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Opinion discussion

Reactive oxygen species may play an essential role in driving biological evolution: The Cambrian Explosion as an example

Dong Yang[1], *Xuejun Guo*[2,*], *Tian Xie*[2], *Xiaoyan Luo*[2]

1. *Gene Engineering and Biotechnology Beijing Key Laboratory College of Life Sciences, Beijing Normal University, Beijing 100875, China*
2. *State Key Laboratory of Environment Simulation, School of Environment, Beijing Normal University, Beijing 100875, China*

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ABSTRACT

The Cambrian Explosion is one of the most significant events in the history of life; essentially all easily fossilizable animal body plans first evolved during this event. Although many theories have been proposed to explain this event, its cause remains unresolved. Here, we propose that the elevated level of oxygen, in combination with the increased mobility and food intake of metazoans, led to increased cellular levels of reactive oxygen species (ROS), which drove evolution by enhancing mutation rates and providing new regulatory mechanisms. Our hypothesis may provide a unified explanation for the Cambrian Explosion as it incorporates both environmental and developmental factors and is also consistent with ecological explanations for animal radiation. Future studies should focus on testing this hypothesis, and may lead to important insights into evolution.

Contents

* *Corresponding author*. E-mail: guoxj@bnu.edu.cn (Xuejun Guo).

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Introduction

Although the first animals may have evolved during the Ediacaran Period (about 580 million years ago (mya)) or even earlier, essentially all easily fossilizable animal body plans emerged within about 35 mya in the early Cambrian (Marshall, 2006; Briggs, 2015; Valentine, 2004; Butterfield, 2015). This phenomenon is called the Cambrian Explosion, and is one of the most important evolutionary events in Earth history.

There have always been questions about the reliability of fossil evidence, because of the incompleteness of the fossil record and conflicts with molecular clock estimates. However, recent evidence strongly supports the conclusion that the Cambrian Explosion was a real evolutionary phenomenon as opposed to an artifact of taphonomy. The occurrence of trace fossils is independent of the presence of hard parts, and can provide information on soft-bodied animals that were rarely preserved. Studies have shown that trace fossils became larger and more complex throughout the Cambrian Period, which is consistent with the rapid radiation of animals (Valentine, 2004). Although earlier molecular clock analyses often conflicted with fossil data by suggesting many phyla evolved several hundred million years before the Cambrian, recent advances in molecular analyses have significantly reduced Precambrian divergence time estimates between phyla (Lee et al., 2013; Erwin et al., 2011; Rota-Stabelli et al., 2013), and current results from molecular analyses and are more or less reconcilable with the fossil record.

One explanation for the Cambrian Explosion is that the rate of animal evolution may have been much higher during that period. One simulation study has indicated that rates of evolution would have to increase by a factor of five to recreate the observed divergences that were then compressed into 35 million years (Levinton et al., 2004). Recent analyses of molecular and morphological data of arthropods have suggested that their rates of evolution indeed increased by 4- to 5.5-fold in the early Cambrian (Lee et al., 2013).

Several possible mechanisms for the Cambrian explosion have been proposed (Marshall, 2006; Valentine, 2004); some are based on environmental changes, such as increased atmospheric oxygen levels or Snowball Earth events. However, it is difficult to directly correlate environmental change with new levels of developmental and morphological organization. Another theory is that the evolution of a new genetic circuit was the primary cause. However, evidence suggests that the genes governing bilaterian development evolved at least tens of millions years before the Cambrian Explosion (Valentine, 2004). Finally, there are ecological explanations whereby predation and grazing are suggested to have been the major causes of the rapid radiation of animals. Although ecological factors are expected to play important roles in evolution, these theories fail to explain the duration and uniqueness of the Cambrian Explosion. Furthermore, none of these theories directly address why the rate of evolution increased.

It has long been established that oxygen can produce reactive oxygen species (ROS), and that the resulting oxidative stress may cause genomic damage and mutations (Schieber and Chandel, 2014; Puente et al., 2014; Cadet and Wagner, 2013). Furthermore, because ROS are also important signaling molecules, their increased abundance could also provide new regulatory mechanisms for development (Covarrubias et al., 2008). Therefore, we proposed that ROS may have been a central factor in the environmental, developmental and ecological mechanisms that caused the radiation of early bilaterians. In the following sections of this article, we will discuss this model in greater detail.

