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Opinion

Cells discover fire: Employing reactive oxygen species in development and consequences for aging

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Abstract

The free radical theory of aging states that aging results from the accumulated damage caused by reactive oxygen species (ROS). Herein, we provide a critique of the theory that aims to point out the theory's weaknesses and put forward ideas for how future experiments must adjust to several emerging concepts. In the same way fire is dangerous and nonetheless humans learned how to use it, it now appears that cells evolved mechanisms to control and use ROS. The way ROS are used as signaling molecules in many crucial biological functions suggests ROS are not unwanted by-products of metabolism. We hypothesize that the connection between ROS and cellular processes like growth, proliferation, and apoptosis may explain why long-lived animals appear to have lower levels of ROS production: the longer development of long-lived animals may lead to lower steady state levels of ROS. With age, antioxidant systems become deregulated, just like so many other cellular components, and so oxidative damage occurs. Therefore, the production of ROS is not merely a cause of havoc but rather a complex and critical system whose disruption in disease and aging leads to oxidative damage. Potential roles of ROS in aging are discussed under this model.

Keywords: Apoptosis; Cell proliferation; Free radicals; Longevity; Redox signaling; Senescence

1. Introduction

Aging can be defined as a progressive functional decline and increasing mortality with time. About 50 years ago, Denham Harman proposed the free radical theory of aging (Harman, 1956). Briefly, the theory states that the aging process results from the accumulated damage caused by reactive oxygen species (ROS), highly reactive molecules that, among other sources, are normal by-products of cellular metabolism (Harman, 1981; Beckman and Ames, 1998; Finkel and Holbrook, 2000; Balaban et al., 2005). This work provides a critique of the free radical theory of aging. Based on what we have learned in recent years concerning free radical biology and the link between ROS and aging, our aim is to point out the theory's weaknesses and put forward ideas for how future experiments must adjust to several emerging concepts.

Opinions in biology are changing from seeing ROS and redox state—the oxidation–reduction potential—as mere sources of damage to players in signal transduction (Darley-Usmar and Starke-Reed, 2000). ROS and redox state act as messengers in the regulation of gene expression in development, growth, and apoptosis. Herein, we review recent observations relating oxidative damage to aging from the emerging perspective that ROS are signaling molecules crucial in numerous cellular functions and under strict control. Lastly, we derive a model attempting to explain observations linking ROS to multiple cellular processes, including development. Potential roles of ROS in aging are discussed under this model.

This work, in line with the above definition of aging, is related to the fundamental causes of aging, not age-related diseases. Many factors may affect general health and hence influence longevity without being involved in aging (Hayflick, 2000; de Magalhaes et al., 2005). For example, average longevity in industrial nations increased roughly 50% in the past century and yet there is no evidence of a slower human aging process (Finch, 1990; Hayflick, 1994). Similarly, multiple factors may contribute to specific diseases and impact on longevity but not be involved in aging. To determine whether a given intervention altered aging different parameters must be evaluated. Changes in maximum longevity, for instance, are considered a more reliable indicator of changes in the aging process than average longevity. A significant change in the rate at which mortality increases with age may also signal changes in rate of aging. Still, a retardation of multiple age-related changes and pathologies is probably

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the best indicator of a change in the aging process (Finch, 1990; de Magalhaes et al., 2005). This is a crucial, but often misinterpreted, concept in aging research (Hayflick, 2000). Herein, we discuss the free radical theory in the context of the aging process, not specific age-related diseases.

Fundamental causes of aging determine why different species age at radically different paces. For instance, even under the best housing conditions, a mouse will age 25–30 times faster than a human being (Finch, 1990). For the free radical theory of aging, or any other theory, to be accepted, it must explain why similar species, such as mammals or even primates, age at such markedly different rates. Therefore, a comparative biology of aging perspective was also taken into consideration in this review.

2. A critique of the free radical theory of aging

2.1. The connection between oxidative damage and aging

Various measurements of oxidative damage seem to correlate with age in a variety of organisms, at least in some tissues (Beckman and Ames, 1998; Fukagawa et al., 2000; Barja, 2002), and tend to be associated with organismal longevity (Sohal et al., 2002). In fact, oxidative damage has been widely used as a biomarker of aging and disease. Succinctly, several short- and long-lived rodent cohorts feature, respectively, increased and decreased oxidative damage (Jolly et al., 2001; de Boer et al., 2002; Holzenberger et al., 2003; Quarrie and Riabowol, 2004). Oxidative damage and disruption of oxidative-pathways have also been described in numerous human pathologies (Halliwell, 1997), including neurodegenerative diseases, AIDS (Kannan and Jain, 2000), diabetes, and atherosclerosis (Fukagawa et al., 2000).

