A synopsis on aging—Theories, mechanisms and future prospects

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ABSTRACT

Answering the question as to why we age is tantamount to answering the question of what is life itself. There are countless theories as to why and how we age, but, until recently, the very definition of aging – senescence – was still uncertain. Here, we summarize the main views of the different models of senescence, with a special emphasis on the biochemical processes that accompany aging.

Though inherently complex, aging is characterized by numerous changes that take place at different levels of the biological hierarchy. We therefore explore some of the most relevant changes that take place during aging and, finally, we overview the current status of emergent aging therapies and what the future holds for this field of research.

From this multi-dimensional approach, it becomes clear that an integrative approach that couples aging research with systems biology, capable of providing novel insights into how and why we age, is necessary.

Contents

1. Introduction

Aging is a topic that has captivated both scientists and philosophers throughout history. For Plato (428–347 BC), those who lived longer reached a philosophical understanding of mortal life, which lead to the desire in understanding everlasting ideas and truths, beyond the mortal world (Baars, 2012): "for wisdom and assured true conviction, a man is fortunate if he acquires them even on the verge of old age" (Cary et al., 1852). But perhaps the most accurate depiction of the human perception of aging comes from Giacomo Leopardi (1798–1837): "Old age is the supreme evil, because it deprives us of all pleasures, leaving us only the appetite for them, and it brings with it all sufferings. Nevertheless, we fear death, and we desire old age" (Leopardi et al., 1905).

In its broadest sense, aging merely refers to the changes that occur during an organisms' life-span, though the rate at which these take place varies widely (Kirkwood, 2005). Consequently, such definition comprises changes that are not necessarily deleterious, such as wrinkles and graying hair in humans, which do not affect the individual's viability. As Anton and co-workers put it (Anton et al.,

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2005), the phenotype is the end result of the interaction between genotype and external factors:

[phenotype] = [genotype] + [(diet, lifestyleandenvironment)].

To differentiate these innocuous changes from those leading to increased risk of disease, disability or death, biogerontologists tend to use a more precise term – senescence – when describing aging (Dollemore, 2002). Senescence is, therefore, the progressive deterioration of bodily functions over time and normal human aging has been associated with a loss of complexity in a wide range of physiological processes and anatomic structures (Goldberger et al., 2002), including blood pressure (Kaplan et al., 1991), stride intervals (Hausdorff et al., 1997; Terrier and Dériaz, 2011), respiratory cycles (Peng et al., 2002; Schumann et al., 2010) and vision (Azemin et al., 2012), among others, such as postural dynamics (Manor et al., 2010), ultimately leading to decreased fertility and increased risk or mortality (Chesser, 2015; Lopez-Otin et al., 2013). Herein, however, we will refer to the more inclusive term "aging", due to its extensive use in the literature. Though aging may be defined as the breakdown of self-organizing systems and reduced ability to adapt to the environment (Vasto et al., 2010), this is still a rather complex biological process with poorly understood mechanism(s) of regulation. Explanations of the aging mechanisms have become unexpectedly complicated. Where gerontologists once looked for a single, all-encompassing theory that could explain aging, such as a single gene or the decline of the immune system, they are now finding that multiple processes, combining and interacting on many levels, are on the basis of the aging process (Dollemore, 2002; Guarente, 2014) These processes take place not only at a cellular and molecular level, but also on tissues and organ systems. The relatively young science of aging is now becoming increasingly aware of the biochemical mechanisms that cause or react to aging (Yin and Chen, 2005). Hence, gerontology research currently stands on chemistry and biochemistry, as these are at the core of the aging processes. Advanced analytical studies are underway to observe and identify age-related changes in living organisms. Simultaneously, new synthetic and medicinal chemistry methodologies are yielding small molecule tools for the complete elucidation of complex biological pathways, as well as potential lifespan extending therapeutics (Ostler, 2012). However, to better understand how these could contribute to extend the knowledge of the mechanisms of aging, it is necessary to explore what are the prevailing theories as to why and how we age. Thus, we will extensively review and evaluate the prevalent theories of aging focusing on the major chemical, biological, psychological and pathological aspects of the process. The discussion of the different models of senescence will highlight the urgent need for system-wide approaches that provide a new, integrative view on aging research.