1. Increased oxygen level before the Cambrian set the stage for animal evolution

Before the Great Oxygenation Event (GOE) at about 2.45 Ga, any oxygen molecules in the atmosphere were captured by oxygen sinks such as dissolved iron and organic matter (Canfield, 2005, 1998; Holland, 2006). Between 1.8 and 0.85 Ga, the oxygen level in the atmosphere remained low, no more than 10% PAL (present atmospheric level) (Canfield, 2005, 1998; Holland, 2006; Sperling et al., 2015; Mills and Canfield, 2014). Some researchers have estimated that during much of the Proterozoic Eon, atmospheric oxygen could have been as low as 0.1% PAL (Sperling et al., 2015), in which case the oxygen content of seawater would have been exhausted as it passed from the sea surface downward to the seafloor, and thus caused the deep-ocean anoxia (Canfield, 2005). Canfield and colleagues (Canfield, 2005, 1998; Holland, 2006) have shown that the oxygen content in the atmosphere and the anoxic conditions in the deep ocean could cause the buildup of H_2S in the ocean, and form a so-called "Canfield Ocean", which may explain why complex multicellular organisms did not evolve during this period of Earth's history (termed the "Boring Billion").

Between 850 and 540 mya, there was a rapid increase in atmospheric oxygen content. The causes of this rise remain uncertain, although plausible explanations have been proposed, such as increased burial of organic carbon associated with continental breakup (Canfield, 2005, 1998; Holland, 2006). At the end of this period, the level of oxygen in the atmosphere was close to that of the present (Holland, 2006), which could lead to oxygenation of the deep ocean. A recent geochemical study on sedimentary rocks from the late Ediacaran revealed that these rocks formed under a more oxygenated environment than the underlying Cryogenian deposits. This finding suggests that immediately before the Cambrian Explosion, water of the deep ocean had already transitioned from anoxic to fully oxygenated (Chen et al. 2015; Johnston et al., 2012).

A high oxygen level is essential for metabolically active animals. It is also necessary for synthesizing collagen. Because collagen is essential for the formation of tissues, a minimum level of oxygen is required for the evolution of complex animals. Furthermore, because of their thick muscle layers and mesodermally derived internal organs, it is more difficult for triploblastic animals to obtain sufficient oxygen via diffusion, and their maximum body sizes should be related to oxygen availability. Therefore, the diversification of bilaterians could only take place after the oxygen level at the seafloor increased (Marshall, 2006; Valentine, 2004; Chen et al., 2015; Johnston et al., 2012; Mills et al., 2014; Knoll and

Carroll, 1999). All variants of such "oxygen theories" suggest that the major role of oxygen was to remove an environmental barrier to the evolution of larger, metabolically active animals and release the morphological potential of developmental genes that had already evolved. In these theories, oxygen did not directly cause animals to evolve.

One critical issue is the minimum oxygen requirements of animals. Calculations suggest that a worm 600 μm in length could survive at the 0.36% PAL (Mills and Canfield, 2014). Studies of extant animals have also revealed considerable tolerance for hypoxic conditions. For example, in an oceanic environment where the oxygen level was only about 0.3–0.5 mL/L, dense communities of animals, such as starfish, crabs, sponges and shrimp, were identified (Sperling et al., 2015). Therefore, the question remains of why animals did not evolve for most of the Proterozoic Eon.

However, if the role of oxygen is not simply to overcome an environmental barrier, but to directly enhance the evolutionary rate, this question can be resolved, because although extant animals can survive in low-oxygen environments, higher oxygen levels would be required to promote evolutionary radiation. As discussed in the next section, by creating ROS, oxygen may indeed affect evolution more directly.

2. Increased oxygen consumption generates more ROS and contributes to evolution

ROS include a series of reactive compounds, such as superoxide anions ($O_2.^-$), hydroperoxyl radicals ($HO_2.$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (.OH), all of which are derived from the reduction of molecular oxygen. The primary ROS is the superoxide anion ($O_2.^-$), which is continuously produced as a byproduct of the mitochondrial electron transport chain (Sabharwal and Schumacker, 2014; Fisher-Wellman and Bloomer, 2009) (Fig. 1). Superoxide anions are generally converted to hydrogen peroxide by superoxide dismutases (SODs). In the presence of partially reduced metal ions, hydrogen peroxide is converted to the highly reactive hydroxyl radical through Fenton and Haber–Weiss reactions (Betteridge, 2000). Normally, "redox homeostasis" is maintained (Schieber and Chandel, 2014; Mailloux et al., 2013; Ray et al., 2012; D'Autreaux and Toledano, 2007) because cells have evolved a sophisticated system to counterbalance oxygen radicals (Fig. 1). This system encompasses superoxide dismutase, glutathione peroxidase, catalase and non-enzymatic chemicals (Ray et al., 2012).

