea level. This factor is considered physiologically and clinically relevant because at this altitude arterial oxygen saturation begins to drop in most people (Julian, 2011). Groups like Andeans and Tibetans present different adaptations to hypoxia (Bigham *et al.*, 2013). However, the data regarding the effect on pregnancy and particularly in human development is controversial. In the case of native Andeans, some studies suggest an increase of intrauterine growth restriction (IGR) incidence and a decrease of birth weight by an average of 120 grams per every 1000 of meter elevation increase (Julian, 2011). Although other research groups disagree with these reports (Vitzthum, 2013), what appears to be consistent are the effects observed in lowland immigrants moving into highlands, like Europeans, who present IGR levels five times more frequently than native Andes inhabitants (Julian, 2011). Evidently native organisms of high altitude places live and reproduce under those conditions, and some even in more extreme ones, for example the Himalayan

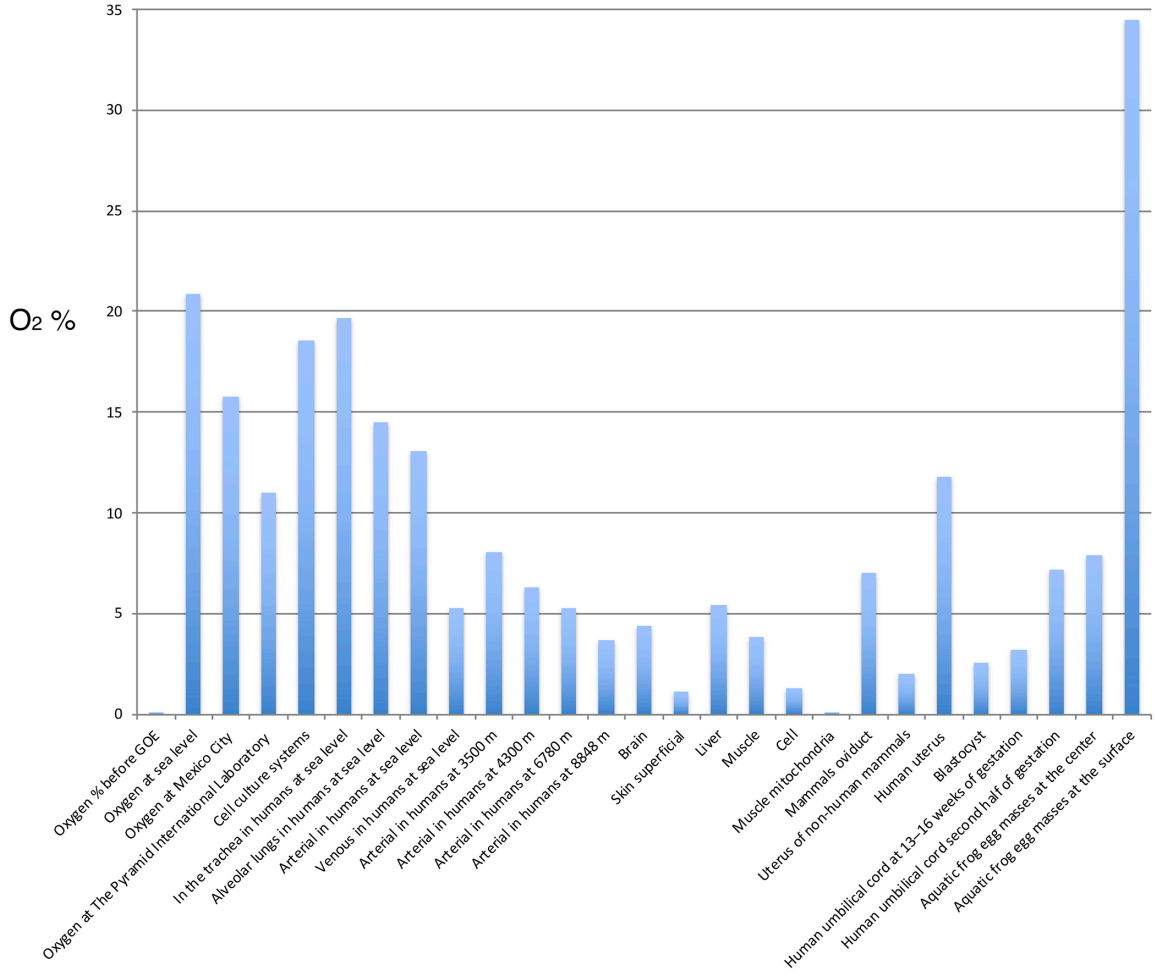

Fig. 2. Graphical representation of the estimated PO_2 values in atmospheric air and in different animal tissues *indicated with numerical values in Table 1.*

bumble-bees of the genus *Bombus* that are commonly found above 4000 m (Williams *et al.*, 2010) where oxygen can be expected to be around 12.52%.

The particular cellular, biochemical, and physiological adaptations to low oxygen in different adult animals are beginning to be discerned (McClelland and Scott, 2019). Nevertheless, the adaptations of the developmental mechanisms to low levels of O_2 can still represent an interesting area of research.

But let's go back to "normoxia". It is important to have an understanding of normal oxygen levels in different tissues and organs in our bodies. For the purpose of the present review continues to explain the expected oxygenation conditions during normal development.

The environmental oxygen contained in air is taken in through the respiratory system and then carried to the organs of the body through the oxygenated blood in the vasculature. The estimated PO_2 in different tissues in organisms including humans has been shown to present a declining gradient from high concentration in the environment to decreasing amounts in the alveoli, the blood, organs, cells and down to mitochondria (Fig. 2, Table 1) (Carreau *et al.*, 2011, Hsia *et al.*, 2013, Ortiz-Prado *et al.*, 2019). Now the particular oxygen levels in physiological and normal conditions for different tissues, cells and organelles are called "physioxia" and should be clearly distinguished from the idea of normoxia. Therefore, we can say with some confidence that normoxia, hypoxia and hyperoxia are relative terms, and should be defined taking as a reference the specific physioxia oxygen levels of the organisms or tissues used as models of study. It will also be important to experimentally determine them, to eventually perform the experiments in a more controlled environment. However, other factors should still be considered.

In addition to altitude, another fundamental factor is the water vapor contained in the air that evidently contributes with its partial pressure and affects the partial pressure of the other gases in the atmosphere (Wenger *et al.*, 2015). In the real-world atmospheric water vapor concentration varies throughout the day (Chepfer *et al.*, 2019). It can also vary depending on geographic location since it is not the same to live in the most humid regions of the world like the Amazon rainforest or in the driest deserts like the Atacama in South America. In the case of tissue culture systems, the atmosphere is, in most cases, saturated with water vapor, representing 6.2% of the atmosphere to avoid media evaporation. In addition to water vapor, there is also CO_2 which is commonly added at a concentration of 5% (Wenger *et al.*, 2015), and is required in conjunction with bicarbonate, to maintain tissue culture media pH (Freshney, 2010). Consequently, the more-realistic O_2 concentration in the atmosphere of tissue culture incubators is likely to be around 18.6% (Wenger *et al.*, 2015), although some tissue culture media do not require the additional 5% CO_2 in the atmosphere. But what is the O_2 concentration in the tissue culture media? O_2 behaves differently in air compared to water. When a gas comes in contact with a liquid the gas dissolves until equilibrium is reached. The gas concentration in the liquid depends mostly on the gas' partial-pressure and in its solubility in the liquid. The solubility of any gas in water (solubility coefficient), for example O_2 or CO_2, is measured for the pure gases in pure water at a pressure of 760 mmHg at a specific temperature. For example, the oxygen solubility coefficient at 0°C is 48.9 milliliters (ml) of oxygen in one liter of water. Compared to the coefficient at 35°C is 24.4 ml, we see an almost 50% decrease in solubility. Also, the salts contained in the water affect oxygen solubility, decreasing about 20% in sea water when compared to pure water (Hainsworth, 1981).