Given the importance of ROS in biology, a variety of mechanisms evolved to deal with ROS (Beckman and Ames, 1998): from antioxidants, such as vitamins C and E, passing by enzymes that detoxify ROS such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, to a number of enzymes that catalyze the repair of damage caused by ROS, such as 8-oxo-dGTPase and methionine sulfoxide reductase A (MSRA). The mere existence of enzymes to prevent and repair damage by ROS is a strong indicator that ROS are biologically important, potentially dangerous molecules (Beckman and Ames, 1998). It is not surprising then that ROS have been implicated in a number of pathologies, including age-related pathologies, and in aging itself. Overall, the association between oxidative damage and disease, including aging, is clear, as reviewed by many others (Beckman and Ames, 1998; Cadenas and Davies, 2000; Finkel and Holbrook, 2000; Barja, 2002; Sohal et al., 2002).

2.2. The free radical theory of aging: trial by fire

Correlation, of course, does not imply causation. Maybe oxidative damage is a consequence of aging rather than a cause. To test the free radical theory of aging numerous manipulations of oxidative damage have been attempted in model organisms using a variety of techniques. So far, however, the results have been largely disappointing (reviewed in Sohal et al., 2002; de Magalhaes, 2005). For instance, by feeding antioxidants to rodents it is possible to decrease oxidative damage and sometimes even slightly increase longevity, but aging is not delayed (Comfort et al., 1971; Heidrick et al., 1984; Hagen et al., 2002). In fact, as argued previously (Hayflick, 1994; Arking, 1998), there is no clear evidence that ROS inhibitors delay mammalian aging.

If the optimization of antioxidant defenses was able to delay the aging process that would strongly suggest that ROS are a causative factor in mammalian aging. In mice, however, ubiquitous overexpression of SOD1 (Huang et al., 2000), overexpression of glutathione peroxidase (McClung et al., 2004), and overexpression of catalase in the nucleus (Schriner et al., 2000) all failed to increase longevity. Transgenic mice overexpressing the human thioredoxin gene, which regulates the redox content of cells and is thought to be anti-apoptotic (Kwon et al., 2003), featured an increased resistance to oxidative stress and an increase in longevity (Mitsui et al., 2002). Similarly, mice knock-out for $p66^{shc}$ lived about 30% longer and their cells were more resistant to oxidative stress (Migliaccio et al., 1999). $p66^{shc}$ may regulate intracellular oxidant levels in mammalian cells and appears to be pro-apoptotic (Nemoto and Finkel, 2002). Since both $p66^{shc}$ and thioredoxin are not known to be antioxidants, however, it is unclear how these findings relate to oxidative damage and anti-oxidant defenses. Moreover, it is not known whether life-extension in these experiments was due to delayed aging or delayed disease (de Magalhaes et al., 2005). Mice over-expressing catalase in mitochondria have been recently shown to live about 20% longer than controls (Schriner et al., 2005). Nevertheless, the extended lifespan of these animals, termed MCAT, appeared to be related to a lower incidence of cardiac pathology, which has been associated with functional abnormalities in mitochondria. The rate at which mortality increased with age did not indicate MCAT mice as aging slower than controls (Schriner et al., 2005). Therefore, MCAT mice may well be a case of a life-extending intervention due to a lower incidence of a specific pathology across the entire lifespan, not due to changes in rate of aging.

In some cases, disruption of antioxidant pathways has resulted in increased oxidative damage and, sometimes, in decreased longevity, but it is not clear aging was accelerated. For instance, knocking out the gene responsible for 8-oxo-dGTPase in mice, although resulting in an increased cancer incidence, did not alter the aging process (Tsuzuki et al., 2001), and mice deficient for glutathione peroxidase do not age faster (Ho et al., 1997). Mice without MSRA have a decreased longevity (Moskovitz et al., 2001), but whether their aging process is affected remains a subject of debate (de Magalhaes et al., 2005). Disruption of mitochondrial DNA polymerase resulted in an accelerated aging phenotype in mice (Trifunovic et al., 2004), but an increase in ROS production or higher levels of oxidative damage were not observed (Kujoth et al., 2005). Lastly, mice heterozygous for SOD2 showed increased oxidative damage at various levels,

including DNA oxidative damage, but did not show significant changes in longevity or rate of aging (Van Remmen et al., 2003), clearly arguing that oxidative damage alone does not drive aging in mammals.

Antioxidants have been related to aging in invertebrates (Beckman and Ames, 1998; Finkel and Holbrook, 2000). The most compelling evidence so far was the delayed aging observed in flies (*Drosophila melanogaster*) overexpressing SOD1 and catalase (Orr and Sohal, 1994), even though the same authors have recently suggested that their earlier findings may have been overestimated (Orr et al., 2003). Overexpression of human SOD1 in the motorneurons of flies extended lifespan by up to 40% (Parkes et al., 1998), so it is possible that antioxidant protection plays a role in aging in some species. Since humans are mammals, however, findings in invertebrates are likely to be less relevant to understand human aging—the ultimate goal of gerontology—than studies in mammalian models. Indeed, since invertebrate models of aging like flies are mostly composed of post-mitotic cells, that may make them more susceptible to oxidative damage and thus not accurately represent human aging (Hayflick, 1994; de Magalhaes, 2005).