An imbalance between generation and removal of ROS causes oxidative stress (Fig. 1). Excessive ROS levels damage DNA by

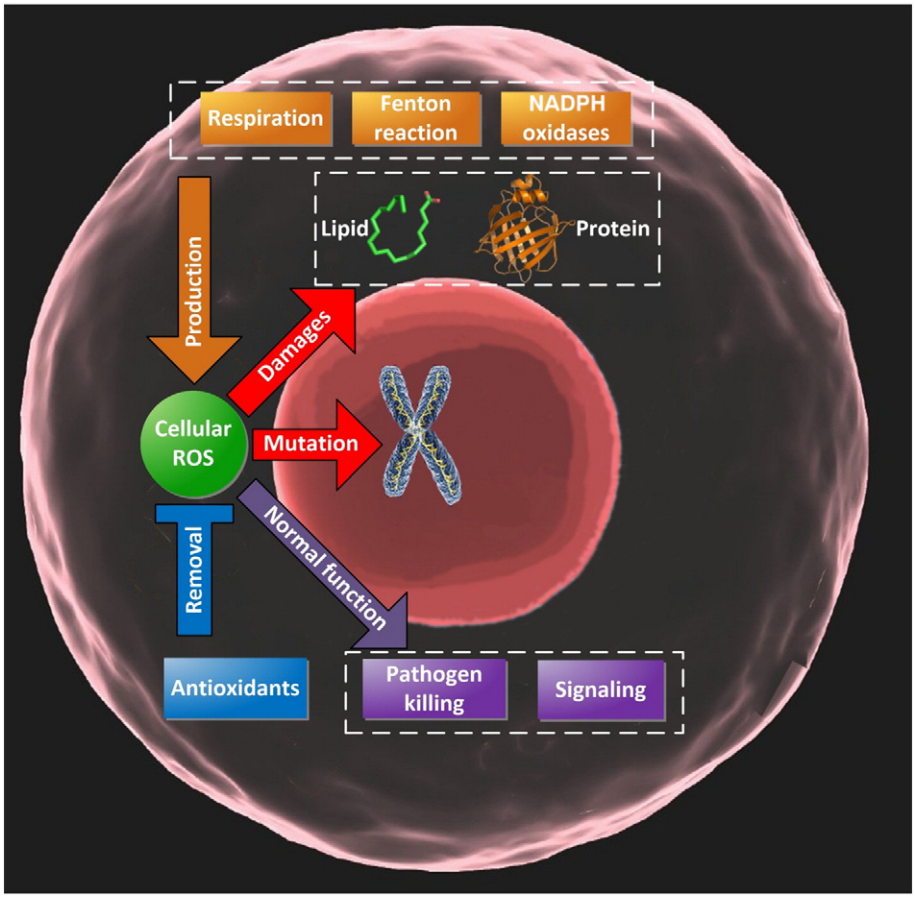

Fig. 1 – Cellular reactive oxygen species (ROS) homeostasis. Homeostasis of ROS is maintained by the balancing effects of ROS production through respiration, NADPH oxidase and the Fenton reaction (orange boxes) and removal by antioxidants (blue boxes). ROS may have normal functions (purple boxes) when homeostasis is maintained or abnormal effects (red arrows) when homeostasis is disrupted.

producing single- or double-stranded breaks, purine, pyrimidine, or deoxyribose modifications, DNA crosslinks and modification of DNA methylation patterns, which can have mutagenic effects. For example, the oxidative DNA adduct8-oxo-deoxyguanosine (8-oxo-dG) can cause G/T transversions and double-strand breaks that may lead to chromosome fusions and thus create novel genes.