TABLE 1

ESTIMATED PO_2 VALUES IN ATMOSPHERIC AIR AND IN DIFFERENT ANIMAL TISSUES

	% O_2	mmHg	Reference
Oxygen % before GOE	0.001	0.007	(Lyons et al., 2014)
Oxygen at sea level	20.9	159	(Wenger et al., 2015)
Oxygen at Mexico City	15.8	120	
Oxygen at The Pyramid International Laboratory	11.04	84	
Cell culture systems	18.6	141	(Wenger et al., 2015)
In the trachea in humans at sea level	19.7	150	(Carreau et al., 2011)
Alveolar lungs in humans at sea level	14.5	110	(Carreau et al., 2011)
Arterial in humans at sea level	13.10	99.8	(Ortiz-Prado et al., 2019)
Venous in humans at sea level	5.3	40	(Carreau et al., 2011)
Arterial in humans at 3500 m	8.04	61	(Ortiz-Prado et al., 2019)
Arterial in humans at 4300 m	6.30	48	(Ortiz-Prado et al., 2019)
Arterial in humans at 6780 m	5.27	40	(Ortiz-Prado et al., 2019)
Arterial in humans at 8848 m	3.68	28	(Ortiz-Prado et al., 2019)
Brain	4.4	33.8	(Carreau et al., 2011)
Skin superficial	1.1	8	(Carreau et al., 2011)
Liver	5.4	40.6	(Carreau et al., 2011)
Muscle	3.8	29.2	(Carreau et al., 2011)
Cell	1.3	9.9	(Carreau et al., 2011)
Muscle mitochondria	0.002 to 0.02	0.02 to 0.2	(Hsia et al., 2013)
Mammals oviduct	5 to 7	37.9 to 53.1	(Ottosen et al., 2006)
Uterus of non-human mammals	2	15.2	(Ottosen et al., 2006)
Human uterus	11.8	89.5	(Ottosen et al., 2006)
Blastocyst	2.6	20	(Fathollahipour et al., 2018)
Human umbilical cord at 13–16 weeks of gestation	3.2	24	(Jauniaux et al., 2006)
Human umbilical cord second half of gestation	4.6 to 7.2	35 to 55	(Jauniaux et al., 2006)
Aquatic frog egg masses at the center	7.9	59.9	(Seymour and Roberts, 1991)
Aquatic frog egg masses at the surface	34.5	262	(Seymour and Roberts, 1991)

Therefore in the different water bodies (seawater vs freshwater) O_2 concentration will show different solubility due to environmental and physical differences like temperature, pressure, atmospheric precipitation, changes in sunlight incidence, and biochemical activities such as respiration, organic material decomposition and photosynthesis (Odum, 1956). The day/night cycle will cause temperature variation and oxygen liberation generated by photosynthetic activity; on the contrary respiration will consume oxygen which has a deeper impact in small water bodies like ponds during nighttime, favoring hypoxic environments (Ginot and Hervé, 1994). Different animals like *C. elegans*, *D. melanogaster*, amphibians and *D. rerio* can tolerate anoxia during early embryonic development, being able to survive for 24 hours under these harsh conditions by suspending their development and resuming it once they are exposed to normal air (Padilla and Roth, 2001). Particularly, zebrafish embryos tolerate 24-hour periods of anoxia during early cleavage, gastrulation and early somite segmentation, with survival rates between 83 to 98%. However, studies have shown that when 1-day embryos (during the process of straightening over the yolk), are exposed to anoxia for 24 hours, their survival rate decreases to 64% and can drop to 4.4% and cero when exposed to 24 hour anoxia starting at 30 and 50 hours of development respectively

(Padilla and Roth, 2001). The anoxia tolerance during early development in zebrafish embryos suggests that the mechanism that promotes the suspended development has been selected during the evolution of these organisms to cope with environmental oxygen variation. Other fish species inhabit even more extreme conditions, like the annual killifishes, that are freshwater teleost from Africa and America. The regions where they live cycle from rainy season with floods to dry season when most ponds dry up. During the dry season, all the juvenile and adult killifish die, while the embryos enter into a developmental arrest or diapause. The dormant states occur at particular developmental stages: Diapause I (facultative) at the stage of dispersion of blastomeres after epiboly is finished, Diapause II (obligate) during somitogenesis, and Diapause III (obligate) at pre-hatching embryo. The embryos rest in the mud until the next rain comes; embryos then resume development or the embryos most advanced in their development simply hatch and reach sexual maturity in just a few weeks (Wourms, 1972b). Interestingly, in the killifish *Austrofundulus limnaeus* embryos that are at developmental stages from early up to completion of epiboly tolerate hypoxia for 24 hours. The hypoxia tolerance in this species dramatically increases up to 62~8 days in embryos that are in somitogenesis (around 38 somite stage) and then drops after approximately 50 somite stage or four days after diapause II (Podrabsky *et al.*, 2007, Wourms, 1972a). Although zebrafish and killifish have notable developmental and habitat differences, they show the capacity to enter into developmental dormancy to protect the embryo and increase survival rates when exposed to environmental stressors like hypoxia, mostly during early development epiboly-gastrulation-somitogenesis. These are notable adaptations to extreme oscillating environments.

Another interesting strategy is found in the Australian aquatic frogs *Limnodynastes tasmaniensis*, which lay eggs in gelatinous envelopes that protect and improve the egg clutch oxygenation by virtue of maintaining them near the water surface and to avoid sinking to the anaerobic pond bottom. Some of these egg masses are truly large groups averaging 368 eggs! This kind of structures show steep PO_2 gradients from 0 kPa, but most commonly from 8 kPa (59.9 mmHg or 7.9%), up to 35 kPa (262 mmHg or 34.5%) from the center up to the surface of the egg masses. The particular high PO_2 at the clutch surface shows that oxygen-oversaturated water can exist when sunlight reaches the most intense incidence, which in turn increases the photosynthetic activity from the aquatic phototrophs (Seymour and Roberts, 1991). Other frog and salamander species like *Ambystoma gracile* present alternative strategies like being associating with green algae that grow inside the peri-vitelline membrane, providing oxygen and protection from UV-radiation, thus contributing to embryo survival and growth thanks to this symbiotic interaction (Marco and Blaustein, 2000). These examples clearly show how relative the concepts of physioxia, hypoxia and hyperoxia appear in natural environments due to the extreme oscillations in physicochemical variables, and how difficult it is to determine the range or limits of each. However, what is important to highlight are the impressive required physiological, anatomical, biochemical, genetic and behavioral adaptations that have been selected to guarantee the development of diverse species under such extreme environments that are even more variable for organisms with external development.

Another important aspect affecting oxygen solubility is that water is about 1000 times denser than air and 50 times more viscous. Oxygen takes approximately one second to diffuse 0.1 millimeter (mm) in water, but 100 seconds in 1mm and 3 hours in 1 cm. These diffusion times explain why multicellular organisms larger than a one mm develop specialized mechanisms to increase the oxygen supply to the different tissues (Hainsworth, 1981).

In organisms with internal development like mammals, a finer control of the embryo's environment exists thanks to the different anatomical and physiological adaptations; a gradient of oxygen availability to inner organs is found. The reported normal oxygen at the mammalian oviduct under physiological conditions is around 5% to 7% (37.9 to 53.1 mmHg) or even lower in the uterus reaching 2% (15.2 mmHg) in non-human mammals. In humans oxygen in the uterus has been determined to be around 11.8% (89.5 mmHg) (Ottosen *et al.*, 2006). In embryos at blastocyst stage, before implantation, oxygen is at 2.6% (20 mmHg) (Fathollahipour *et al.*, 2018). In contrast, after implantation during gestational weeks 13 to 16 the fetal blood PO_2 is 24 mmHg (3.2 %) and during the second half of the gestation is around of 35 to 55 mmHg (4.6 to 7.2 %) (Jauniaux *et al.*, 2006) (Table 1).

The data presented up to this point shows the enormous variability of oxygen availability to organisms and tissues at the different stages of their lifecycle. Now we will explore in general terms what is known about the functions of oxygen and ROS in different developmental processes and some of the potential scenarios of their evolutionary emergence.