Overall, and although it is possible that multiple antioxidants must be optimized for aging to be delayed, these results cast doubts that antioxidant protection is linked to aging rates in mammals, thus arguing that antioxidant protection does not explain why different species age at different rates. In fact, correlations between rate of aging and antioxidant levels in mammals are, if they exist, very weak (reviewed in Finch, 1990; Sohal and Weindruch, 1996), suggesting that antioxidant protection is already optimized in mammals.

Even though the sources of ROS are numerous, including endogenous and exogenous sources, some advocates of the free radical theory of aging focus on ROS derived from the cellular metabolism taking place in mitochondria (Beckman and Ames, 1998). One hypothesis is that ROS production or leakage in mitochondria, rather than antioxidant defense systems, determines the rate of the aging process (Cadenas and Davies, 2000; Barja, 2002). Supporting this concept is a correlation between mitochondrial ROS production and longevity, observed at least for a few species (reviewed in Barja, 2002; Sohal et al., 2002): short-lived mammals, such as mice (*Mus musculus*) and rats (*Rattus norvegicus*), have an increased ROS production when compared to metabolically-equivalent longer-lived birds such as parakeets (*Melopsittacus undulatus*), pigeons (*Columba livia*), and canaries (*Serinus canarius*). Likewise, bats (*Myotis lucifugus*) have a lower production of ROS when compared to mice and live much longer despite similar metabolic rates and body sizes (Brunet-Rossinni, 2004). Mice under caloric restriction (CR), which delays aging in many species, including rodents, feature a lower production of ROS and a slower accumulation of oxidative damage (Weindruch and Walford, 1988; Weindruch, 1996; Barja, 2002). On the other hand, there are several pathologies in mice and humans derived from mutations affecting the mitochondrion, which often involve an increase in ROS leakage, that do not yield an accelerated aging phenotype (Wallace, 1999; DiMauro and Schon, 2003). One example is Friedreich's ataxia, which appears to derive from increased oxidative stress in mitochondria (Wong et al., 1999) but at a pathophysiological level does not resemble accelerated aging (Martin, 1978).

Free radical generation is often considered a deleterious by-product of oxygen metabolism (Harman, 1981; Nemoto and Finkel, 2004). Not surprisingly, metabolic rate, which is usually estimated by measuring oxygen consumption at rest, has been directly related to the rate of free radical generation in mitochondria (Sohal and Allen, 1990; Sohal et al., 2002), as supported by experimental studies in mammals (Ku et al., 1993). For instance, it has been suggested that metabolic rates may influence the rate at which tissues are bombarded with ROS (Austad, 2005). A number of results, however, suggest that metabolic rates do not influence longevity in endotherms like birds and mammals (Trevelyan et al., 1990; Harvey et al., 1991; de Magalhaes et al., unpublished data). Of course, it is not O_2 consumption alone that determines ROS production but a complex number of systems that may differ from species to species. Nonetheless, if ROS production due to energy production in the mitochondria is linked to aging then O_2 consumption should, to some degree, impact on longevity, as predicted in many formulations of the free radical theory of aging (see Sohal et al., 2002 for arguments), including those of its conceiver (Harman, 1981). Moreover, a number of studies in mice did not find a negative correlation between metabolic rates and longevity. For example, one study found that C/EBP β/β mice burned more fat, had bigger mitochondria, pumped out larger quantities of mitochondrial proteins, and nonetheless lived longer than controls (Chiu et al., 2004). Another study reported that in individual mice high metabolic rates were associated with a longer lifespan (Speakman et al., 2004). Together, these results argue that oxygen metabolism and thus aerobic energy production in mitochondria are not related to aging, making it necessary to re-asses some formulations of the free radical theory of aging.

In conclusion, manipulating components of antioxidant systems does not appear to affect aging in mammals (Sohal et al., 2002; de Magalhaes, 2005). This is in direct contrast with other systems, such as endocrine systems, in which single gene manipulations—e.g. in *GHR*, *GHRHR*, *IGF1R*, *PIT1*, and *PROP1*—can in some cases increase longevity by roughly 50%, delay age-related changes, and significantly alter rate of aging (de Magalhaes, 2005; de Magalhaes et al., 2005). Since it appears that antioxidant protection does not affect aging rates, the best explanation offered by the free radical theory of aging for why different species age at different rates is thus the hypothesis that ROS production varies between species and determines rate of aging. As we will discuss ahead, however, this notion may also be incorrect.

