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LETTER TO THE EDITOR

The Free Radical Theory of Aging Is Dead. Long Live the Damage Theory!

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Abstract

The free radical theory of aging posits that aging is caused by accumulation of damage inflicted by reactive oxygen species (ROS). Although this concept has been very useful in defining the contribution of oxidative damage to the aging process, an increasing number of studies contradict it. The idea that oxidative damage represents only one of many causes of aging also has limitations, as it does not explain causal relationships and inevitability of damage accumulation. Here, it is discussed that infidelity, heterogeneity, and imperfectness of each and every biological process may be responsible for the inevitable accumulation of by-products and other damage forms. Although ROS are prototypical by-products, their contribution to aging is governed by the metabolic organization of the cell, its protective systems, and genotype. These factors are controlled by natural selection and, like dietary and genetic interventions that extend lifespan, change the composition of cumulative damage and the rates of accumulation of its various forms. Oxidative damage, like other specific damage types viewed in isolation or in combination, does not represent the cause of aging. Instead, biological imperfectness, which leads to inevitable accumulation of damage in the form of mildly deleterious molecular species, may help define the true root of aging. Free radical and other specialized damage theories served their purpose in the understanding of the aging process, but in the current form they limit further progress in this area. *Antioxid. Redox Signal.* 20, 727–731.

Free Radical Theory of Aging

The free radical theory of aging (26) was originally described by Denham Harman in the 1950s (11). It proposes that organisms age because they accumulate oxidative damage. This damage comes from reactive oxygen species (ROS), which are partially reduced metabolites of molecular oxygen generated as products of metabolic reactions or as by-products of various cellular processes, such as respiration. For many years and to this day, this theory has been the most popular concept in the area of aging, with thousands of publications every year. There are numerous studies that demonstrate that ROS and oxidative damage increase with age (28) and that reducing oxidative damage extends the lifespan of various model organisms (yeast, nematodes, fruit flies, mice, *etc.*), as well as that increased production of ROS shortens lifespan (16).

Domination of the free radical theory has been little affected by an increasing number of studies that seem to contradict it (4, 6–8, 17, 18, 21, 23, 24, 27, 30, 31). For example, although in some experimental systems, antioxidant proteins extend lifespan, their overexpression in other systems was found to be ineffective (21). These findings also hold when the system is controlled for appropriate regulation and expression levels of these proteins (24). Increased antioxidant protection may even lead to shortened lifespan, whereas decreased antioxidant function may extend it (31). Evidence against the idea of the universal role of ROS in the aging process also includes the observation that aging still occurs under anaerobic conditions, where there is little ROS. Specifically, the lifespan of anaerobically grown yeast cells is shorter compared with the cells grown aerobically (rather than longer as would be expected if ROS are the cause of aging), not regulated by antioxidant enzymes (whereas these enzymes may regulate it under aerobic conditions), and is further shortened (rather than extended as commonly observed for this dietary regimen) by caloric restriction (17).

To reconcile the free radical theory with the observations that contradict it, researchers have proposed that ROS may

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serve signaling functions, thereby activating protective and adaptive programs (25, 32). It was also proposed that it is necessary to consider positional effects of ROS generation and primary targets of these reactive species (16). Indeed, if oxidative stress occurs in localized areas of the cell, analyses of total oxidative damage may not represent the actual damage inflicted by ROS. Whereas these arguments help address many experimental contradictions, many other questions remain. For instance, these arguments do not explain the fact that the utilization of molecular oxygen, the precursor for ROS, is not universally required for the aging process. However, these arguments may not even be necessary, if oxidative damage is viewed in the context of a model that considers aging as a product of biological imperfectness leading to inevitable accumulation of the myriad damage forms (see below).