Oxygen and ROS in animal development

Oxygen's relevance in cellular physiology not only resides in the function of respiration, oxygen also participates in more than a thousand biochemical reactions in the metabolism of aerobic organisms not present in anaerobes; many of which are fundamental in the biosynthesis of diverse biomolecules (Raymond and Segre, 2006, Thannickal, 2009). As mentioned previously, oxygen can also be partially reduced generating ROS as metabolic by-products or by specific enzymatic activities. Traditionally, ROS have been considered as mostly harmful metabolites for living organisms, however increasing evidence indicates that ROS are formed in physiological amounts (physioxia) during normal metazoan development and play essential roles (Covarrubias *et al.*, 2008, Rampon *et al.*, 2018) in stages or processes like fertilization, oocyte to embryo transition, cleavage, blastocyst, blastocyst hatching, gastrulation, morphogenesis, differentiation and stem cell maintenance among many others (Table 2).

In 1908, Otto Warburg described that oxygen consumption increases during fertilization in the sea urchin *Arbacia pustulosa* (Warburg, 1908). A hundred years later, in alternative sea urchin species, it was found that the consumed oxygen was massively converted into H_2O_2 by Nox (Udx1) activity. The oxidative burst served for the cross-linking of the fertilization envelope components to block polyspermy (Wong *et al.*, 2004). In mouse, an equivalent process is observed were the zona pellucida hardens in response to ovoperoxidase activity (Schmell and Gulyas, 1980). These examples emphasize the functional relevance of cell surface and extracellular matrix biochemical modifications generated by ROS in normal physiological processes. Later in sea urchin development, Udx1 continues to be expressed and participates in the regulation of blastomere cleavage, since treatment with the pan-Nox inhibitor DPI interferes with cell divisions, an effect that is reversible when

TABLE 2

Oxygen / ROS	Detection	ROS / redox inhibited / modulated by	ROS formation mechanism / redox system	Not sensitive to the inhibitors	Stage	Effect / ROS target	References
Sea urchin (Arbacia pustulosa)							
Oxygen	Winkler method	nd	nd	nd	Fertilization	Increase in oxygen consumption	(Warburg, 1908)
Sea urchin *(Strongylocentrotus purpuratus* or *Lytechinus variegatus)*							
H_2O_2	Luminol Amplex red	DPI	Udx1	Apo	Fertilization	Fertilization envelope cross-linking	(Wong *et al*., 2004)
Oxidative eustress, potentially H_2O_2	H2DCFDA	DPI	Udx1		Cleavage	Cell cleavage is inhibited or delayed	(Wong and Wessel, 2005)
			Mitochondria				
Fruit fly *(Drosophila melanogaster)*							
H_2O_2	roGFP	DTT, Thioredoxin Deadhead (DHD), GSH/GSSG, NADPH/ $NADP^+$	nd		Oocyte to embryo transition	Reactive cysteine-containing proteins	(Petrova *et al*., 2018)
Frog (Xenopus laevis or tropicalis)							
H_2O_2	HyPer Amplex Red	Ca2+ ionophore A23187 Malonate, antimycin A and sodium azide	Mitochondria complex II, III and IV	DPI, Apo	Fertilization, egg activation		(Han *et al*., 2018)
		Malonate and sodium azide	Mitochondria complex II and IV		Cleavage	Cell cycle arrest Cdc25C phosphatase, a key regulator of the cell cycle	(Han *et al*., 2018)
Zebrafish (Danio rerio)							
H_2O_2	HyPer	VAS2870	Nox, catalase		From blastula up to 72 hpf		(Gauron *et al*., 2016)
Oxidative eustress, possibly H_2O_2	CM-H2DCFDA	VAS2870, Apo, EUK-134, Tempol, *cyba* and *duox* splice morpholinos	Nox	DPI	Sphere, Shield, up to 24hpf	Cell motility. Potentially F-actin, tubulin, E/cadherin	(Mendieta-Serrano *et al*., 2019)
Mice (Mus musculus)							
H_2O_2	Catalase	Catalase	nd		Unhatched blastocyst	Cell death of some inner cell mass cells	(Pierce *et al*., 1991)
$O_2^{\bullet-}$	PBN spin trap.	PBN, menadione, SOD	nd	DMPO	Unhatched blastocyst	Hatching / zona pellucida	(Thomas *et al*., 1997)
Oxidative eustress, $O_2^{\bullet-}$	DCDHF-DA, MTT, DHE, DHR	Antioxidants DMSO, DCDHF-DA, phenol, NAC, DTT, EUK-134	nd			Apoptosis interdigital tissue. And correlation with regions of cell death	(Salas-Vidal *et al*., 1998, Schnabel *et al*., 2006)
C. pyrrhogaster, E. coqui, X. laevis, G. gallus							
Oxygen, oxidative eustress	DHE, CellROX Deep Red	Hyperoxia, hypoxia, NAC	Hyperoxia		Embryonic stages with limbs with interdigital tissue	Apoptosis interdigital tissue.	(Cordeiro *et al*., 2019)

Experimental evidence for the formation of reactive oxygen species (ROS) or redox changes that play important roles in developmental process in different animal models. Hydrogen peroxide (H_2O_2). $O_2^{\bullet-}$, superoxide. Diphenyleneiodonium (DPI). Apocinin (Apo). Dithiothretiol (DTT). N-acetylcysteine (NAC). N-tert-Butyl- a-phenylnitrone (PBN). Superoxide dismutase (SOD). Sod/catalase mimetic (EUK-134). 4-Hydroxy-2,2,6,6-tetramethylpiperidine 1-oxyl (Tempol). 2,7-dichlorodihydrofluorescein diacetate (DCDHF-DA). Dihydroethidium (DHE). Dihydrorhodamine (DHR). 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyl tetrazolium bromide (MTT). Dimethyl sulfoxide (DMSO). nd, not determined.

DPI is removed or can be rescued by the addition of H_2O_2 to the media (Wong and Wessel, 2005). Interestingly, following fertilization in *Xenopus*, an increase in ROS production occurs and ROS generation continues during early development. *Xenopus* fertilization induces Ca^{2+} waves that stimulate the formation of mitochondria derived H_2O_2 that not only activates the zygote but continues to be required during cleavage. The identified ROS target is the Cdc25C phosphatase, a key cell cycle regulator with conserved cysteines in the catalytic domain that are regulated by oxidation (Han *et al*., 2018). Therefore H_2O_2, generated from different sources, participates in equivalent process that are evolutionary conserved.

Another example found in sea urchin is the breaking of axial symmetry that specifies the oral-aboral axis and is regulated by a redox gradient established by the asymmetrical distribution of active mitochondria that generate an H_2O_2 gradient, which is in turn important for the regulation of *nodal* asymmetrical localization (Coffman *et al*., 2009, Coffman and Davidson, 2001, Coffman *et al*., 2004, Coffman *et al*., 2014). Nodal is a member of the TGFβ family that acts as an extracellular signalling ligand and, together with Wnt signalling, establishes the dorsal organizer, involved in axial patterning in vertebrates (Zinski *et al*., 2018). Recently it was found that the control of DNA epigenetic modifications plays important roles in mouse embryonic gastrulation through its effects on Nodal signalling (Dai *et al*., 2016). One type of modification is the prevalent methylation of the C5-position of cytosine to 5-methylcytoine (5mC) that is catalysed by DNA methyltransferases; a modification that is reversed by Ten-eleven translocation (TET) dioxygenases which oxidase 5mC to 5-hydroxymethyl cytosine (5hmC). TET dioxygenases are a family of oxygen sensitive enzymes that are important in the regulation of embryonic stem cells differentiation and, as mentioned above, have been proved to be relevant for development. It is known that under graded levels of available O_2 (0.5 to 5%) TET shows differential activity. These O_2 concentrations can be expected in different developing tissues (Lamadema *et al*., 2019). In contrast to the described phenotype of the triple mutant in mice, in zebrafish the tripe mutant of *tet1*, *tet2* and *tet3* are able to advance further in gastrulation (Li *et al*., 2015) contrary to what can be expected from the observed effects of the triple TET mutants in mouse. However, in the zebrafish model, two of the *tet* genes mutants have only the last coding exon deleted,

consequently still partial activity might be possible, potentially showing an incomplete loss of function that might explain the lack of negative effects on gastrulation of the triple mutants, an aspect that should be explored in the future. These results indicate that despite Nodal's relevance for patterning along the antero-posterior axis in zebrafish (Tuazon and Mullins, 2015) the regulation of its activity or localization may not depend on TET dioxygenases activity as reported in mice. Still these examples show that, at least in two phylogenetic distant organisms, O_2 or its derivative H_2O_2 regulate by different mechanisms a common final target Nodal that is important for the determination of embryonic axes.