3. ROS and redox signaling as communication systems

Despite their potential to cause damage, ROS have been shown in recent years to be critical components of a variety of

cellular processes (Fig. 1). In fact, eukaryotes have taken advantage of the high reactivity of ROS and adapted these molecular species, particularly H_2O_2 and nitric oxide—though the latter is not commonly related to aging—as signaling molecules in multiple biological processes (Remacle et al., 1995; Nose, 2000; Soberman, 2003; Esposito et al., 2004). For example, ROS can play specific roles in the active sites of enzymes, and redox reactions can affect the activity of transcription factors such as NF-κB, JUN, and FOS (Remacle et al., 1995; Aw, 1999). As such, it is now clear that ROS can have a great impact on gene expression (Gamaley and Klyubin, 1999; Allen and Tresini, 2000) and on global cellular behaviors like proliferation, apoptosis, and senescence.

Redox state and ROS can act as messengers during cellular growth and proliferation. In mammalian cells, a number of extracellular stimuli like growth factors have been shown recently to induce a transient increase in intracellular ROS (Pani et al., 2001; Soberman, 2003; Berner and Stern, 2004). It now appears that ROS induced by growth factors are part of a downstream propagation of mitogenic and anti-apoptotic signals. Exemplifying, epidermal growth factor can induce a transient increase in H_2O_2 generation through the tyrosine kinase activity of the epidermal growth factor receptor (Bae et al., 1997). Although the mechanisms are not completely understood, ROS appear to exert their effects through the reversible oxidation of active sites in protein tyrosine phosphatases. The inhibition caused by ROS helps the propagation of receptor tyrosine kinase signals mediated by protein tyrosine phosphorylation in turn associated with the proliferative stimulus (Chiarugi and Cirri, 2003). Similarly, low amounts of hydroperoxides can stimulate cell growth (Baker and He, 1991). Furthermore, cancer cells often have increased levels of ROS and yet proliferate vigorously (Szatrowski and Nathan, 1991). In fact, ROS have been widely implicated in mitogenic signaling and cancer (Suh et al., 1999; Ha et al., 2000).

On the other hand, and even though the exact mechanisms are still unknown, apoptosis, necrosis, and growth arrest can all be regulated by ROS (Aw, 1999; Chandra et al., 2000; Kannan and Jain, 2000; Kwon et al., 2003). Briefly, apoptosis itself is largely based on free radicals released from mitochondria (Green and Reed, 1998). In fact, ROS generation in mitochondria no longer appears to be an unwanted random process, but rather a precise mechanism used in signaling pathways such as apoptosis (Duchen, 1999; Carmody and Cotter, 2001; Fleury et al., 2002), as demonstrated in cardiomyocytes (Duranteau et al., 1998). Moreover, it is intriguing that the oncogenic protein ras increases the intracellular levels of ROS, thus inducing senescence (Lee et al., 1999), and likewise $p21^{waf1}$ (Macip et al., 2002) and p53 (Macip et al., 2003) have been shown to trigger ROS. Indeed, even though details are still lacking, oxidative signaling influences the way p53 mediates apoptosis and growth arrest (Polyak et al., 1997; Martindale and Holbrook, 2002).

ROS also appear to serve as messengers in many developmental stages. For example, a burst of ROS occurs at fertilization in sea urchins (Heinecke and Shapiro, 1989) and prenatal and embryonic development in mammals has been suggested to be regulated by redox state (Dennery, 2004). It is also noteworthy that H_2O_2 is required for thyroxine synthesis which accelerates developmental processes dramatically. In thyroid cells, regulation of H_2O_2 concentration is critical for thyroxine synthesis since H_2O_2 is needed to catalyze the binding of iodine atoms to thyroglobulin.

Finally, ROS are used by the immune system. For example, ROS have been shown to trigger proliferation in T cells through NF-κB activation (Ginn-Pease and Whisler, 1998; Tatla et al., 1999). Macrophages and neutrophils generate ROS