Oxidative Damage is Just One Type of Damage

Oxidative damage received much attention in the field of aging because it could be monitored by using established analytical techniques (28) and be regulated by designated enzyme systems (superoxide dismutase, catalase, peroxiredoxin, glutathione peroxidase, methionine sulfoxide reductase, *etc.*). ROS are continuously generated as a consequence of respiration and other metabolic processes, and their damaging activity is easy to comprehend from a chemical point of view. Molecular oxygen is a prototypical reactive compound, which adventitiously reacts with the active sites of various enzymes, resulting in partially reduced oxygen species, the ROS, which damage cellular biomolecules (12). However, there is no reason to think that any other cellular process could not generate by-products. Many by-products escape researchers' attention because experimental analyses mostly focus on the primary functions of proteins, RNAs, and cellular metabolites, whereas their indirect functions are rarely examined. Nevertheless, there is abundant literature on damage accumulation during aging that considers processes beyond oxidative damage (28). It started with the Orgel's idea of errors in transcription and translation leading to error catastrophe due to errors in protein function (22) and expanded to other types of damage, including damage to DNA, proteins, and metabolites. The idea of damage accumulation as the causal factor in the aging process is currently favored by many researchers. It has been unclear, however, what the actual spectrum of damage in the cell is and why a balance between damage accumulation and clearance cannot be maintained over time in an organism. For example, if superoxide anion radical is a damaging species, why do not cells completely remove it with superoxide dismutase or decrease its generation during metabolic processes? Why brain, being a highly active metabolic organ, has lower antioxidant protection? How could the impact of oxidative damage be compared with the damage from other processes, such as metabolite damage, translational errors, transcriptional heterogeneity, mistargeting proteins to cellular compartments, and imbalance in the levels of interacting factors?

Biological Imperfectness and the Aging Process

We suggest that all biomolecules and biological processes are imperfect, manifesting in unintended activities and functions. Thus, damage, in the form of by-products, errors of all sorts, misbalance in cellular components, *etc.*, is produced by each and every cellular process (9, 10). For example, consider enzymes. They have impressive specificity, but they are not perfect and generate minor reaction products and other unwanted by-products (29, 20). Enzymes' fidelity is restricted by the fact that they are flexible polymers that exist in various conformations and are made of a limited set of amino acids and cofactors. It is further compromised by errors in protein sequence and structure resulting from errors in transcription, translation, folding, and post-translational modifications, by mutations and genetic variability, and by other factors. In other words, not a single enzyme is perfect, no matter how well its active site is built by the combined action of its amino acid residues and cofactors. Besides making the main product from its substrate through its direct (evolved) function, the enzyme produces a little bit of something else and occasionally reacts with molecules other than its natural substrate, which are the manifestations of its indirect (not evolved) functions. It should be noted that such by-products are largely not random. They are governed by catalytic properties of each enzyme; their chemical identity and rates of accumulation can be changed during evolution. Thus, a gene encoding an enzyme codes for both direct and indirect functions of this enzyme, both of which are genetically controlled.

However, the enzyme-generated by-products only account for a fraction of the damage produced in cells because all other cellular components and systems are also imperfect and heterogeneous. It may be expected that each cellular reaction and all macromolecular interactions generate damage through indirect functions of biomolecules. More broadly, damage will necessarily arise from imperfectness, heterogeneity, and noise of biological systems. Similarly, variability in gene and protein expression will result in cell-to-cell differences as well as differences among individual organisms of the same species. Many minor products of cellular metabolism are simply not detectable because methods do not exist that can analyze them, or because an averaged signal is analyzed. The concept of by-products of catalytic reactions is well accepted in chemistry, but biologists tend to operate in terms of perfect biological systems, overlooking this fundamental principle. Biology increases complexity, but nothing disappears from chemistry when it comes to biology.

Cellular damage generated as a result of imperfectness would certainly include oxidative damage. However, the latter, like any other damage form, would only represent a subset of total damage, which, regardless of its contribution to the regulation of lifespan, would have nothing to do with the cause of aging (9, 10). How does the cell deal with the damage? Much of the damage remains confined within the space surrounded by cellular and organelle membranes. Many cellular by-products that represent more severe damage and immediate danger can be cleared up by the protection and repair systems that metabolize or export damage from the cell. There are also related strategies, such as the so-called Maxwell's demons, which represent the processes that by generating the progeny from within the old (*e.g.*, budding process in yeast) result in an unequal distribution of molecular damage between cells (3). However, irrespective of the specific strategies that help clear and redistribute the damage, the number of damage forms would always be greater than the number of protective systems. This is because each biological

process generates damage and because clearance systems, while removing certain damage types, generate other damage types. Thus, the damage will inevitably accumulate in the cell, unless the cell divides, diluting its damage. Sooner or later, depending on the regulation imposed by natural selection, damage accumulating in postmitotic cells will compromise cellular function and the cell will senesce and die. Nondividing cells can modulate the time to senescence by altering their metabolism and by the selective use of designated protective systems, but cannot completely stop the process of damage accumulation, and therefore cannot avoid cell death.