In mammalian early development ROS participate in additional events. For example, in the mouse blastocyst some cells of the inner cell mass die, apparently to avoid their differentiation into trophectodermal cells and in order to maintain a balance among these two cell lineages. H_2O_2 contained in the blastocoel fluid appears to be the cell death inducer since the effect can be down regulated by catalase (Pierce *et al*., 1991). Presumably trophectodermal cells present Nox activity that is responsible for the H_2O_2 production in the non-hatched blastocyst (Manes, 1992). Later on in their development blastocyst hatch from the zona pellucida, a process that requires the formation of superoxide since hatching is inhibited *in vitro* by the superoxide scavengers PBN, SOD, and menadione, but not by the hydroxyl radical scavenger DMPO (Thomas *et al*., 1997). The H_2O_2 production from the surface of trophectodermal cells is proposed to participate in the interaction of hatched blastocyst and the endometrium in the decidual response (Manes and Lai, 1995) due to Nox activity. All the components required for the Nox complex are expressed in the trophectodermal cells (Gomes *et al*., 2012) supporting the proposal that ROS also participate in the implantation of the blastocyst (Bevilacqua *et al*., 2012).

However, ROS are involved in other developmental processes in which cell death regulation is important. More than 20 years ago we showed that in mouse embryos different tissues undergo intense oxidative eustress and that these areas correlate with regions were abundant apoptosis occurs, like in the otic vesicles at 9.5 days post coitum (dpc), in the developing eyes at 10.5-dpc, in the sternum fusion line, at the interdigital tissue of the autopod in the limbs of 13.5-dpc embryos, and along the fusion line of palatal shelves and in the prominent rugae of 14.5-dpc embryos (Salas-Vidal *et al*., 1998). Most of these events of cell death were known to occur in organs undergoing important morphogenetic remodelling (Glucksmann, 1951, Saunders, 1966). In particular the amniote limb is a classical model for the study of the remodelling function of cell death (Salas-Vidal *et al*., 2001, Saunders and Gasseling, 1962). We found that in embryonic mouse limbs the interdigital tissues present strong oxidative eustress which correlates with the regions were most cell death occurs. We also observed that the *in vitro* treatment with different antioxidants in dissected limbs significantly decreases the interdigital cell death, interfering with digit individualization (Salas-Vidal *et al*., 1998). Remarkably the interdigital–digit differential pattern of oxidative eustress and cell death was shown to depend on the enzymatic activity of glutathione peroxidase 4 (GPx4), activity that is more pronounced at the digit tissue (Schnabel *et al*., 2006). More recent studies from other research groups showed that during mouse limb development vascularization undergoes important remodelling; in particular the interdigital limb tissue shows vascular enrichment before the onset of massive cell death. Inactivation of vascular endothelial growth factor (*Vegf*) expression decreased interdigital vascularization, ROS formation, and cell death. On the contrary, *Vegf* over expression at the interdigital tissue increased vascularization, ROS formation and cell death. This chain of effects was shown to depend on the oxygen availability, since a rise in oxygen partial pressure increased ROS formation and cell death, an effect that was down regulated when limbs are exposed to hypoxic conditions (Eshkar-Oren *et al*., 2015). It is particularly relevant that the evolutionary transition of tetrapods from an aquatic environment to a terrestrial life increased the exposure to environmental oxygen, leading to an increase in ROS and in interdigital cell death (Cordeiro *et al*., 2019). These reports represent a beautiful example of the impact of changing levels of oxygen and ROS on a particular developmental morphogenetic process in the evolution of organisms during the colonization of land.

An important feature in many of these events in which oxygen, ROS, or reduction-oxidation processes are involved are the highly defined patterns in which they occur. More than a century ago Charles Manning Child described the formation of metabolic and redox axial gradients during the earliest stages of development in different animal species (Child, 1914, Child, 1941, Child, 1942). Child speculated that the observed patterns might relate to "embryonic axes and prospective regions", ideas that were possibly inspired by his previous observations on the sensitivity gradients to potassium cyanide (KCN), that upon exposure cause in planarians tissue disintegration that started at the "head" region (Child, 1913). Although Child's work did not address whether these gradients are an "effect" of development or have a "causal" link with the control of early development, his work demonstrated that dynamic patterns of chemical activities occur during development. Recently, his work has gained renewed interest by many authors in part because some of the vital dyes that were used at that time, janus green and indophenol blue, are known to stain mitochondria and therefore the gradients observed by Child can be related to the mitochondrial redox gradients that regulate oral-aboral axis specification in the sea urchin (Coffman and Denegre, 2007) and could have envisioned other developmental processes in which redox gradients participate in the determination of prospective regions. An interesting example of redox gradients was found in early zebrafish embryos that present dynamic and heterogeneous H_2O_2 levels of accumulation in space and time during early development. Particularly during gastrulation, they found a ventral-dorsal H_2O_2 gradient that primarily appears to be controlled by the degradation of H_2O_2, by catalase activity (Gauron *et al*., 2016). Unfortunately, the observed H_2O_2 ventral-dorsal gradient was not further characterized, but it is tempting to propose that the gradient is somehow related to the determination of this axis, a possibility worth exploring in the future. However, oxidative eustress has been shown to be important in other aspects in the process of gastrulation, particularly in affecting cell motility during epiboly, an event in which the blastoderm cells cover the massive cell of the yolk. In our group we recently demonstrated that oxidative eustress displays interesting dynamics during epiboly in early zebrafish embryos and we found that Nox activity is responsible for the generation of the observed oxidative eustress, presumably due to H_2O_2. Even more interesting was the finding that pharmacological inhibition of Nox decreases oxidative eustress, delays epiboly progression, alters E-cadherin and cytoskeleton patterns and, by 24 hours post-fertilization, decreases embryo survival, effects that are rescued by exogenous

hydrogen peroxide treatment (Mendieta-Serrano *et al.*, 2019).

Catalase, an enzyme that catalyzes the transformation of H_2O_2 into water and oxygen, has been recently shown to be important in regulating proliferation to differentiation transition of retinal progenitor cells in the retinal epithelium in 2 days post fertilization zebrafish (Albadri *et al.*, 2019), highlighting the relevance of redox regulation in cell differentiation. The relevance of catalase antioxidant activity has been demonstrated also during early zebrafish development, where catalase participates in the regulation of H_2O_2 temporal dynamic levels during the first seven days of development (Gauron *et al.*, 2016).

It is interesting that in the examples discussed above oxidative eustress or ROS show defined dynamic localization and/or temporal patterns. But how are these patterns established? In principle, the mechanisms responsible for ROS formation and degradation/inactivation should contribute to define the observed patterns of ROS accumulation or gradients.

One remarkable example are the constituents of the glutathione redox system that include all the genes and proteins involved in its synthesis, utilization, and recycling that show complex dynamics during development and are required for proper metazoan development. Glutathione (GSH) and glutathione disulfide (GSSG) constitute a redox couple, and the ratio of GSH to GSSG is used as an indicator of the redox cellular status (Timme-Laragy *et al.*, 2013, Timme-Laragy *et al.*, 2018). Interestingly, the periods of development (gastrulation, organogenesis and pharyngula periods) in zebrafish when more oxidized GSH levels and less reducing capacity are found (Timme-Laragy *et al.*, 2018) are the same periods when higher H_2O_2 levels and lower catalase activity are observed (Rampon *et al.*, 2018). Therefore, catalase and the GSH to GSSG ratio are fundamental players for the establishment of oxidative eustress localization, temporality, and intensity dynamics of ROS developmental patterns.

In mouse limbs the patterns of oxidative eustress and GPx4 expression and activity are complementary, indicating that GPx4 activity is involved in setting at least part of the oxidative eustress patterns that regulate cell death and tissue regression patterns relevant for the limb morphogenesis (Schnabel *et al.*, 2006). In zebrafish GPx4 shows interesting expression and protein localization patterns during early embryonic development (Mendieta-Serrano *et al.*, 2015). Preliminary unpublished work from our group suggest that GPx4 localization is important for generating the tissue boundaries of ROS or of oxidative eustress and therefore can be an additional mechanism that determines ROS spatial patterns.