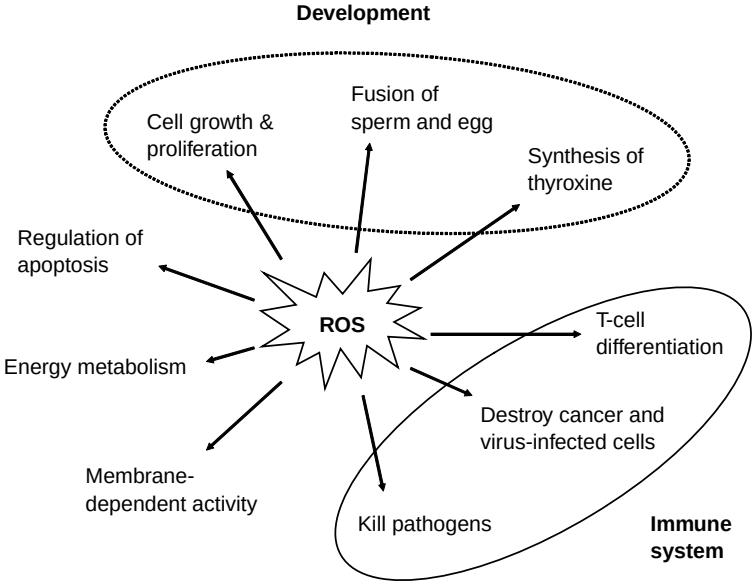

Fig. 1. ROS in normal cellular functions. The uses of ROS in the immune system and development are highlighted with, respectively, a straight and a dashed ellipse. Where possible, an effort was made to relate ROS directly to the end result of their actions. Since nitric oxide is not commonly related to aging its functions are not represented.

in order to kill certain bacteria that they engulf by phagocytosis. Oxidants can also activate lymphocytes, which then use ROS as weapons against infection (Reth, 2002). Furthermore, TNF-α mediates cytotoxicity of tumor and virus infected cells; it has been proposed that, at least partly, TNF-α does not affect normal cells by inducing SOD2 (Remacle et al., 1995; Katama et al., 2005).

4. A model of ROS in development and aging

4.1. ROS in cells: the discovery of fire

Clearly, ROS are potentially dangerous molecules. The free radical theory of aging assumes free radical reactions cause damage and thus constitute the bulk of deleterious events that lead to aging (Harman, 1981; Weindruch, 1996; Beckman and Ames, 1998; Finkel and Holbrook, 2000; Cadenas and Davies, 2000; Barja, 2002). In other words, ROS escape the control of the cellular machinery and become a catalyst for damage. Yet it appears bizarre that in the billions of years of aerobic evolution eukaryotic cells failed to tame ROS. To quote Max Delbruck: "Any living cell carries with it the experience of a billion years of experimentation by its ancestors".

Our interpretation is that in the same way fire is dangerous and humans learned how to control and use it, cells control and use ROS. The amazing cellular machinery involved in oxidative pathways and redox regulation, such as antioxidants, scavengers, and repair enzymes, evolved to control ROS and is much more efficient than previously thought (Gamaley and Klyubin, 1999; Soberman, 2003; Thorpe et al., 2004). Not surprisingly then, ROS production is under strong control (Esposito et al., 2004), and ROS are used by cells in a variety of functions (Fig. 1). It now appears that contrary to most formulations of the free radical theory of aging, which argue ROS are uncontrolled and thus cause damage that accumulates with age, ROS are under strict control. In fact, a compartmentalization of oxidative events appears to exist in terms of physiological stimuli, signaling mechanisms, and functional consequences (Pani et al., 2001; Soberman, 2003), clearly arguing in favor of seeing ROS as tamed in cells.

Even ROS originating in mitochondria are no longer seen as unintentional by-products of metabolism but as players in signaling pathways (Carmody and Cotter, 2001). One appropriate example comes from mice with a faulty proof-reading version of mitochondrial polymerase age. As mentioned above, these animals appear to age faster than controls, yet this phenotype is not a result of ROS damage or leakage but instead it appears to be linked to an induction of apoptosis (Kujoth et al., 2005). These results are in line with our argument linking mitochondria to apoptosis as part of a signaling cascade that might be involved in aging but is not necessarily a result of damage caused by ROS.

Overall, it now seems that ROS mostly impact on cells through their actions on signaling pathways rather than via nonspecific damage, as reviewed before (Maher and Schubert, 2000). As mentioned earlier, cancer cells often have increased ROS levels (Suh et al., 1999) while oxidative stress can induce senescence or apoptosis in normal cells (Aw, 1999; Martindale and Holbrook, 2002). The control of cell proliferation by ROS thus appears to be unrelated to damage but rather due to signaling pathways: low amounts of ROS can trigger cell proliferation while disruption of redox state by external factors can induce growth arrest, on a first stage, apoptosis if the stress is higher than a given threshold, and even necrosis if the stress is overwhelming (Fig. 2). Different types of human cells cultured above 20% O_2 display a reduced growth rate and endure fewer cumulative population doublings, but the same effect is not witnessed in tumor cell lines (Saito et al., 1995). What we hypothesize is that this phenomenon, as well as the high ROS levels often present in cancer cells, may be unrelated to damage. Instead, maybe anti-cancer mechanisms are activated by ROS signaling in normal but not in transformed cell lines. After all, cancer cells do not seem to feature any novel anti-oxidant defenses.