Increased oxygen consumption can also produce excess ROS (Fisher-Wellman and Bloomer, 2009; Radak et al., 2013; Gomes et al., 2012; Bouzid et al., 2015) that can cause DNA damage and mutations. In mammals, thymine glycol and 8-oxoguanine, derived from the oxidized bases deoxythymidine and 8-oxo-deoxyguanosine (8-oxo-dG), can be detected and quantified in urine, and thus provide means to measure the degree of DNA oxidation. Ames and colleagues have shown that the yield of these compounds is directly proportional to oxygen consumption, which indicates that increasing the rate of oxidative metabolism causes greater DNA damage (Ames, 1989; Ames and Gold, 1991). Furthermore, Gillooly and colleagues have shown that organisms with more active metabolisms tend to be associated with higher mutation rates (Gillooly et al., 2001, 2007).

Trace fossil evidence suggests that primitive bilaterians were active burrowers (Valentine, 2004), which indicates that they consumed more oxygen than their diploblastic ancestors. However, the evolution of the antioxidant system in animals may have been a gradual process. For example, the comparison of glutathione peroxidases (GPOX) between invertebrates and vertebrates suggests that invertebrates lack Se-dependent glutathione peroxidase. In vertebrates, GPOX protects the organism from oxidative damage by removing cytotoxic H_2O_2 and other lipid or organic hydroperoxides (ROOH) formed in aerobic metabolism (Ahmad et al., 1989). Rapid increase in the mobility of early bilaterians may have produced excess ROS, which may have caused oxidative stress and led to an increased mutation rate (Figs. 2 and 3). Because of their ability to cause mutations, ROS have been proposed to be universal constraints on evolution (Dowling and Simmons, 2009). However, could ROS also directly drive evolution?

According to neo-Darwinian theory, natural selection is the driving force of evolution, and mutations provide the raw genetic materials from which advantageous alleles are selected. Nei (2007) suggested an alternative theory: because a majority of amino acid substitutions in proteins are neutral, a considerable portion of morphological evolution is caused by neutral mutations that are not greatly affected by natural selection. Therefore, a significant portion of evolution is likely driven by mutations. Based on this theory, increased rates of evolution may be explained by mutations caused by ROS.

Direct evidence for evolution driven by ROS may be observed looking inside an individual organism. Cancer development is a process of Darwinian evolution where the evolving units are individual cancer cells. Studies have suggested that carcinogenesis can be described as a succession of clonal expansions. Cancer development is a multi-stage process that includes initiation, promotion and progression stages. ROS have been shown to play an important role in the initiation of cancer cells by causing DNA damage. For example, elevated levels of oxidative DNA lesions such as 8-oxo-dG have been observed in many cancers (Valko et al., 2006). The clonal expansion of the initiated cells characterizes the promotion stage, which requires the continual presence of proliferative and