2. Theories of aging and how they shape the definitions of senescence

Many widespread theories as to why aging takes place abound. Generally, these consider it a programmed development (Tower, 2015a), though many disagree and the debate is still ongoing (Blagosklonny, 2013; Goldsmith, 2014, 2012, 2013). By 1990, Medvedev attempted to rationally classify the numerous theories of aging, which exceeded 300 (Medvedev, 1990). Aging has been attributed to molecular cross-linking (Bjorksten, 1968), free radical-induced damages (Harman, 1993), changes in immunological functions (Effros, 2005), telomere shortening (Kruk et al., 1995) and the presence of senescence genes in the DNA (Warner et al., 1987). More recently, however, a unified theory encompassing genes, the performance of genetic maintenance and repair systems, milieu and chance is becoming increasingly accepted (Rattan,

2006), highlighting the need for a systematic and integrative analysis of the aging process. The vast amount of research carried out concerning aging and aging-related processes makes it almost impossible to give a complete overview of the aging theories that have been put forth. Most of these, if not all, can, however, be classified into two categories: error theories and program hypotheses, which will be explored in the following sections. A third category – combined theories –, which contains certain elements of both groups, can be considered (Fig. 1). Such categorization is subjective and others have been suggested (Baltes et al., 2012; de Magalhães, 2005; Jin, 2010; Vina et al., 2007; Weinert and Timiras, 2003). As such, only a brief description of these prevailing theories will be discussed. However, despite whatever the theory, all aim at answering one question: what is the cause of aging? No matter the working hypothesis, one must consider that the underlying assumption that there is *one single* cause for aging may not be correct. Moreover, gerontologists may have to face the possibility that there may not be a universal cause of aging valid for all living organisms.

(1) Program theories

Programmed aging theories, sometimes referred to as active or adaptive aging theories, suggest that there is a deliberate deterioration with age because a limited life span results in evolutionary benefits (Goldsmith, 2012).

For many years, programmed aging has been debated and some studies have substantiated this hypothesis. For example, Ünal et al. (2011) have suggested that there are mechanisms that preserve the integrity of spores of aging diploid yeast cells. Through these mechanisms, aging diploid cells that are induced to sporulate appear to lose all age-associated damage to a point that is no longer detectable, though the assumption that these findings can be extrapolated to higher organisms has been put into question (Biliński et al., 2012).

Yet, though development and morphogenesis can be easily understood as programmed, as they are the end-result of a determined sequence of molecular and cellular events designed to produce a given phenotype (Austad, 2004), aging is mostly thought of as decay. If aging is indeed programmed, the purposes of such program remain unclear. Some have suggested that aging may constitute an altruistic plan (Longo et al., 2005), by eliminating post-reproductive age individuals, who would compete for resources, by avoiding overpopulation and by promoting adaptation through a succession of generations (Kirkwood Thomas and Melov, 2011). The supporters of this view underscore that the similarities between the biochemical pathways that regulate aging in organisms such as yeasts, flies and mice, together with evidence consistent with programmed death in salmon and other organisms, hint at the possibility that programmed aging can occur in higher eukaryotes (Longo et al., 2005). Moreover, this plan could be the result of "aging genes" (de Magalhães, 2013). Nonetheless, if this was the case, than certainly such mechanisms would be susceptible to inactivation, and, despite many gene mutations have been described as life-extending mutations (Barbieri et al., 2003; Fontana et al., 2010; Friedman and Johnson, 1988; Meléndez et al., 2003) none has been reported that abolishes the process of aging (Kirkwood, 2011). It should be noted that, in some model organisms, genes have been demonstrated to play a pivotal role in aging. In fact, the first described mutation to yield a significant extension in the lifespan of *Caenorhabditis elegans* was in the *age-I* gene, which was shown to result in a 65% increase in mean lifespan and a 110% increase in maximum lifespan of this organism (Johnson, 1990). Since then, many mutations that result in lifespan extension in *C. elegans* have been identified, most of which involving genes that are homologs of the of components of the insulin/IGF (insulin-like growth factor) pathway (Mattson, 2003), namely, *daf-2/daf-16* (Kenyon, 2010) and *sir2.1* (Guarente and Kenyon, 2000),
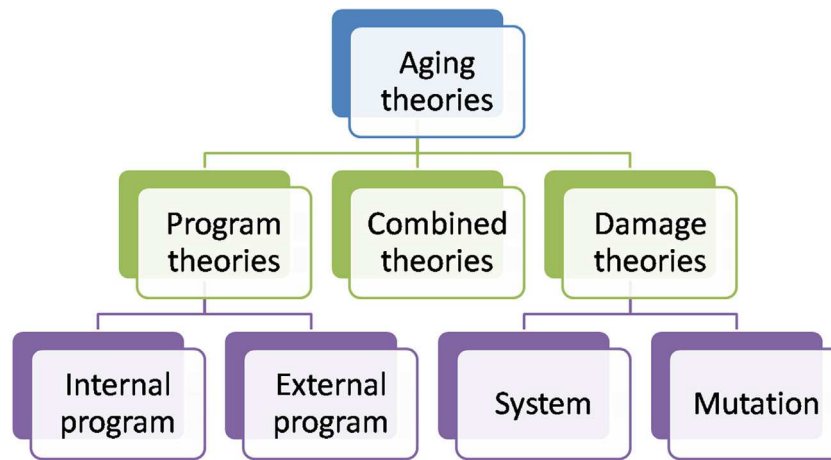
Fig. 1. Categorization of the main theories of aging. Classification based on the worked developed by (Semsei, 2000) and (de Magalhães, 2013).