Experimentally, the notion that ROS are not just causes of havoc but actually tamed in cells is relevant. So far, most experiments aiming to test the free radical theory of aging do so based on the notion that ROS are damaging, destructive compounds that must be tamed or reduced. On the other hand, it now appears that, with the exception of disease states (Halliwell, 1997; Kannan and Jain, 2000), ROS are under strict control and are regulated by a number of mechanisms that use ROS in normal cellular processes. This does not invalidate a possible role of ROS in aging (see below), but it challenges the view that disruption (Sohal and Allen, 1990) or damage

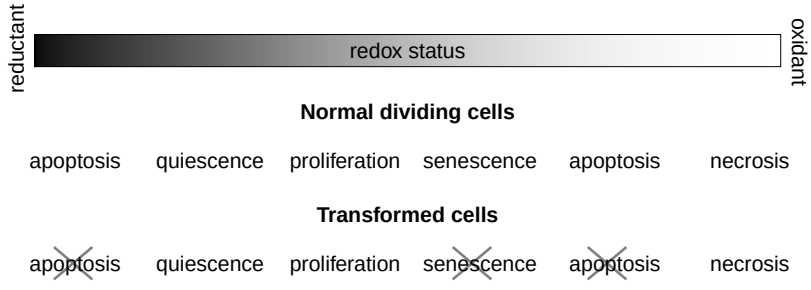

Fig. 2. Proposed cellular responses to oxidative stress and oxidation–reduction (redox) status in mitotic cells. Some quiescent cells can be stimulated with low amounts of ROS to proliferate. In normal cells, high levels of ROS can trigger senescence or apoptosis, probably due to the activation of anti-tumor mechanisms. In transformed cells, however, high levels of ROS do not typically cause senescence or apoptosis and so these cells proliferate vigorously even with high ROS production. Overwhelming oxidative stress leads, of course, to cytotoxicity. Adapted from Aw (1999).

(Nemoto and Finkel, 2004; Balaban et al., 2005) caused by uncontrolled or toxic ROS is the primary mechanism of aging.

4.2. ROS in growth and development

The idea that ROS are used as signaling molecules during development, cellular proliferation, and differentiation has been suggested by many others (Allen and Tresini, 2000; Rollo, 2002). What we hypothesize is that the connection between ROS and growth and development may explain why long-lived animals have lower levels of ROS production without ROS production being necessarily a causal factor in aging. Among vertebrates there is a strong correlation between developmental time and longevity (Finch, 1990; Charnov, 1993; de Magalhaes et al., unpublished data). Our hypothesis is that the lower ROS production and decrease in oxidative damage observed in long-lived animals results from their delayed development and/or growth which impacts on cell turnover, cell growth, and apoptosis (Fig. 3).

Rodents, bats, and birds have similar adult body sizes and metabolic rates but the way rodents age considerably faster and have higher ROS production has been hailed as evidence in favor of the free radical theory of aging (Barja, 2002). Our interpretation, however, is that differences in ROS production are due to the faster development and/or growth of rodents which is a product of cellular proliferation—in turn a result of rates of cell division and the proportion of dividing cells—and apoptosis. For example, gestation is about three times longer in bats (*Myotis lucifugus*) than in mice and bats take five to seven times longer to reach sexual maturity (Nowak, 1997). Age at sexual maturity is an established measurement of rate of development (Charnov, 1993), even if it is a simplification that does not take into account variations in growth rates during the developmental period. Nonetheless, our argument is simple: for animals of the same size that mature at radically different ages there must be differences in the processes controlling growth and development, such as cell proliferation and apoptosis. Since ROS are part of signaling pathways in cell proliferation and apoptosis, differences in ROS production between these organisms may merely reflect differences in developmental schedules which happen to be associated with longevity. It is not surprising then that, for instance, mice have higher levels of ROS generation than bats (Brunet-Rossinni, 2004). Similar results are observed when birds are compared to rodents (Table 1). Furthermore, while cellular stress resistance, including oxidative stress, has been correlated with animal longevity (Kapahi et al., 1999; Finkel and Holbrook, 2000), this may be unrelated to aging but rather a consequence of different developmental schedules, allometric effects, or differences in tumor-suppressor mechanisms.

Growth in rodents is negatively correlated with lifespan (Miller et al., 2002; Rollo, 2002). Moreover, in many genetic manipulations of endocrine systems that extend longevity, often retarding aging, animals are dwarf or at least are smaller than controls. Frequently, these long-lived strains also feature lower levels of oxidative damage, even though precise studies of the multiple sources of ROS are lacking (Bartke et al., 2003; Brown-Borg and Rakoczy, 2003; 2005; Holzenberger et al., 2003; Madsen et al., 2004; Brown-Borg et al., 2005). Therefore, our interpretation is that, as above, the slower or impaired growth of long-lived rodents may impact on cell proliferation, growth, or turnover, and consequently on ROS metabolism, without the need for ROS to play a causal role in life-extension.