As oxidative damage represents only a subset of total damage, its behavior and impact on cellular function will characterize cumulative damage under some conditions, but not under other conditions. Therefore, oxidative damage and the associated clearance systems may regulate lifespan, or they may not, depending on the contribution of oxidative damage to the overall damage. We may expect much variability in the role of oxidative damage in aging among different cell types, genotypes, metabolic states (*e.g.*, depending on the use of molecular oxygen), various species, and different environmental conditions. These considerations obviate the need to consider localized ROS or a balance between generation and removal of oxidative damage as well as contradictory data on the role of ROS and their clearance in regulating lifespan. ROS may simply be irrelevant to aging under certain conditions, such as anaerobic growth, but may be relevant under other conditions, such as hypoxia. As such, the ROS contribution will be greatly influenced by other processes and will be dependent on numerous other factors that regulate cellular life. More importantly, neither ROS nor any other damage forms would represent the actual cause of aging, since the underlying reason the damage is generated, and cannot be fully cleared, is biological imperfectness.

Cumulative Damage Defines Lifespan

Many genetic manipulations and nutrient conditions are known to extend the lifespan (2, 13, 14), consistent with the evolutionary underpinning of the aging process (15). Moreover, interventions that lead to lifespan extension in one organism can often be successfully applied to other organisms. For example, caloric restriction or inhibition of target of rapamycin (TOR) function affect the lifespan in many organisms. These lifespan extension effects are thought to modulate the rate of aging through hormesis and other mechanisms. In the context of this discussion, this would mean that these treatments regulate the rate of damage accumulation by targeting molecules that make it. However, the forms of accumulated damage will also change depending on the metabolic organization of the cell (1). Upon changes in environmental conditions, nutrients, or other factors, the cell will respond by changing its metabolism, signaling programs, gene expression, *etc.* The new metabolic state will be accompanied by the new landscape of damage accumulation, that is, different damage forms will be accumulating, and the rates of accumulation of the common damage may also be different. Whereas the overall effect of the lifespan extending treatments may be the decrease of cumulative damage, these treatments will do so principally by restructuring metabolism to generate a different damage spectrum, which will accumulate at different rates, compared with the untreated state.

Experimental data appear to be consistent with this idea. For example, caloric restriction and TOR deficiency in yeast increase respiration (5, 19). Although ROS and some forms of oxidative damage may be increased by these interventions, other damage forms may be decreased. More generally, it would be insufficient to demonstrate a decrease in the accumulation of any single damage type upon certain treatment, as other damage forms may become more abundant or more relevant to aging, under these conditions. The use of oxidative damage as a marker of cumulative damage may be misleading in assessing aging and senescence, although under some conditions it may well correlate with the aging process.

It is also important to distinguish the cause of aging from the control of lifespan (9, 10). Natural selection can control lifespan by influencing the rate of damage generation and the rate of clearance of its severe forms, thereby regulating chemical identity of various damage forms and their rates of accumulation. On the other hand, imperfectness, infidelity, and heterogeneity are fundamental properties of biological systems. They may be viewed as the true root of aging. Thus, natural selection can slow down or accelerate damage accumulation and the onset of damage overload, but cannot stop them or postpone indefinitely. We do not know the limits to lifespan extension because, in the natural setting, what matters is the ability to pass genes to the next generation rather than achieving the maximal lifespan. These considerations do not exclude a possibility of exceptional lifespan and even immortality for certain species that can constantly grow or generate all their somatic cells from stem cells. As long as their cells divide diluting mild damage or are regenerated, a balance between damage generation and clearance may be achieved. However, it cannot be achieved in organisms with postmitotic nonrenewable cells (*e.g.*, in mammals). This also means that the aging process appeared and disappeared many times during evolution.