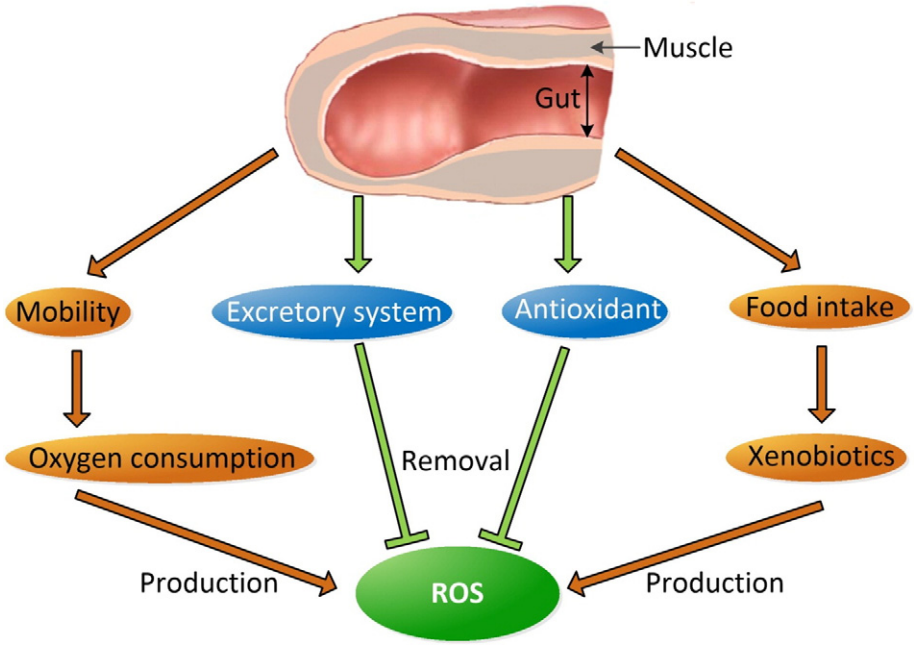

Fig. 2 – The primitive bilaterian may have produced more ROS. The primitive bilaterian (drawn at the top) had evolved a relatively well-developed gut and functional muscle layer, which resulted in higher mobility and greater oxygen consumption, as well as increased food intake, which caused accumulation of xenobiotics. Because its antioxidant and excretory system were relatively unchanged, cellular ROS levels increased. Factors that contribute to ROS production are indicated in orange, and those that promote ROS removal are in blue.

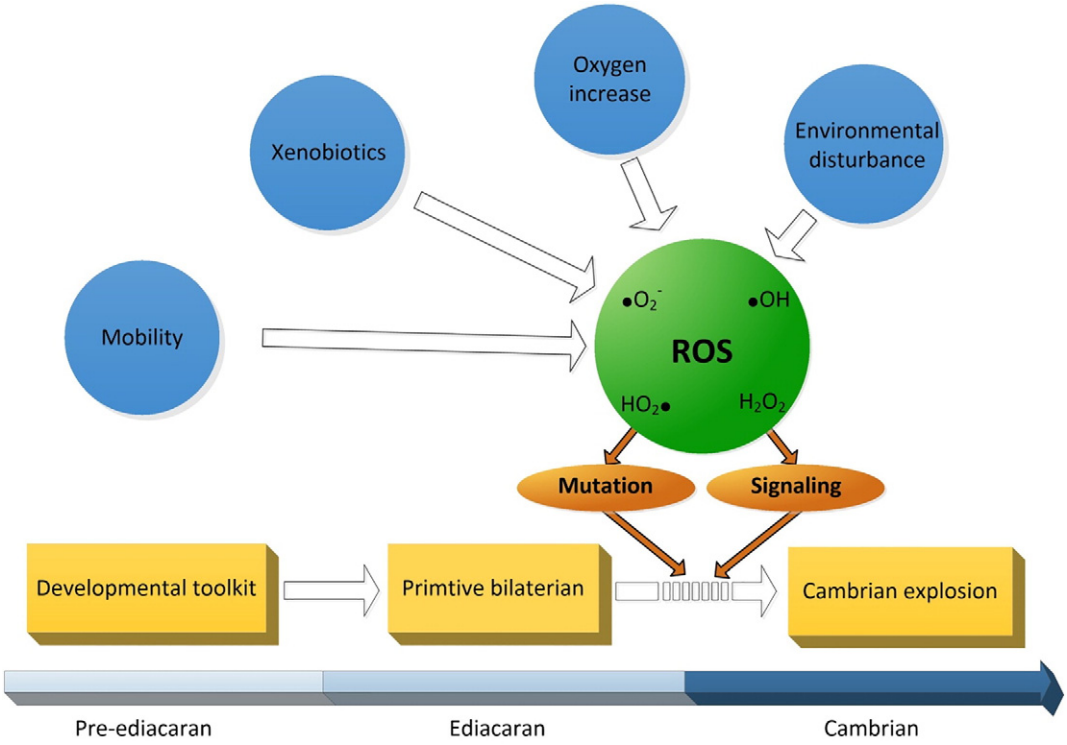

Fig. 3 – An oxidative model for the Cambrian Explosion. The developmental toolkit evolved before the Ediacaran Period, whereas the first primitive bilaterians evolved during the Ediacaran. During the early Cambrian, many factors (in blue circles) contributed to increasing cellular ROS levels, which drove evolution by causing more frequent mutations and by providing new signaling mechanisms, which caused the Cambrian Explosion.

anti-apoptotic signals (Valko et al., 2006). ROS also contribute in this stage, as discussed below. Although the evolution of cancer cells differs from macroevolution, this analogous process demonstrates that ROS can drive evolution.