Consequently, the diversity of antioxidant activities not only protects life during oxidative distress events, these mechanisms should be considered as active regulators of developmental redox signaling networks.

Redox signaling networks: origin and evolution

So far only some actors in redox status regulation have been highlighted. However, the composition of redox networks is by far much more complex and its targets are important pieces of this multidimensional puzzle. Targets of action are different macromolecules, but we will focus on proteins. Protein targets of oxidative eustress are diverse and include receptors, protein tyrosine kinases, protein tyrosine phosphatases (Corcoran and Cotter, 2013, Dustin *et al.*, 2020), components of diverse signaling pathways like members of the superfamily of small Ras GTPases (Heo, 2011), cytoskeleton components (Fiaschi *et al.*, 2006, Wilson *et al.*, 2016) and transcription factors (Brigelius-Flohe and Flohe, 2011) among many others. Most literature is dedicated to the redox sensitivity of cysteine (Cys) residues, although other amino acids like methionine are known to be susceptible to redox modifications, as well. Some Cys residues can be direct targets of H_2O_2 when proteins are found close to the microenvironment were localized and transient high levels of H_2O_2 are formed, or as recent evidence suggest, the "final" target proteins are not directly oxidized by H_2O_2 and Cys modifications result from the action of intermediate protein relays, like peroxiredoxins and thioredoxins. The intermediary proteins are highly sensitive to H_2O_2 and successively will modify the proteins that are the "final" targets (Netto and Antunes, 2016, Travasso *et al.*, 2017). About 214,000 Cys are encoded in the human genome and most proteins have at least one in their sequence (Go *et al.*, 2015). Cys residues are known to be underrepresented in the protein sequences in different organisms like humans and other mammals (2.26%), fruit flies (1.90%), worms (*C. elegans*) (1.97%), yeast (1.25%) or eubacteria like *E. coli* (1.13%), and the thermophil *T. aquaticus* (0.41%) from the theoretically expected abundance of 3.28% calculated for an amino acid (Cys) coded by 2 out of 61 codons. This suggests that there is a selection against Cys utilization in proteins (Go *et al.*, 2015, Miseta and Csutora, 2000). However, the observed increase in Cys residues representation in the proteomes from the different organisms analyzed positively correlate with the increase of complexity of the organisms (Miseta and Csutora, 2000), stressing the importance of Cys in the evolution of redox regulated signaling and control functions, and the increase of potential targets that participate in the redox networks important for development.

But when did these networks appear in evolution? Are redox networks evolutionary novelties exclusive of eukaryotes and in particular of the ones that gave rise to multicellular organisms?

It has been hypothesized for long time that free oxygen in the atmosphere was a prerequisite for the origin and evolution of metazoans (Nursall, 1959) based on the assumption that the high energy demand imposed constraints to the origin of multicellular organisms and that they were only liberated from these constrains once free oxygen was available in sufficient amounts in the environment.

Current evidence suggests that life originated around 4.2 to 3.8 billions of years ago (bya) in an anoxic environment. Oxygenic photosynthesis evolved much later around 3.8 to 2.15 bya (Lyons *et al.*, 2014). Eukaryotes are estimated to have evolved about 1.8 and 1 bya (Eme *et al.*, 2014) in an environment that still contained low levels of free oxygen, since it is considered that well-oxygenated oceans were only possible between 1 to 0.54 bya (Canfield, 1998). Evidence based on paleontological samples and molecular clocks indicate that the possible origin of multicellular organisms occurred around 1 to 0.72 bya (Dohrmann and Worheide, 2017) implying that the line that originated metazoans enjoyed a more oxygenated environment and most evidence suggest that multicellularity was only possible or was limited by the available free oxygen. However, the appearance of some of the proteins discussed in previous sections that are involved in the regulation of ROS and oxidative eustress have been dated long before the origin of eukaryotes in environments presumably lacking free oxygen. In spite of this data, is common to assume that proteins with antioxidant enzymatic activities should have arouse after or during the oxygen rise in the

atmosphere. This view has been challenged thanks to information generated in the last twenty years of research.

The origin of fundamental antioxidant enzymes like superoxide dismutase, catalase, peroxiredoxin, ferric reductase/oxidoreductase, and catalase/peroxidase is proposed to have occurred about 4.1–3.5 billion years ago and their appearance was followed by ancestral forms of Nox (Inupakutika *et al*., 2016) (Fig. 1), 1 billion years before the great oxidation event (GOE), that is considered the period when free oxygen presence was in sufficient amounts to leave undisputable evidence in the geologic record (Lyons *et al*., 2014). Nonetheless, some trace amounts of free oxygen were possibly present before the GOE, around 0.001% that could have originated due to inorganic photolysis and peroxy hydrolysis, which could also generate ROS (Hsia *et al*., 2013) exposing primordial living organisms to oxygen that could have acted as sufficient environmental pressure necessary for the selection of antioxidant activities. Some groups have performed sequence analysis to reconstruct the potential sequences of ancestral thioredoxins in the Last Bacterial Common Ancestor (LBCA), Last Archaeal Common Ancestor (LACA), Archaeal–Eukaryotic Common Ancestor (AECA), Last Eukaryotic Common Ancestor (LECA), Last Animalia and Fungi Common Ancestor (LAFCA), and tested experimentally the activity of these "resurrected proteins". Interestingly, they found through the activity assays that resurrected thioredoxins show evidence that thioredoxins served as catalysts of cellular reduction reactions from the beginning of evolution, even before the rise of oxygen in the GOE (Napolitano *et al*., 2019). By aligning the sequences of resurrected thioredoxins with the sequences of human, zebrafish, the cnidaria *N. vectensis*, and the porifera *A. queenslandica* it is shown that the cysteines Cys32 and Cys35 in the respective active sites are conserved and also show that the Cys residues are conserved between organisms. It should be emphasized that some thioredoxin targets are NF-κB (p50) and redox factor-1 (Ref-1) (Go *et al*., 2015), therefore ancestral thioredoxin systems persist in metazoans that function not only as antioxidants but as relays in the regulation of transcription factors important for development.

Importantly we have also found that the Cys residues 330 and 377 of Cdc25C, phosphatase that is redox sensitive in *Xenopus* and that participates in the control of early blastomere cleavage (Han *et al*., 2018) are conserved in human, zebrafish, *N. vectensis* and *A. queenslandica*. These two last organisms represent metazoan early branching taxa and therefore represent that the redox control mechanism apparently appeared early in the origin of metazoan. We have found that other proteins relevant in the control of development have deep evolutionary conservation of Cys residues that are potentially redox regulated, indicating that these redox control systems were established early in the origin of metazoans and some apparently even before and are inherited from metazoan ancestors.

The information discussed in the present review shows that redox EcoEvoDevo is a nascent but expanding area that will give us incredible discoveries in the near future, and will uncover the degree in which such humble molecules like dioxygen and ROS play in the complex regulation developmental networks.

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References

ALBADRI, S., NASO, F., THAUVIN, M., GAURON, C., PAROLIN, C., DUROURE, K., VOUGNY, J., FIORI, J., BOGA, C., VRIZ, S. *et al.,* (2019). Redox Signaling via Lipid Peroxidation Regulates Retinal Progenitor Cell Differentiation. *Dev Cell* 50: 73-89 e6.

AMES, B.N., SHIGENAGA, M.K. and HAGEN, T.M. (1993). Oxidants, antioxidants, and the degenerative diseases of aging. *Proc Natl Acad Sci USA* 90: 7915-7922.

BABIOR, B.M. (1984). The respiratory burst of phagocytes. *J Clin Invest* 73: 599-601.

BABIOR, B.M., KIPNES, R.S. and CURNUTTE, J.T. (1973). Biological defense mechanisms. The production by leukocytes of superoxide, a potential bactericidal agent. *J Clin Invest* 52: 741-744.

BALDRIDGE, C.W. and GERARD, R.W. (1932). The extra respiration of phagocytosis. *Am. J. Physiol.* 103: 235-236.

BEVILACQUA, E., GOMES, S.Z., LORENZON, A.R., HOSHIDA, M.S. and AMARANTE-PAFFARO, A.M. (2012). NADPH oxidase as an important source of reactive oxygen species at the mouse maternal-fetal interface: putative biological roles. *Reprod Biomed Online* 25: 31-43.

