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Minireview

Increased molecular damage and heterogeneity as the basis of aging

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Abstract

Aging at the molecular level is characterized by the progressive accumulation of molecular damage. The sources of damage act randomly through environmental and metabolically generated free radicals, through spontaneous errors in biochemical reactions, and through nutritional components. However, damage to a macromolecule may depend on its structure, localization and interactions with other macromolecules. Damage to the maintenance and repair pathways comprising homeodynamic machinery leads to age-related failure of homeodynamics, increased molecular heterogeneity, altered cellular functioning, reduced stress tolerance, diseases and ultimate death. Novel approaches for testing and developing effective means of intervention, prevention and modulation of aging involve means to minimize the occurrence and accumulation of molecular damage. Mild stress-induced hormesis by physical, biological and nutritional methods, including hormetins, represents a promising strategy for achieving healthy aging and for preventing age-related diseases.

Keywords: biogerontology; gerontogenes; homeodynamics; homeostasis; reactive oxygen species; stress.

Introduction

A wealth of information has been gleaned by biogerontologists regarding changes during aging at all levels of biological organization. Whereas several of these age-related normal and pathological changes may be widely observed across species, other changes are specific to specific species, organs, systems, tissues, cells, organelles and macromolecules. A common molecular characteristic of aging to emerge from such studies is the occurrence and accumulation of damage to macromolecules, and progressive accumulation of damaged macromolecules inside and outside cells. Table 1 lists the major categories of damage to macromolecules that have been observed to accumulate in various cells, tissues and organs during aging. Although different types of molecular damage accumulate at different rates and to different extents in different cells, the fact remains that there is progressive accumulation of molecular damage, which is the universal characteristic of aging.

All small and large molecules are prone to damage, but the source and biological consequences of various types of molecular damage vary widely. Furthermore, whereas the action of a damaging agent is essentially stochastic, the vulnerability of a macromolecule to the damaging agent and the final occurrence of damage are determined by the chemical sequence, structure and accessibility of the macromolecule in the presence of several other interactive macromolecules. Therefore, some macromolecules may be preferentially damaged, whereas others may not be easily damaged.

What is more important is to realize that it is not a straightforward and simple matter to relate any particular type of damage and its level in cells to a specific biological consequence. However, it is generally agreed that increased molecular damage and heterogeneity are the fundamental basis of aging and age-associated pathologies (Holliday, 2006, 2007; Rattan, 2006; Hipkiss, 2007). This article reviews the causes and mechanisms of molecular damage during aging, the consequences of progressive accumulation of molecular damage during aging, and anti-aging strategies for intervention in and prevention of age-related diseases.

Molecular damage during aging

There are three major sources of damage within a cell: (i) reactive oxygen species (ROS) and other free radicals (FRs) formed by the action of external inducers of damage (e.g., UV rays) and as a consequence of intrinsic cellular metabolism involving oxygen, metals and other metabolites; (ii) nutritional glucose and its metabolites, and their biochemical interactions with ROS and FRs; and (iii) spontaneous errors in biochemical processes, such as DNA duplication, transcription, post-transcriptional processing, translation, and post-translational modifications.

The occurrence of molecular damage has led to the formulation of at least two mechanistic theories of biological aging, which have been the basis of most of the experimental aging research during the last 50 years (Rattan, 2006). The first is the so-called free radical theory of aging (FRTA), originally proposed in 1954 but first published in 1956, which arose from consideration of the aging phenomenon from the premise that a single common biochemical process may be responsible for the aging and death of all living beings (Harman, 2006). There is abundant evidence to show that a variety of ROS and other FRs are indeed involved in molecular damage that

Table 1 Main categories of molecular damage during biological aging.

Macromolecule	Examples of damage	Selected references
DNA	Mutations, epimutations, base modifications, deletions and strand breaks	Loeb et al., 2005; Lombard et al., 2005; Wallace, 2005; Singh, 2006
RNA	Base modifications, miscoding and missplicing	Rattan, 2003
Protein	Amino acid modifications, misincorporation, misfolding and aggregation	Rattan, 1995; Baynes, 2000; Grune, 2000; Rattan, 2003; Stadtman and Levine, 2003; Cloos and Christgau, 2004; Grune et al., 2004
Carbohydrates, lipids, and molecular conjugates	Advanced glycation end products, lipofuscin and aggrosomes	Dukic-Stefanovic et al., 2001; Hallén, 2002; Suji and Sivakami, 2004; Niki et al., 2005; Stroikin et al., 2005

can lead to structural and functional disorders, diseases and death.