3. Global environmental disturbances causing large-scale ecological oxidative stress

The well-documented presence of unusually large negative carbon isotopic excursions at the Neoproterozoic–Cambrian boundary points to environmental disturbances (Knoll and Carroll, 1999). One possible cause is that the relatively rapid movement of continents may have led to immense methane burps that induced significant temperature increases (Kirschvink and Raub, 2003; Peters and Gaines, 2012). Furthermore, a long period of oxygen erosion in the Neoproterozoic Era may have resulted in low-relief continental interiors with highly weathered rock and associated soil sat the surface (Smith and Harper, 2013). The major subsequent sea-level rise in the early Cambrian could then have led to flooding of these interiors and triggered extensive erosion and mobilization of weathered rock and regolith, which would cause the rapid input of calcium, phosphate, and other ions into the oceans (Peters and Gaines, 2012). Other disturbances, such as Snowball Earth events and true polar wander, may also explain these negative carbon isotopic excursions (Marshall, 2006; Kirschvink and Raub, 2003).

Although it is clear that the environment must be conducive to the evolution of large animals, none of the proposed environmental explanations have addressed why a permissive environment should necessarily lead to the evolution of such complexity (Marshall, 2006). These environmental perturbations should be related to the increased biological disparity and diversity through some intrinsic biochemical mechanisms. Oxidative stress resulting from the imbalance of ROS is a common consequence of environmental stress (Snoeijs et al., 2012; Zhang et al., 2009a, 2009b; Quaiser et al., 2016). Therefore, we hypothesize that major environmental disturbances resulted in considerable oxidative stress at a large ecological scale, which promoted mutations in the genomes of primitive bilaterians, and may have laid the foundation for the Cambrian Explosion (Fig. 3).

4. ROS regulate key developmental genes and signaling pathways that affect cell renewal and differentiation

In addition to causing oxidative stress and producing mutations, ROS can also directly regulate the embryonic developmental process (Puente et al., 2014; Covarrubias et al., 2008; Brautigam et al., 2011). Some of the most important developmental genes, such as *Hox*, *TGF-β*, *Otd*, and *Pax*, initiate morphogenesis and are inferred to have been present in stem-group bilaterians (Covarrubias et al., 2008;

Kamata et al., 2005). ROS play an important role in regulating these key genes. For example, Pax proteins bear two conserved cysteines (Cys 37 and 49), which make them ideal targets for redox regulation (Covarrubias et al., 2008). The *Hox* gene family is critical in development because the nested, overlapping domains of expression subdivide the anterior/posterior (A/P) axis. Products of many *Hox* genes (*e.g.*, *Ant, Scr, HoxA5, HoxB5, HoxC5, HoxA6, HoxB6, HoxC6, HoxA7,* and *HoxB7*) are redox-regulated through oxidation of a highly conserved Cys39 residue (Covarrubias et al., 2008). Experimental evidence has also demonstrated that Oct4, a transcription factor critical for the maintenance of embryonic stem cells, is redox sensitive, as its binding to the DNA promoter region of Fgf4 is regulated by ROS (Covarrubias et al., 2008).

As signaling molecules, ROS can regulate a variety of cellular functions, including proliferation, differentiation, migration and apoptosis. ROS are thought to interact with several pathways (*e.g.*, Wnt, Hedgehog, and FOXO signaling cascades) to affect the transcription machinery required for stem cell differentiation (Wang et al., 2013a, 2013b; Bigarella et al., 2013; Jang and Sharkis, 2007). From the perspective of the Cambrian Explosion, it is notable that a small increase in the complexity of a combinatorial developmental system can lead to an extraordinary range of stable spatial patterns of gene expression, and thereby release rich morphogenetic potential (Sole et al., 2003).Thus, through redox regulation of key genes that affect embryonic development and stem cell renewal and differentiation, ROS may have played a central role in creating the disparity and diversity of Cambrian animals (Fig. 3).

The signaling roles of ROS are also supported by studies of carcinogenesis. In cancer cells, ROS provide essential proliferative and survival signals that make continual clonal expansions possible. For example, ROS can activate NF-κB and AP-1 family transcription factors (Valko et al., 2006; Hsu et al., 2000). In addition, ROS can activate tyrosine protein kinases either by inhibiting protein tyrosine phosphatases (PTPs) or by directly activating the kinase (Tauzin et al., 2014; Zimmer, 2009).