which, interestingly, have been shown to interact to extend lifespan in *C. elegans* (Berdichevsky et al., 2006).

Composed mostly of post-mitotic cells, *C. elegans* is one of the most widely studied model organisms. With a lifespan ranging from days to a few weeks, it has been noted that, under caloric restriction (CR) and/or crowded conditions, *C. elegans* can enter an alternative stasis-like developmental pathway, called *dauer* (Riddle et al., 1981). This pathway consists of a developmental arrest, leading to an increased adult phase (de Magalhães, 2013; Kenyon et al., 1993; Meléndez et al., 2003). This arrest suggests that, at least partially, aging and development are coupled in *C. elegans*, as well as in other invertebrates (Brakefield et al., 2005). However, in addition to the severity of the restriction (30–70% fewer calories than the control group), the degree of lifespan lengthening in *C. elegans* depends on numerous factors, namely, age at onset of restriction (Weinert and Timiras, 2003). Though providing some key insights into longevity, invertebrates are, nevertheless, distant animal models and are likely unrepresentative of human biology and physiology.

The endocrine system has also been viewed as involved in "telling the time". Because the levels of hormones such as growth hormone (GH) and its corresponding downstream target insulin-like growth factor I (IGF-1) decline with age, the idea that such changes cause aging has been suggested a few decades ago (Hammerman, 1987; Ho et al., 1987; Rudman, 1985), and, in rats, deficiency in growth hormone production (loss of function mutations at the *Pit-I* locus) has been linked to lifespan extension and delayed immune aging (Flurkey et al., 2001). Due to the fact that the brain regulates the endocrine system, the neuroendocrine theory of aging has emerged as the main hormone-based theory of aging (Finch, 2014; Meites, 2012), and, not surprisingly, many anti-aging products aim at restoring the levels of specific hormones in older people (Elewa and Zouboulis, 2014; Sah et al., 2013). Some studies have supported the idea that the insulin pathway is associated with human longevity, as individuals with mutated *Prop-I* gene – a pituitary transcription factor whose mutation causes dwarfism (Kržišnik et al., 2010) – may live longer and patients with GH and IGF-1 deficiencies have shown signs of early aging, despite actually living longer (Anisimov and Bartke, 2013; Brownborg et al., 1996). Some have proposed that such mechanisms could be activated by decreasing cellular replication (Kushner, 2013) or that it may operate on the basis of antioxidant regulation (Vitale et al., 2013). Whatever the mechanism, it is now clear that the early assumption that the aging process is driven by hormone changes that occur with age is unsubstantiated. If anything, the decrease in GH/IGF-1 signaling increases lifespan, not the contrary (de Magalhães, 2013) and,

more broadly, hormonal changes may regulate aging as an indirect consequence of the developmental program. The imbalance on chemical processes caused by differential gene expression and hormonal changes may contribute to aging, but, so far, such assertions remain in the realm of speculation. Furthermore, the significant lifespan differences observed in numerous species, under identical conditions, seems to indicate that there is no pre-determined time-line for aging. Thus, under certain conditions, it may be possible to prolong or to curtail lifespan, leading to the hypothesis that aging is not predetermined, but rather the end-result of a "wear-and-tear" mechanism.