The chemistry and biochemistry of FRs are very well worked out, and the cellular and organismal consequences are well documented (Sitte and von Zglinicki, 2003). However, the main criticism raised against FRTA involves its lack of incorporation of the essential and beneficial role of FR in the normal functioning and survival of biological systems (Holliday, 1995; Linnane et al., 2007). Furthermore, FRTA presents FRs as the universal cause of damage without taking into account differences in the wide range of FR-counteracting mechanisms in different species, which effectively determine the extent of damage occurrence and accumulation. In addition, a large body of data showing the contrary and/or lack of predictable and expected beneficial results of antioxidant and FR-scavenging therapies has restricted the application of FRTA (Le Bourg and Fournier, 2004; Le Bourg, 2005; Howes, 2006).

The second major mechanistic theory that incorporates the crucial role of macromolecular damage is the so-called protein error theory of aging (PETA). The history of PETA, also known as the error catastrophe theory, is often marked by controversy (Holliday, 1996; Rattan, 1996, 2003). Since the spontaneous error frequency in protein synthesis is generally several orders of magnitude higher than that in nucleic acid synthesis, the role of protein errors and their feedback in biochemical pathways is considered crucial in aging. Several attempts have been made to determine the accuracy of translation in cell-free extracts, and most studies show that there is an age-related increase in the misincorporation of nucleotides and amino acids (Holliday, 1996, 2005; Rattan, 1996, 2003; Hipkiss, 2003). It has also been shown that there is age-related accumulation of aberrant DNA polymerases and other components of the transcriptional and translational machinery (Holliday, 1996; Rattan, 1996, 2003; Fukuda et al., 1999; Srivastava et al., 2000; Srivastava and Busbee, 2002).

Further evidence in support of PETA comes from experiments demonstrating that induction of and increases in protein errors can accelerate aging in human cells and bacteria (Holliday, 1996; Rattan, 1996, 2003; Nyström, 2002a,b). Similarly, an increase in the accuracy of protein synthesis can slow aging and increase life span in fungi (Silar and Picard, 1994; Silar et al., 2000; Holbrook and Menninger, 2002). Therefore, it cannot be ruled out that several types of error in various components of the protein synthetic machinery and in mitochondria indeed have long-term effects on cellular stability and survival (Kowald and Kirkwood, 1993a,b; Hipkiss, 2003; Holliday, 2005). However, almost all of the methods used relied on indirect *in vitro* assays, and direct, realistic and accurate estimates of age-related changes in errors in cytoplasmic and mitochondrial proteins, as well as their biological relevance, have not yet been made. Similarly, application of methods such as two-dimensional gel electrophoresis, which can resolve only some types of misincorporations, remains insensitive and inconclusive (Holliday, 1996; Rattan, 1996, 2003). It will be necessary to combine several methods, such as electrophoresis, mass spectrometry, protein-protein interactions and antibody-based detection of molecular heterogeneity, to identify the extent of protein errors and their biological role in aging.

Both FRTA and PETA provide mechanisms for the occurrence of molecular damage. In addition, nutritional components, especially sugars and metal-based micronutrients, can induce, enhance and amplify molecular damage, either independently or in combination with other inducers of damage. It is important to point out that although the action of damaging agents is mainly stochastic, whether a specific macromolecule is damaged and whether the damage persists depend on its structure, localization and interactions with other macromolecules, and on the activity and efficiency of a complex series of maintenance and repair pathways, as discussed below.

Homeostasis, homeodynamics and survival

All living systems have the intrinsic ability to respond to, counteract and adapt to external and internal sources of damage and disturbance. The traditional conceptual model to describe this property is homeostasis, which has dominated biology, physiology and medicine since the 1930s. However, tremendous advances in our understanding of the processes of biological growth, development, maturation, reproduction, and, finally, of aging, senescence and death have led to the realization that a homeostasis model as an explanation is incomplete. The main reason for the incompleteness of the homeostasis model is its defining principle of 'stability through constancy', which does not take into account new themes, such as cybernetics, control theory, catastrophe theory, chaos theory, and information and interaction networks, that describe the complexity of biology. Since the 1990s,

the term homeodynamics (Yates, 1994) has been increasingly used, especially in the context of aging. The concept of homeodynamics accounts for the fact that the internal milieu of complex biological systems is not permanently fixed, is not at equilibrium, and is subject to dynamic regulation and interaction among various levels of organization.