5. The evolutionary lag between the digestive and excretory systems in early metazoans may also cause oxidative stress

The history of animal evolution is accompanied by constant improvement of the digestive tract (Valentine, 2004). For example, sponges and placozoans lack an enteron (Valentine, 2004). A primitive gut evolved in cnidarians. Triploblastslater evolved an increasingly sophisticated digestive system with two openings and digestive glands (Valentine, 2004; Vannier et al., 2014). In comparison, the evolution of the excretory system lagged behind significantly. For example, the acoel flatworm, the presumed basal bilateral animal, lacks nephridia (Valentine, 2004), which indicates that stem-group bilaterians may also have lacked nephridia and had limitations in excretion. Furthermore, in extant animals, toxic xenobiotics can be removed through the feces route. Because the digestive tract of the acoel flatworm has only one opening, it is likely that stem bilaterians had not evolved an anus and may have been limited in their ability to remove toxic xenobiotics from their diet (Fig. 2) (Hejnol and Martindale, 2008).

The lack of an efficient excretory system is understandable from an evolutionary perspective; prior to the emergence of effective digestive and locomotive mechanisms, animals were limited in their ability to gather nutrients and would not accumulate significant amounts of toxic xenobiotics from their diet. Therefore, pressure to evolve a more efficient excretory system was not present. However, primitive bilaterians were able to burrow into the seafloor, actively prey on microbial mats, and digest food using a well-developed enteron, which greatly improved their ability to gather food. Consequently, accumulation of toxic xenobiotics became a potential problem. For example, a study of early arthropod fossils revealed that the guts of these animals, with complex digestive systems, were enriched in iron oxides (Vannier et al., 2014). This finding indicates that the diets of early bilaterians may have contained significant amounts of metals that may have accumulated in their tissues.

Bioconcentration is the process by which the concentration of a chemical in an aquatic organism exceeds that in water as a result of exposure to waterborne chemicals, whereas biomagnification is the process whereby xenobiotic substances are transferred from food to the organism and result in higher concentrations compared with the source. Both processes are thought to be wide spread phenomena in marine food webs (Gray, 2002; Kelly et al., 2007). Living animals with more primitive excretory systems, such as bivalves and arthropods, show bioconcentration and biomagnification of heavy metals (Olmedo et al., 2013; Wang et al., 2013a, 2013b). In the early Cambrian, the evolutionary lag between the digestive and excretory systems of primitive bilaterians may have caused more severe bioconcentration and biomagnification of xenobiotics (Fig. 2). These compounds, particularly heavy metals, produce ROS through Fenton and Haber–Weiss reactions and thus cause severe oxidative stress (Fig. 3).

6. An oxidative model for the evolution of early bilaterians

Here we propose a revised model for the Cambrian Explosion (Fig. 3). During the Ediacaran Period, oxygen levels at the bottom of shallow seas were elevated above a threshold sufficient to support larger and more active animals, which enabled the rise of the Ediacarabiota, some of which may have been triploblastic and possibly stem-group bilaterians. Because of the increased mobility of these animals, they would have consumed more oxygen and produced greater amounts of ROS. In addition, their mobility increased their efficiency in foraging, which may have caused them to ingest more xenobiotics than their simple excretory systems could remove, and could also have produced excessive amounts of ROS. Therefore, early bilaterians may have produced large amount of ROS that could not be counterbalanced by their primitive antioxidant systems, which would have resulted in oxidative stress and mutations. Furthermore, these increased amounts of ROS may also have provided new mechanisms for regulating embryonic development. These two effects could

have promoted increased evolutionary rates among early bilaterians, and ultimately led to the Cambrian Explosion.

Our model is well positioned to address several issues associated with the Cambrian Explosion (Marshall, 2006). For example, our model requires an adequate level of oxygen for the emergence of metabolically active triploblasts, which controls the timing of the rapid radiation of animals. Furthermore, if the driving force of the Cambrian Explosion was ROS, the subsequent evolution of a more complex excretory systems and antioxidant mechanisms would subsequently have decreased ROS levels and caused the pace of evolution to slow, which may explain the relatively short duration of the event.

Our model is also consistent with many theories about the Cambrian Explosion. For example, we agree with the oxygen hypothesis, because increased oxygen concentration is critical for the first appearance of the metabolically active triploblasts. However, in our model, oxygen did not simply remove a barrier to evolution but also produced ROS, which, by causing mutations and functioning as new signaling mechanisms, directly drove the rapid diversification of triploblasts. Our model is also in agreement with a number of other hypotheses based on the environmental disturbances that took place during the late Neoproterozoic Era.