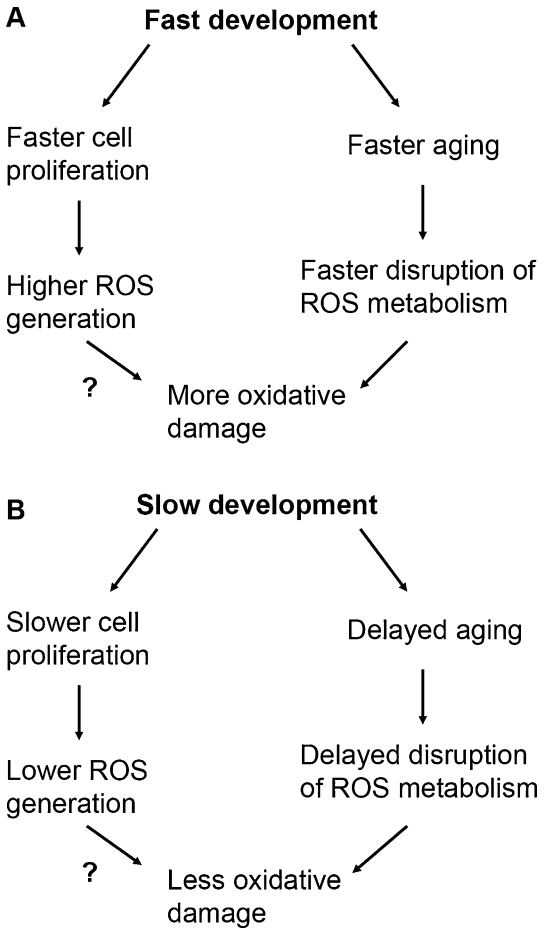

Fig. 3. Overview of the model linking development, aging, ROS generation, and oxidative damage. The pace of development correlates with the pace of aging (Charnov, 1993; de Magalhaes et al., unpublished data), and so slow development is associated with a longer lifespan and vice versa. In species with fast growth and development (A), such as mice and rats, cellular growth, proliferation, and/or turnover occur faster, which in turn involve ROS. Since aging will also occur faster in these species, so will the disruption of homeostasis that characterizes aging, including the deregulation of pathways associated with ROS metabolism that leads to a faster accumulation of oxidative damage. On the other hand, in long-lived species (B), such as many bats and birds, and even humans, development occurs slower, as does cell proliferation and/or turnover. In these species, the rate of ROS production will be lower due to the slower pace of development. Since aging will also occur slower, the disruption of pathways involved in ROS metabolism will be delayed and so oxidative damage will accumulate slower. Therefore, our suggestion is that the correlation between oxidative stress/ROS production and longevity is a consequence of changes at a cellular level driven by different developmental schedules. It is theoretically possible, however, that the way ROS production is optimized during development ends up playing a role in aging (see text).

Interestingly, the growth signals linked to the regulation of growth and aging in these rodents, such as the mitogens growth

Table 1
Relation between maximum longevity and length of development, herein measured as age at sexual maturity, in species previously used in analyses of ROS leakage and aging

Animal (species)	Maximum longevity in years	Age at sexual maturity in weeks	Adult body size in grams	Reference
Parakeets (*Melopsittacus undulatus*)	21	At least 26	30	Sibley (2001)
Pigeons (*Columba livia*)	35	20–25	300	Sibley (2001)
Canaries (*Serinus canara*)	24	±40	20	Sibley (2001)
Bats (*Myotis lucifugus*)	34	30–40	10	Nowak (1997)
Mice (*Mus musculus*)	<5	5–7	20	Nowak (1997)
Rats (*Rattus norvegicus*)	±5	±10	400	Nowak (1997)

Age at sexual maturity and adult body size represent typical values for the species.

hormone (GH) and insulin-like growth factor 1 (IGF-1), modulate components of the anti-oxidant defense system (Rollo, 2002; Brown-Borg and Rakoczy, 2003). Succinctly, GH suppressed H_2O_2-induced apoptosis in neonatal rat cardiomyocytes due to the activation of a signaling cascade involving extracellular signal-regulated kinases (Gu et al., 2001), and IGF-1 appears to exert its neuroprotective actions by influencing oxidative defenses (Gustafsson et al., 2004). Mice treated with GH and insulin, which increase plasma IGF-1 levels, featured a lower ROS production in the liver, despite conflicting results concerning the effects of such treatment on oxidative damage (Sanz et al., 2005). We propose that the impact of GH and IGF-1 on ROS is an example of ROS acting as signaling molecules in cell populations while part of the developmental cascade. These results establish a cause–effect relation in which the GH/IGF-1 axis impacts on development, growth, and aging but also on ROS production and antioxidant systems. As expected, rodents under caloric restriction, whose life-extension may be due to an altered GH/IGF-1 axis (de Magalhaes, 2005), also feature delayed growth (Weindruch and Walford, 1988; Hayflick, 1994).