BIGHAM, A.W., WILSON, M.J., JULIAN, C.G., KIYAMU, M., VARGAS, E., LEON-VELARDE, F., RIVERA-CHIRA, M., RODRIQUEZ, C., BROWNE, V.A., PARRA, E. *et al.,* (2013). Andean and Tibetan patterns of adaptation to high altitude. *Am J Hum Biol* 25: 190-197.

BIPM. (2019). The International System of Units (SI), ed., pp. 216. Cedex France: Bureau International des Poids et Mesures.

BRIGELIUS-FLOHE, R. and FLOHE, L. (2011). Basic principles and emerging concepts in the redox control of transcription factors. *Antioxid Redox Signal* 15: 2335-2381.

BURDON, R.H. (1995). Superoxide and hydrogen peroxide in relation to mammalian cell proliferation. *Free Radic Biol Med* 18: 775-794.

CAMPBELL, N.A., REECE, J.B. and MITCHELL, L.G. (1999). *Biology*. Addison Wesley Longman, Inc.

CANFIELD, D.E. (1998). A new model for Proterozoic ocean chemistry. *Nature* 396: 450-453.

CANUTO, V.M., LEVINE, J.S., AUGUSTSSON, T.R. and IMHOFF, C.L. (1982). UV radiation from the young Sun and oxygen and ozone levels in the prebiological palaeoatmosphere. *Nature* 296: 816-820.

CARREAU, A., EL HAFNY-RAHBI, B., MATEJUK, A., GRILLON, C. and KIEDA, C. (2011). Why is the partial oxygen pressure of human tissues a crucial parameter? Small molecules and hypoxia. *J Cell Mol Med* 15: 1239-1253.

CHENG, G., CAO, Z., XU, X., VAN MEIR, E.G. and LAMBETH, J.D. (2001). Homologs of gp91phox: cloning and tissue expression of Nox3, Nox4, and Nox5. *Gene* 269: 131-140.

CHEPFER, H., BROGNIEZ, H. and NOEL, V. (2019). Diurnal variations of cloud and relative humidity profiles across the tropics. *Sci Rep* 9: 16045.

CHILD, C.M. (1913). Certain dynamic factors in experimental reproduction and their significance for the problems of reproduction and development. *Roux Archiv. Dev. Biol.* 35: 598-641.

CHILD, C.M. (1914). Susceptibility Gradients in Animals. *Science* 39: 73-76.

CHILD, C.M. (1941). Formation and Reduction of Indophenol Blue in Development of an Echinoderm. *Proc Natl Acad Sci USA* 27: 523-528.

CHILD, C.M. (1942). Oxidation-Reduction Patterns in Amphibian and Teleost Development. *Proc Natl Acad Sci USA* 28: 339-343.

COFFMAN, J.A., COLUCCIO, A., PLANCHART, A. and ROBERTSON, A.J. (2009). Oral-aboral axis specification in the sea urchin embryo III. Role of mitochondrial redox signaling via H2O2. *Dev Biol* 330: 123-130.

COFFMAN, J.A. and DAVIDSON, E.H. (2001). Oral-aboral axis specification in the sea urchin embryo. I. Axis entrainment by respiratory asymmetry. *Dev Biol* 230: 18-28.

COFFMAN, J.A. and DENEGRE, J.M. (2007). Mitochondria, redox signaling and axis specification in metazoan embryos. *Dev Biol* 308: 266-280.

COFFMAN, J.A., MCCARTHY, J.J., DICKEY-SIMS, C. and ROBERTSON, A.J. (2004). Oral-aboral axis specification in the sea urchin embryo II. Mitochondrial distribution and redox state contribute to establishing polarity in Strongylocentrotus

purpuratus. *Dev Biol* 273: 160-171.

COFFMAN, J.A., WESSELS, A., DESCHIFFART, C. and RYDLIZKY, K. (2014). Oral-aboral axis specification in the sea urchin embryo, IV: hypoxia radializes embryos by preventing the initial spatialization of nodal activity. *Dev Biol* 386: 302-307.

COMMONER, B., TOWNSEND, J. and PAKE, G.E. (1954). Free radicals in biological materials. *Nature* 174: 689-691.

CORCORAN, A. and COTTER, T.G. (2013). Redox regulation of protein kinases. *FEBS J* 280: 1944-1965.

CORDEIRO, I.R., KABASHIMA, K., OCHI, H., MUNAKATA, K., NISHIMORI, C., LASLO, M., HANKEN, J. and TANAKA, M. (2019). Environmental Oxygen Exposure Allows for the Evolution of Interdigital Cell Death in Limb Patterning. *Dev Cell* 50: 155-166 e4.

COVARRUBIAS, L., HERNÁNDEZ-GARCÍA, D., SCHNABEL-PERAZA, D., SALAS-VIDAL, E. and CASTRO-OBREGON, S. (2008). Function of reactive oxygen species during animal development: Passive or active?. *Dev Biol* 320: 1-11.

CROSS, A.R. and JONES, O.T. (1991). Enzymic mechanisms of superoxide production. *Biochim Biophys Acta* 1057: 281-298.

DAI, H.Q., WANG, B.A., YANG, L., CHEN, J.J., ZHU, G.C., SUN, M.L., GE, H., WANG, R., CHAPMAN, D.L., TANG, F. *et al.,* (2016). TET-mediated DNA demethylation controls gastrulation by regulating Lefty-Nodal signalling. *Nature* 538: 528-532.

DILLON, M.E., FRAZIER, M.R. and DUDLEY, R. (2006). Into thin air: Physiology and evolution of alpine insects. *Integr Comp Biol* 46: 49-61.

DOHRMANN, M. and WORHEIDE, G. (2017). Dating early animal evolution using phylogenomic data. *Sci Rep* 7: 3599.

DUSTIN, C.M., HEPPNER, D.E., LIN, M.J. and VAN DER VLIET, A. (2020). Redox regulation of tyrosine kinase signalling: more than meets the eye. *J Biochem* 167: 151-163.

EME, L., SHARPE, S.C., BROWN, M.W. and ROGER, A.J. (2014). On the age of eukaryotes: evaluating evidence from fossils and molecular clocks. *Cold Spring Harb Perspect Biol* 6: a016139.

ESHKAR-OREN, I., KRIEF, S., FERRARA, N., ELLIOTT, A.M. and ZELZER, E. (2015). Vascular patterning regulates interdigital cell death by a ROS-mediated mechanism. *Development* 142: 672-680.

FABRY, C. and BUISSON, H. (1913). L'absorption de l'ultra-violet par l'ozone et la limite du spectre solaire. *J. Phys. Theor. Appl.* 3: 196-206.

FATHOLLAHIPOUR, S., PATIL, P.S. and LEIPZIG, N.D. (2018). Oxygen Regulation in Development: Lessons from Embryogenesis towards Tissue Engineering. *Cells Tissues Organs* 205: 350-371.

FIASCHI, T., COZZI, G., RAUGEI, G., FORMIGLI, L., RAMPONI, G. and CHIARUGI, P. (2006). Redox regulation of beta-actin during integrin-mediated cell adhesion. *J Biol Chem* 281: 22983-22991.

FRESHNEY, R.I. (2010). *Culture of Animal Cells.* John Wiley & Sons, Inc.

FRIDOVICH, I. (1978). The biology of oxygen radicals. *Science* 201: 875-80.

GAURON, C., MEDA, F., DUPONT, E., ALBADRI, S., QUENECH'DU, N., IPENDEY, E., VOLOVITCH, M., DEL BENE, F., JOLIOT, A., RAMPON, C. *et al.,* (2016). Hydrogen peroxide (HO) controls axon pathfinding during zebrafish development. *Dev Biol* 414: 133-141.

GERSCHMAN, R., GILBERT, D.L., NYE, S.W., DWYER, P. and FENN, W.O. (1954). Oxygen poisoning and x-irradiation: a mechanism in common. *Science* 119: 623-626.

GINOT, V. and HERVÉ, J.-C. (1994). Estimating the parameters of dissolved oxygen dynamics in shallow ponds. *Ecological Mod.* 73: 169-187.