In our model, predation plays a wider role than that proposed in the traditional ecological models (Marshall, 2006; Valentine, 2004; Bengtson, 2002). In addition to exerting selective pressure, the emergence of predation greatly enhanced the mobility of both predators and prey, which increased consumption of oxygen and led to greater production of ROS. Finally, our model is in agreement with developmental hypotheses. Recent advances in the study of the molecular developmental basis of evolution have indicated that body plan diversification likely occurred through changes in developmental regulatory networks rather than in the genes themselves. Because ROS play important roles in tissue development, they may have provided primitive bilaterians with new regulatory mechanisms for development and thus contributed to their diversification.

7. ROS may have broader implications in evolution

Evolution driven by ROS may explain other evolutionary events in addition to the Cambrian Explosion, including the origins of eukaryotes and insects.

Different models have been proposed to explain the origin of eukaryotes (Zimmer, 2009). The traditional “archezoa hypothesis” suggests a proto-eukaryotic cell that had already evolved a nucleus, a cellular skeleton and the endomembrane system before it acquired mitochondria through endosymbiosis. The major difficulty facing this model is the failure to identify any living eukaryotes that lack mitochondria or mitochondrion-related organelles (MROs). Therefore, an alternative model has been proposed whereby the fusion of archaea and bacteria preceded the formation of other features of eukaryotes (Zimmer, 2009; Margulis et al., 2000).

Even today, mitochondria are the major sites of ROS production. Therefore, we assume that when archaea first acquired mitochondria, they would have faced intense oxidative pressure because primitive mitochondria produced more ROS than new cells could scavenge. This oxidative stress may have led to DNA damage and mutations, which would not only drive the diversification of eukaryotes but also cause the vast expansion of their genomes. New eukaryotic cells would be subject to selective pressure to limit the effects of ROS. This may have led to the evolution of the endomembrane system, particularly the nuclear envelope, to decrease the diffusion of ROS and protect the genomic DNA from damage.

Insects are the most diverse group of animals, with at least one million described species. It has been estimated that there may be as many as 6 to 10 million species within this class, which represents more than 90% of all animal species. The evolutionary history of insects has been studied in detail (Engel, 2015). Genomic data indicate that insects evolved during the Ordovician, roughly at the same time as the first land plants, although the oldest known insect fossils occur in Devonian strata. A major radiation took place during the Carboniferous when pterygotes first appeared. The Endoptergota underwent a radiation in the Permian Period.

The flourishing of plants on land led increased atmospheric oxygen content, which reached 35% toward the end of the Carboniferous. This event coincided with the early history of insects. The high concentration of atmospheric oxygen may have supported the evolution of insect flight, an activity that is metabolically intense and consumes a large amount of oxygen. Consequently, when primitive insects first gained the ability to fly, they may have produced greater amounts of ROS, and thus caused mutations and influenced their regulatory networks, which may explain at least the initial diversification of insects.

8. Conclusions

We propose that ROS were important factors in driving the Cambrian Explosion. Several other factors, including rising oceanic oxygen levels and the appearance of primitive bilaterians that were more metabolically active and had improved digestive systems, could lead to the accumulation of ROS in tissues. Excess ROS can cause genomic mutations, which may increase the rate of evolution. In addition, ROS may have provided new regulatory mechanisms that were incorporated into the genetic circuit for embryonic development, which may have contributed to the evolution of new body plans.

Our hypothesis resolves many questions associated with the Cambrian Explosion. For example, it explains how environmental stresses affected evolution. It also explains the timing and duration of the Cambrian Explosion. Furthermore, our hypothesis provides a connection between environmental factors and developmental mechanisms. It is also consistent with other proposed models, such as the predation theory. Therefore, our hypothesis may provide a unified explanation for the rapid radiation of animals during the early Cambrian. In addition, ROS may have played more roles in evolution both before and after the Cambrian Period. For example, they may have been involved in the rises of eukaryotes and insects. Therefore, this ROS-based hypothesis may have deep implications in the evolutionary history of life.

Future studies should focus on testing this hypothesis, which may lead to important new insights into evolution. For example, mutations could be introduced to enhance ROS production in animals, and evolutionary rates could be measured and compared with predictions from our hypothesis. Alternatively, phylogenetic studies could be performed to look for possible relationships between antioxidant genes and evolutionary rates.

Author disclosure statement

No competing financial interests exist.

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