Aging, senescence and death are the final manifestations of unsuccessful homeodynamics. Table 2 lists the key molecular pathways and processes operating in cells that are quintessential components of the homeodynamic machinery. All these maintenance and repair processes involve genes whose products and their interactions give rise to a 'homeodynamic space', which is a type of buffering capacity and the ultimate determinant of an individual's chance and ability to survive and maintain a healthy state. However, our knowledge of the number of genes and their variants, their multiple interactions and consequences is too meager at present to identify, define and manipulate the homeodynamic machinery in any sensible way.

Consequences of increased molecular heterogeneity

The biological consequences of increased levels of molecular damage are wide-ranging, including altered gene expression, genomic instability, mutations, loss of cell division potential, cell death, impaired intercellular communication, tissue disorganization, organ dysfunctions, and increased vulnerability to stress and other sources of disturbance. A common mechanistic basis for all these consequences is increased molecular heterogeneity.

Since there is extremely low probability that any two molecules will be damaged in exactly the same way and to the same extent, an increase in molecular heterogeneity is inevitable. For example, if there are a thousand protein molecules freshly translated from a newly transcribed mRNA, and all these molecules are equally prone to post-translational stochastic damage as a function of their dwell time, very soon molecular heterogeneity will emerge within the molecular population. Furthermore, the nature, site and extent of damage will give rise to a population of that specific protein with alterations in structure and function ranging from being fully active to totally inactive molecules. Of course, among the thousands of types of proteins in a cell, some may be preferentially damaged in a particular context. For example, it has been reported that among 1000–2000 proteins inside mitochondria, aconitase is preferentially oxidatively damaged (Yan et al., 1997; Das et al., 2001). Some other proteins known to be more prone to oxidation include Hsp70, protein elongation factors, glutamine synthetase, glutamate synthetase, and pyruvate kinase (Nyström, 2002b; Stadtman and Levine, 2003). Similarly, vimentin is the specific target of glycation among thousands of other proteins in the skin (Kueper et al., 2007). Whereas altered and abnormal macromolecules are often preferentially degraded in normal, healthy and young cells, this process is progressively impaired with age (Hipkiss, 2006, 2007).

The resulting increase in molecular heterogeneity and dysfunctionality has at least two major consequences, discussed briefly as follows.

Interrupted networks

Since biological macromolecules generally act in scale-free networks, increased molecular heterogeneity is bound to lead to differential network perturbations and interruptions. Such interruptions may first occur at weak links, followed by disorganization, congestion and collapse of strong links and high-degree central hubs (Csermely, 2006; Budovsky et al., 2007; Hallen, 2007; Soti and Csermely, 2007; Szalay et al., 2007). Some of the major consequences of interrupted networks include inhibition of signaling cascade and transcription factor-regulated gene expression, dysregulation of feedback control leading to metabolic instability, and increased sensitivity to stress and other damaging agents.

Illegitimate networks

Damage to macromolecules often leads to changes in structure, function and stability, such as altered folding, mistargeting, and altered epitope exposure. This can result in the formation of novel interactions, hubs and network structures (Budovsky et al., 2007; Szalay et al., 2007), leading to new biological phenotypes and altered hierarchy of various mediators of the network, such as mediator ranking in the immune system (Tieri et al., 2005). Illegitimate networks can also lead to the activation, translocation and binding of transcription factors and other responsive elements, resulting in unwarranted gene expression that is otherwise strictly regulated.

At present, experimental biogerontology cannot be used to design precise experiments for testing the above hypotheses on the consequences of interrupted and ille-

Table 2 Main molecular pathways for maintenance and repair homeodynamics.