GLUCKSMANN, A. (1951). Cell deaths in normal vertebrate ontogeny. *Biol Rev Camb Philos Soc* 26: 59-86.

GO, Y.M., CHANDLER, J.D. and JONES, D.P. (2015). The cysteine proteome. *Free Radic Biol Med* 84: 227-245.

GOMES, S.Z., LORENZON, A.R., VIEIRA, J.S., ROCHA, C.R., BANDEIRA, C., HOSHIDA, M.S., LOPES, L.R. and BEVILACQUA, E. (2012). Expression of NADPH oxidase by trophoblast cells: potential implications for the postimplanting mouse embryo. *Biol Reprod* 86: 56.

HAINSWORTH, F.R. (1981). *Animal Physiology.* Addison-Wesley Publishing Company Inc., USA.

HALLIWELL, B. and GUTTERIDGE, J.M. (1984). Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem J* 219: 1-14.

HALLIWELL, B. and GUTTERIDGE, J.M. (2015). *Free Radicals in Biology and Medicine* Oxford University Press.

HAN, Y., ISHIBASHI, S., IGLESIAS-GONZALEZ, J., CHEN, Y., LOVE, N.R. and AMAYA, E. (2018). Ca(2+)-Induced Mitochondrial ROS Regulate the Early Embryonic Cell Cycle. *Cell Rep* 22: 218-231.

HANSFORD, R.G., HOGUE, B.A. and MILDAZIENE, V. (1997). Dependence of H2O2 formation by rat heart mitochondria on substrate availability and donor age. *J Bioenerg Biomembr* 29: 89-95.

HARDY, M.M., FLICKINGER, A.G., RILEY, D.P., WEISS, R.H. and RYAN, U.S. (1994). Superoxide dismutase mimetics inhibit neutrophil-mediated human aortic endothelial cell injury in vitro. *J Biol Chem* 269: 18535-18540.

HARTLEY, W.N. (1881). XXI.—On the absorption of solar rays by atmospheric ozone. *J. Chem. Soc. Trans.* 39: 111-128.

HEO, J. (2011). Redox control of GTPases: from molecular mechanisms to functional significance in health and disease. *Antioxid Redox Signal* 14: 689-724.

HSIA, C.C., SCHMITZ, A., LAMBERTZ, M., PERRY, S.F. and MAINA, J.N. (2013). Evolution of air breathing: oxygen homeostasis and the transitions from water to land and sky. *Compr Physiol* 3: 849-915.

INUPAKUTIKA, M.A., SENGUPTA, S., DEVIREDDY, A.R., AZAD, R.K. and MITTLER, R. (2016). The evolution of reactive oxygen species metabolism. *J Exp Bot* 67: 5933-5943.

JAUNIAUX, E., POSTON, L. and BURTON, G.J. (2006). Placental-related diseases of pregnancy: Involvement of oxidative stress and implications in human evolution. *Hum Reprod Update* 12: 747-755.

JULIAN, C.G. (2011). High altitude during pregnancy. *Clin Chest Med* 32: 21-31.

KAJIMURA, S., AIDA, K. and DUAN, C. (2005). Insulin-like growth factor-binding protein-1 (IGFBP-1) mediates hypoxia-induced embryonic growth and developmental retardation. *Proc Natl Acad Sci USA* 102: 1240-1245.

KEILIN, D. and HARDY, W.B. (1925). On cytochrome, a respiratory pigment, common to animals, yeast, and higher plants. *Proc. Royal Soc. London - Series B, Containing Papers of a Biological Character* 98: 312-339.

KEILIN, D., HARTREE, E.F. and HARTREE, E.F. (1939). Cytochrome and cytochrome oxidase. *Proc. Royal Soc. London Series B - Biological Sciences* 127: 167-191.

LAMADEMA, N., BURR, S. and BREWER, A.C. (2019). Dynamic regulation of epigenetic demethylation by oxygen availability and cellular redox. *Free Radic Biol Med* 131: 282-298.

LAMBETH, J.D. (2004). NOX enzymes and the biology of reactive oxygen. *Nat Rev Immunol* 4: 181-189.

LEHNINGER, A.L. (1984). *Bioquímica.* Ediciones Omega, S.A., Barcelona.

LI, C., LAN, Y., SCHWARTZ-ORBACH, L., KOROL, E., TAHILIANI, M., EVANS, T. and GOLL, M.G. (2015). Overlapping Requirements for Tet2 and Tet3 in Normal Development and Hematopoietic Stem Cell Emergence. *Cell Rep* 12: 1133-1143.

LYONS, T.W., REINHARD, C.T. and PLANAVSKY, N.J. (2014). The rise of oxygen in Earth's early ocean and atmosphere. *Nature* 506: 307-315.

MANES, C. (1992). Cyanide-resistant reduction of nitroblue tetrazolium and hydrogen peroxide production by the rabbit blastocyst. *Mol Reprod Dev* 31: 114-21.

MANES, C. and LAI, N.C. (1995). Nonmitochondrial oxygen utilization by rabbit blastocysts and surface production of superoxide radicals. *J Reprod Fertil* 104: 69-75.

MARCO, A. and BLAUSTEIN, A.R. (2000). Symbiosis with Green Algae Affects Survival and Growth of Northwestern Salamander Embryos. *J. Herpetol.* 34: 617-621.

MAYOW, J. (1674). *MEDICO-PHYSICAL WORKS.* THE ALEMBIC CLUB, Edinburgh.

MCCLELLAND, G.B. and SCOTT, G.R. (2019). Evolved Mechanisms of Aerobic Performance and Hypoxia Resistance in High-Altitude Natives. *Annu Rev Physiol* 81: 561-583.

MENDIETA-SERRANO, M.A., MENDEZ-CRUZ, F.J., ANTUNEZ-MOJICA, M., SCHNABEL, D., ALVAREZ, L., CARDENAS, L., LOMELI, H., RUIZ-SANTIESTEBAN, J.A. and SALAS-VIDAL, E. (2019). NADPH-Oxidase-derived reactive oxygen species are required for cytoskeletal organization, proper localization of E-cadherin and cell motility during zebrafish epiboly. *Free Radic Biol Med* 130: 82-98.

MENDIETA-SERRANO, M.A., SCHNABEL, D., LOMELI, H. and SALAS-VIDAL, E. (2015). Spatial and temporal expression of zebrafish glutathione peroxidase 4 a and b genes during early embryo development. *Gene Expr Patterns* 19: 98-107.

MISETA, A. and CSUTORA, P. (2000). Relationship between the occurrence of cysteine in proteins and the complexity of organisms. *Mol Biol Evol* 17: 1232-1239.

MUNDI, H., BJORKSTEN, B., SVANBORG, C., OHMAN, L. and DAHLGREN, C. (1991). Extracellular release of reactive oxygen species from human neutrophils upon interaction with Escherichia coli strains causing renal scarring. *Infect Immun* 59: 4168-4172.

NAPOLITANO, S., REBER, R.J., RUBINI, M. and GLOCKSHUBER, R. (2019). Functional analyses of ancestral thioredoxins provide insights into their evolutionary history. *J Biol Chem* 294: 14105-14118.

NETTO, L.E. and ANTUNES, F. (2016). The Roles of Peroxiredoxin and Thioredoxin in Hydrogen Peroxide Sensing and in Signal Transduction. *Mol Cells* 39: 65-71.

NIKI, E. (2016). Oxidative stress and antioxidants: Distress or eustress? *Arch Biochem Biophys* 595: 19-24.

NURSALL, J.R. (1959). Oxygen as a Prerequisite to the Origin of the Metazoa. *Nature* 183: 1170-1172.

ODUM, H.T. (1956). Primary Production in Flowing Waters1. *Limnol. Oceanogra.* 1: 102-117.

ORTIZ-PRADO, E., DUNN, J.F., VASCONEZ, J., CASTILLO, D. and VISCOR, G. (2019). Partial pressure of oxygen in the human body: a general review. *Am J Blood Res* 9: 1-14.

OTTOSEN, L.D., HINDKAER, J., HUSTH, M., PETERSEN, D.E., KIRK, J. and INGERSLEV, H.J. (2006). Observations on intrauterine oxygen tension measured by fibre-optic microsensors. *Reprod Biomed Online* 13: 380-385.