In our model, ROS production and aging rates thus evolved independently from each other. It can be argued, however, that a higher ROS production, regulated by developmental mechanisms, in turn causes aging. In other words, maybe ROS are controlled in cells and used as signaling molecules, and later provoke damage leading to aging. Maybe even endocrine signals like GH and IGF-1 trigger ROS to regulate development and growth but the same ROS later contribute to aging as a form of antagonistic pleiotropy (Williams, 1957). Theoretically, this possibility exists and at least one model has been proposed along these lines (Sohal and Allen, 1990). Contrary to the predictions of Sohal and Allen (1990), however, metabolic rates, as described above, do not influence mammalian longevity, so this variant of the free radical theory of aging has also its weaknesses. On the other hand, maybe ROS leakage in mitochondria is somehow determined by developmental schedules independently of O_2 consumption but will later impact on longevity. Even so, experiments in which oxidative damage, but not developmental schedules, was altered—such as in SOD2 heterozygous mice—failed to influence aging, as thoroughly described above. Therefore, and although further experiments testing whether ROS contribute to both developmental rates and aging may be useful, at present such hypothesis is merely theoretical.

4.3. Why oxidative damage increases with age

Following on the above model, our interpretation is that aging, as many diseases, deregulates the control multiple systems exert over ROS, resulting in oxidative stress at later ages. The keepers of the flame, the antioxidants, become deregulated with age, just like so many other cellular components, and so oxidative damage occurs. Our interpretation is that loss of control over ROS is likely an effect, not a cause of aging, just like numerous pathologies not caused by ROS are often associated with oxidative damage. Since oxidative pathways are so important and must be strongly regulated in human biochemistry, any disruption of homeostasis—independently of its cause—has the potential to affect these pathways resulting in a loss of control over ROS. Indeed, an age-related impairment of the transcriptional response to oxidative stress has been reported in mice (Edwards et al., 2003), and declines in oxidative stress tolerance with aging have also been linked to impairments in signaling events in mouse hepatocytes (Li and Holbrook, 2003). Given the essential role of ROS in so many cellular processes, this increase in oxidative damage is unsurprisingly widespread in several tissues. Just like fire is used by human civilization and yet can be a great source of damage and death, ROS can, uncontrolled or in certain events such as apoptosis or infection, cause widespread damage and even cell death. The accumulation of oxidative damage with age would then, in our model, result from a deregulation of systems linked with the production and elimination of ROS that may be an effect, not cause, of aging.

Even if ROS are not a causal factor in aging, which we interpret as the most likely possibility but concur the topic will remain contentious for a long time, if redox signaling plays a downstream role in pathways that may impact on aging, such as apoptosis, then manipulations of ROS may theoretically have an effect on aging. This could be the case in the life-extending genetic manipulations of $p66^{shc}$ and thioredoxin. Yet since ROS are not just toxic, damaging agents, manipulations of ROS must consider the multiple roles of ROS in biology and thus be more subtle than previously thought. Lastly, and although this was not the subject of this work, ROS may also be relevant in the pathophysiology of specific age-related diseases.

5. Concluding remarks

This work offers only a simplified overview of free radical biology because our understanding of it is still limited. Different ROS impact on different pathways and different

cellular organelles, and so the role of ROS is much more specific than previously thought. This also means that the methodologies used to measure ROS production and oxidative damage are only looking at a fraction of the biological roles of ROS. Moreover, different cell types may have different balances of ROS. For example, different ROS often have antagonistic effects in cellular functions (Chandra et al., 2000) and single proteins that control oxidation and reduction events can have distinct functions under different intracellular microenvironments and contexts (Soberman, 2003). Therefore, the complexity of ROS in human biology is only beginning to be understood.

Contrary to most formulations of the free radical theory of aging, it appears that ROS are actually under strict control in human cells. ROS are not necessarily a cause of havoc but rather critical biological molecules that are used as intra- and extracellular signaling molecules. We argue that the faster growth and/or development of short-lived species explains why they also feature higher levels of ROS production. With age, control over ROS may be deregulated leading to oxidative damage, but this may be an effect, not cause of aging. A pleiotropic role of ROS in development and aging is plausible, though so far merely theoretical, and further experiments testing such concept are warranted. Consequently, our work, even though it points out flaws in the free radical theory of aging, does not eliminate an involvement of ROS in aging. In conclusion, this emerging model linking ROS to multiple functions, such as development and growth, offers a better explanation to recent results than the free radical theory of aging and provides a theoretical framework to better asses the putative roles of ROS in aging.

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