Biological pathway	Selected references
Antioxidative and enzymic defenses against reactive oxygen species	Halliwell, 2000; Sen et al., 2000; Ray and Husain, 2002; Azzi et al., 2004; Le Bourg, 2005
Stress response	Basu and Srivastava, 2000; Gutzeit, 2001; Verbeke et al., 2001; von Zglinicki et al., 2001; Temple et al., 2005
Protein repair and chaperoning	Mary et al., 2004; Krøll, 2005; Brégégére et al., 2006; Daugaard et al., 2007
Removal and turnover of defective proteins and other cellular components	Grune, 2000; Carrard et al., 2002; Grune et al., 2004; Martinez-Vicente et al., 2005
Nucleic acid repair	Lindahl and Wood, 1999; Bürkle, 2001; Bohr, 2002; Lombard et al., 2005; Rao, 2007a,b

gitimate networks. However, significant developments in systems biology and bioinformatics are beginning to provide experimental possibilities of unraveling rearrangements in cellular networks (Budovsky et al., 2007; Szalay et al., 2007), of quantifying the relevance and hierarchy of different mediators in these networks (Tieri et al., 2005), and of systematic intervention in transcriptional and translational networks (Raghothama et al., 2005; Li and Zhan, 2006).

Strategies for aging intervention, prevention and therapies

Scientific and rational anti-aging strategies aim to slow down aging, to prevent and/or delay physiological decline, and to regain lost functional abilities. Strengthening, improving or enlarging the homeodynamic space (or buffer capacity) at the level of all genes comprising the homeodynamic machinery and the molecular networks described above may be the ideal anti-aging remedy. However, such a gene-therapy approach for gerontomodulation requires redesign of the blueprint for structural and functional units of the body at the level of genes, gene products, macromolecular interactions, and molecular-milieu interactions, which is a daunting task.

A more realistic and promising approach in aging intervention and prevention, and which may be applicable in the near future, is based on using an organism's intrinsic homeodynamic property of self-maintenance and repair. The evolved nature of the homeodynamic machinery, in accordance with the life history traits of different species, sets an intrinsic genetic limit on the essential life span (ELS) or the so-called warranty period (Carnes et al., 2003; Rattan, 2006, 2007). Therefore, the main cause of age-related accumulation of molecular damage is the inefficiency and failure of maintenance, repair and turnover pathways. According to the homeodynamics-based explanations of aging and longevity described above, aging in the period beyond ELS and the onset of one or more diseases before eventual death appear to be the default setting so far. This viewpoint makes interventions in aging different from the treatment of one or more specific diseases, by prioritizing prevention over treatment and by aiming at the level of interacting networks instead of individual pathways.

It has been reported that if cells and organisms are exposed to brief periods of stress so that their stress response-induced gene expression is upregulated and the related pathways of maintenance and repair are stimulated, several anti-aging and longevity-promoting effects can be observed. Such a phenomenon, in which stimulatory responses to low doses of otherwise harmful conditions improve health and enhance life span, is known as hormesis (Rattan, 2004, 2005, 2008).

Mild stresses that have been reported to delay aging and prolong longevity in various systems include temperature shock, irradiation (UV, γ- and X-rays), heavy metals, pro-oxidants, acetaldehyde, alcohols, hypergravity, exercise and food restriction. Various nutritional components, especially those of plant origin, such as spices, flavanoids, and polyphenols, are considered to confer beneficial effects through stress-induced hormetic pathways (Ali and Rattan, 2006; Putics et al., 2008). Such natural and synthetic molecules have been termed hormetins (Ali and Rattan, 2006; Rattan, 2006, 2008). Hormesis-like beneficial effects of chronic but mild undernutrition have been reported for humans (Masoro, 2006, 2007; Rattan, 2006, 2008). Intermittent fasting has been reported to have beneficial effects on glucose metabolism and neuronal resistance to injury (Sharma and Kaur, 2005; Liu et al., 2006; Martin et al., 2006; Mattson, 2007). Since the main mode of action of mild stress-induced hormesis appears to prevent and/or remove molecular damage by stimulating pathways of degradation and turnover, this may be a useful strategy for developing novel approaches to aging intervention and prevention of neurodegenerative diseases such as Alzheimer's and Parkinson's diseases.

Finally, successful homeodynamics is a longevity assurance mechanism, whose strength, efficiency and range have evolved in accordance with the evolutionary history of the species. Survival of a species beyond the ELS is necessarily accompanied by the progressive accumulation of random molecular damage. Progressive failure of homeodynamics leads to physiological malfunctioning manifest as a general functional decline, diseases and ultimate death. Strengthening homeodynamics to prevent or slow down the occurrence and accumulation of molecular damage, as well as to maintain the stability of molecular networks, could be the basis of effective anti-aging interventions against age-related diseases and disability.

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