Concluding Comments

The free radical theory of aging is consistent with numerous studies, but many other reports clearly contradict this idea. Collectively, these studies argue against the universal role of oxidative damage in aging. For this reason, many researchers turned to a broader concept that many forms of damage serve as causal factors in the aging process, with ROS representing some of the major causes, but not the only cause. Whereas attractive, this concept itself has limitations, as it does not explain why cells are unable to maintain a balance between damage generation and removal. Although the inevitable nature of damage accumulation is well accepted by many and even considered by some researchers as a dogma, why the damage is inevitable has actually been unclear. This article discusses a different model that considers biological imperfectness, which manifests as indirect functions of biomolecules, as the true root of aging.

Heterogeneity, imperfectness, and infidelity of biological systems generate damage from every biological process, and therefore, they necessarily lead to the accumulation of damage in postmitotic cells. This biological imperfectness-driven damage is the consequence of life itself and specifically of its inherent chemistry. Unless cells divide to dilute the damage, damage accumulation will ultimately drive cells to senescence. The timing for this process would depend primarily on

the metabolic organization of the cell and its genetic program. As more severe damage is removed by the designated protection systems, the slightly deleterious, mild damage forms will gradually accumulate. Many of these damage forms will not be subject to natural selection and no protection systems will evolve against them. Oxidative damage would contribute to the aging process only as a subset of total damage. It may be more relevant to regulating lifespan under some conditions, but less relevant under other conditions. For example, anaerobically grown yeast cells do not generate significant levels of ROS. Thus, these species as well as enzymes that protect against them do not have a significant role under these conditions. However, they do under conditions that utilize oxygen, for example, respiration and hypoxia. Similarly, the contribution of oxidative damage to the total damage would depend on the cell type, species, metabolic state of the cell, nutrients, genotype, *etc.* Thus, ROS, like any other damage form, may affect lifespan serving as mediators of lifespan control, but the actual cause of aging is biological imperfectness. It is the reason damage is produced in the first place and the sheer number of damage forms precludes its clearance.

In addition, any cell perturbation that changes lifespan, such as dietary intervention, knockout, knockdown, and forced gene overexpression, would necessarily affect cellular metabolism by modulating fluxes through various pathways, such as respiration, glycolysis, amino acid and fatty acid metabolism, as well as cellular regulatory mechanisms. Such metabolic reprogramming will be characterized by different metabolic reactions, which will generate a different spectrum of damage forms. In other words, some damage forms will be the same and some will be different, in an organism subjected to a lifespan-extending intervention. In addition, metabolic reprogramming will lead to the accumulation of damage at different rates. Thus, the lifespan extending strategies act by both slowing down damage accumulation and shifting cellular metabolism to the states wherein different damage forms accumulate. This concept also applies to the dietary regimens that affect lifespan, such as caloric restriction and rapamycin treatment. For example, it would be incorrect to compare oxidative damage under *ad libitum* and caloric restriction conditions and conclude on the contribution of oxidative damage to the aging process, because different forms of damage will be accumulating under these conditions. What should be compared is the cumulative damage as well as its contribution to disruption of cellular homeostasis.

Only recently, experimental tools, such as sensitive, high-throughput sequencing, proteomic and metabolite profiling methods, have been developed that may be used to begin assessing the myriad damage forms generated in the cell. With these tools, basic properties of cellular damage, such as chemical identity, quantity, rates of accumulation, and synchronization, may be defined. This is not easy as one may need to distinguish, for instance, between metabolites and metabolic by-products and show the causal role of imperfectness. Many individual mild damage forms would contribute insignificantly to the overall damage, so removal of any one of them may not have any impact on lifespan or fitness.

As a prototypical aging concept, the free radical theory of aging has served its purpose for the development of our understanding of aging, initially as a standalone idea and later as a concept that oxidative damage represents one of the many causes of aging. This theory offered experimentally testable hypotheses and contributed very significantly to our current understanding of aging. However, further research exposed its weaknesses, which cannot be reconciled even if oxidative damage is viewed as a component of cumulative damage or one of the many causes of aging. Sure, oxidative damage may increase as a function of age, and antioxidant enzymes may protect under some conditions, but focusing on these observations distracts from addressing the true root of aging. It is time to conclude that the oxidative (free radical) theory of aging limits further understanding of the aging process.

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Abbreviations Used

ROS = reactive oxygen species
TOR = target of rapamycin