PADILLA, P.A. and ROTH, M.B. (2001). Oxygen deprivation causes suspended animation in the zebrafish embryo. *Proc Natl Acad Sci USA* 98: 7331-7335.

PIERCE, G.B., PARCHMENT, R.E. and LEWELLYN, A.L. (1991). Hydrogen peroxide as a mediator of programmed cell death in the blastocyst. *Differentiation* 46: 181-186.

PODRABSKY, J.E., LOPEZ, J.P., FAN, T.W., HIGASHI, R. and SOMERO, G.N. (2007). Extreme anoxia tolerance in embryos of the annual killifish Austrofundulus limnaeus: insights from a metabolomics analysis. *J Exp Biol* 210: 2253-2266.

PRIESTLEY, J. (1772). XIX. Observations on different kinds of air. *Phil. Trans. Royal Soc. London* 62: 147-264.

RAMPON, C., VOLOVITCH, M., JOLIOT, A. and VRIZ, S. (2018). Hydrogen Peroxide and Redox Regulation of Developments. *Antioxidants (Basel)* 7: 159.

RAYMOND, J. and SEGRE, D. (2006). The effect of oxygen on biochemical networks and the evolution of complex life. *Science* 311: 1764-1767.

RITCHIE, H.E., OAKES, D.J., KENNEDY, D. and POLSON, J.W. (2017). Early Gestational Hypoxia and Adverse Developmental Outcomes. *Birth Defects Res* 109: 1358-1376.

ROSSI, F. and ZATTI, M. (1964). Biochemical aspects of phagocytosis in polymorphonuclear leucocytes. NADH and NADPH oxidation by the granules of resting and phagocytizing cells. *Experientia* 20: 21-23.

SALAS-VIDAL, E., LOMELI, H., CASTRO-OBREGON, S., CUERVO, R., ESCALANTE-ALCALDE, D. and COVARRUBIAS, L. (1998). Reactive oxygen species participate in the control of mouse embryonic cell death. *Exp Cell Res* 238: 136-47.

SALAS-VIDAL, E., VALENCIA, C. and COVARRUBIAS, L. (2001). Differential tissue growth and patterns of cell death in mouse limb autopod morphogenesis. *Dev Dyn* 220: 295-306.

SAUNDERS, J.W., JR. (1966). Death in embryonic systems. *Science* 154: 604-612.

SAUNDERS, J.W., JR. and GASSELING, M.T. (1962). Cellular death in morphogenesis of the avian wing. *Dev Biol* 5: 147-178.

SCHMELL, E.D. and GULYAS, B.J. (1980). Ovoperoxidase Activity in Ionophore Treated Mouse Eggs. II. Evidence for the Enzyme's Role in Hardening the Zona Pellucida. *Gamete Res.* 3: 279-290.

SCHNABEL, D., SALAS-VIDAL, E., NARVAEZ, V., SANCHEZ-CARBENTE MDEL, R., HERNANDEZ-GARCIA, D., CUERVO, R. and COVARRUBIAS, L. (2006). Expression and regulation of antioxidant enzymes in the developing limb support a function of ROS in interdigital cell death. *Dev Biol* 291: 291-299.

SCHÖNBEIN, C.F. (1840). Recherches sur la nature de l'odeur, qui se manifeste dans certaines actions chimiques. *C.R. Hebd. Seances Acad. Sci. Ser. C.* 10: 706–710.

SEVERINGHAUS, J.W. (2016). Eight sages over five centuries share oxygen's discovery. *Adv Physiol Educ* 40: 370-376.

SEYMOUR, R.S. and ROBERTS, J.D. (1991). Embryonic Respiration and Oxygen Distribution in Foamy and Nonfoamy Egg Masses of the Frog Limnodynastes tasmaniensis. *Physiol. Zool.* 64: 1322-1340.

SIES, H. (2017). Hydrogen peroxide as a central redox signaling molecule in physiological oxidative stress: Oxidative eustress. *Redox Biol* 11: 613-619.

SIES, H., BERNDT, C. and JONES, D.P. (2017). Oxidative Stress. *Annu Rev Biochem* 86: 715-748.

SLATER, E.C. (2003). Keilin, cytochrome, and the respiratory chain. *J Biol Chem* 278: 16455-16461.

SOSA TORRES, M.E., SAUCEDO-VAZQUEZ, J.P. and KRONECK, P.M. (2015). The magic of dioxygen. *Met Ions Life Sci* 15: 1-12.

SUH, Y.A., ARNOLD, R.S., LASSEGUE, B., SHI, J., XU, X., SORESCU, D., CHUNG, A.B., GRIENDLING, K.K. and LAMBETH, J.D. (1999). Cell transformation by the superoxide-generating oxidase Mox1. *Nature* 401: 79-82.

THANNICKAL, V.J. (2009). Oxygen in the evolution of complex life and the price we pay. *Am J Respir Cell Mol Biol* 40: 507-510.

THOMAS, M., JAIN, S., KUMAR, G.P. and LALORAYA, M. (1997). A programmed oxyradical burst causes hatching of mouse blastocysts. *J Cell Sci* 110 (Pt 14): 1597-602.

TIMME-LARAGY, A.R., GOLDSTONE, J.V., IMHOFF, B.R., STEGEMAN, J.J., HAHN, M.E. and HANSEN, J.M. (2013). Glutathione redox dynamics and expression of glutathione-related genes in the developing embryo. *Free Radic Biol Med* 65: 89-101.

TIMME-LARAGY, A.R., HAHN, M.E., HANSEN, J.M., RASTOGI, A. and ROY, M.A. (2018). Redox stress and signaling during vertebrate embryonic development: Regulation and responses. *Semin Cell Dev Biol* 80: 17-28.

TRAVASSO, R.D.M., SAMPAIO DOS AIDOS, F., BAYANI, A., ABRANCHES, P. and SALVADOR, A. (2017). Localized redox relays as a privileged mode of cytoplasmic hydrogen peroxide signaling. *Redox Biol* 12: 233-245.

TRIMBLE, V. (1997). Origin of the biologically important elements. *Orig Life Evol Biosph* 27: 3-21.

TUAZON, F.B. and MULLINS, M.C. (2015). Temporally coordinated signals progressively pattern the anteroposterior and dorsoventral body axes. *Semin Cell Dev Biol* 42: 118-33.

VITZTHUM, V.J. (2013). Fifty fertile years: anthropologists' studies of reproduction in high altitude natives. *Am J Hum Biol* 25: 179-189.

WARBURG, O. (1908). Beobachtungen über die Oxydationsprozesse im Seeigelei. In *Hoppe-Seyler´s Zeitschrift für physiologische Chemie*, vol. 57.

WENGER, R.H., KURTCUOGLU, V., SCHOLZ, C.C., MARTI, H.H. and HOOGEWIJS, D. (2015). Frequently asked questions in hypoxia research. *Hypoxia (Auckl)* 3: 35-43.

WILLIAMS, P.H., ITO, M., MATSUMURA, T. and KUDO, I. (2010). The bumblebees of the Nepal Himalaya (Hymenoptera: Apidae). *Insecta Matsumurana* 66: 19-52.

WILSON, C., TERMAN, J.R., GONZALEZ-BILLAULT, C. and AHMED, G. (2016). Actin filaments-A target for redox regulation. *Cytoskeleton (Hoboken)* 73: 577-595.

WONG, J.L., CRETON, R. and WESSEL, G.M. (2004). The oxidative burst at fertilization is dependent upon activation of the dual oxidase Udx1. *Dev Cell* 7: 801-814.

WONG, J.L. and WESSEL, G.M. (2005). Reactive oxygen species and Udx1 during early sea urchin development. *Dev Biol* 288: 317-333.

WOURMS, J.P. (1972a). Developmental Biology of Annual Fishes. *J. Exp. Zool.* 182: 143-168.

WOURMS, J.P. (1972b). The developmental biology of annual fishes. III. Pre-embryonic and embryonic diapause of variable duration in the eggs of annual fishes. *J. Exp. Zool.* 182: 389-414.

ZINSKI, J., TAJER, B. and MULLINS, M.C. (2018). TGF-beta Family Signaling in Early Vertebrate Development. *Cold Spring Harb Perspect Biol* 10: a033274.

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