(2) Damage theories

Evolutionary biologists may argue that aging occurs due to the absence of natural selection at the post-reproductive stage of life (Johnson et al., 1999). Hence, aging is not programmed; instead, it is the absence of selection for maintenance (Medawar, 1952). Although such aging theories are subjectively appealing, as they convey a cure for aging, the accumulation of damage is a spontaneous entropy-driven process, and, as such, its kinetics can be genetically and environmentally modulated, resulting in the wide range of life-spans we observe (Aledo and Blanco, 2015).

Among the damage theories, a prevailing idea is that of oxidative damage (Harman, 1981). Reactive oxygen species (ROS) – partially reduced intermediates of oxygen that can be radical or non-radical molecules (Zelickson et al., 2013) – are generated during metabolism through a number of inter-related reactions Eqs. (1)–(4) (Novo and Parola, 2008) and are considered to lead to the cumulative DNA, protein and lipid damage (Piedrafita et al., 2015; Rinnerthaler et al., 2015; Thanan et al., 2014) (Fig. 2) observed over a lifetime (Freitas et al., 2013) (Fig. 3). Approximately 2–3% of oxygen taken up is chemically reduced by the addition of single electrons. Incomplete reduction of oxygen can generate a variety of biologically relevant ROS such as, hydrogen peroxide, the anion radical superoxide and the hydroxyl radical (Johnson et al., 1999). The electron transport chain in the mitochondria, the nicotinamide adenine dinucleotide phosphate oxidases (NADPH oxidase) and the 5-lipoxygenase as the three major sources of ROS in living cells (Novo and Parola, 2008). Multiple studies have highlighted the relatively haphazard molecular damage that ROS cause to lipids (Shah et al., 2001), proteins (Mishra et al., 2011) and nucleic acids (Dizdaroglu, 1992) and exposure to ROS have been demonstrated to trigger specific mechanisms aimed at neutralizing their effects (Silva et al., 2015).

1) The four electron reduction reactions forming H_2O from O_2.

$$O_2 + e^- \rightarrow O_2^{\bullet-} (+ H^+ \leftrightarrow HO^{\bullet}_2) \qquad (1.1)$$
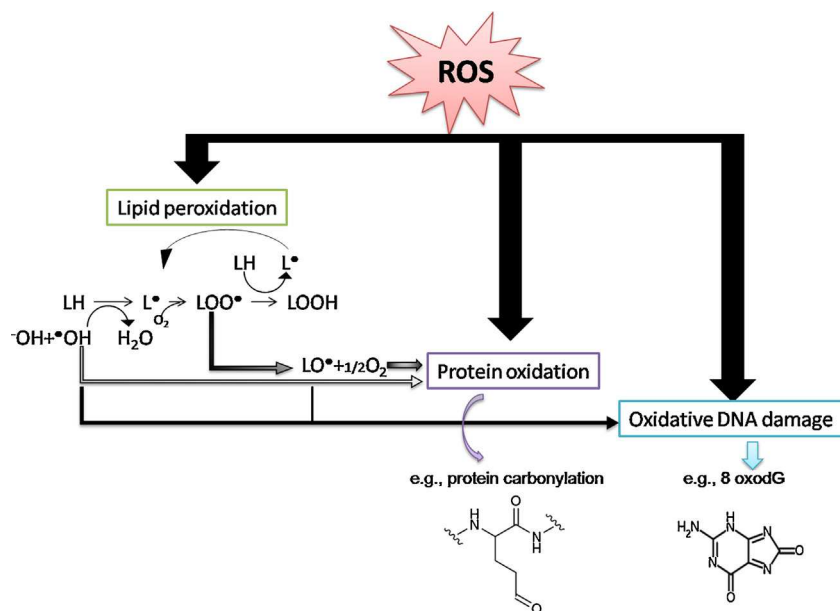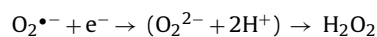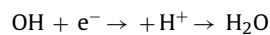
Fig. 2. General mechanism of oxidative damage to biomolecules. Oxidative damage to lipids yields lipid peroxidation products, mainly localized at the cellular membrane, which results in a loss of membrane properties/function. Their reactive end products can induce damage to other molecules, such as proteins and DNA. In nuclear and mitochondrial DNA, 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) is one of the predominant forms of free radical-induced oxidative lesions (Valavanidis et al., 2009). Potential outcomes include dysfunction of the affected biomolecules and interference with signaling pathways. Adapted from (Thanan et al., 2014).
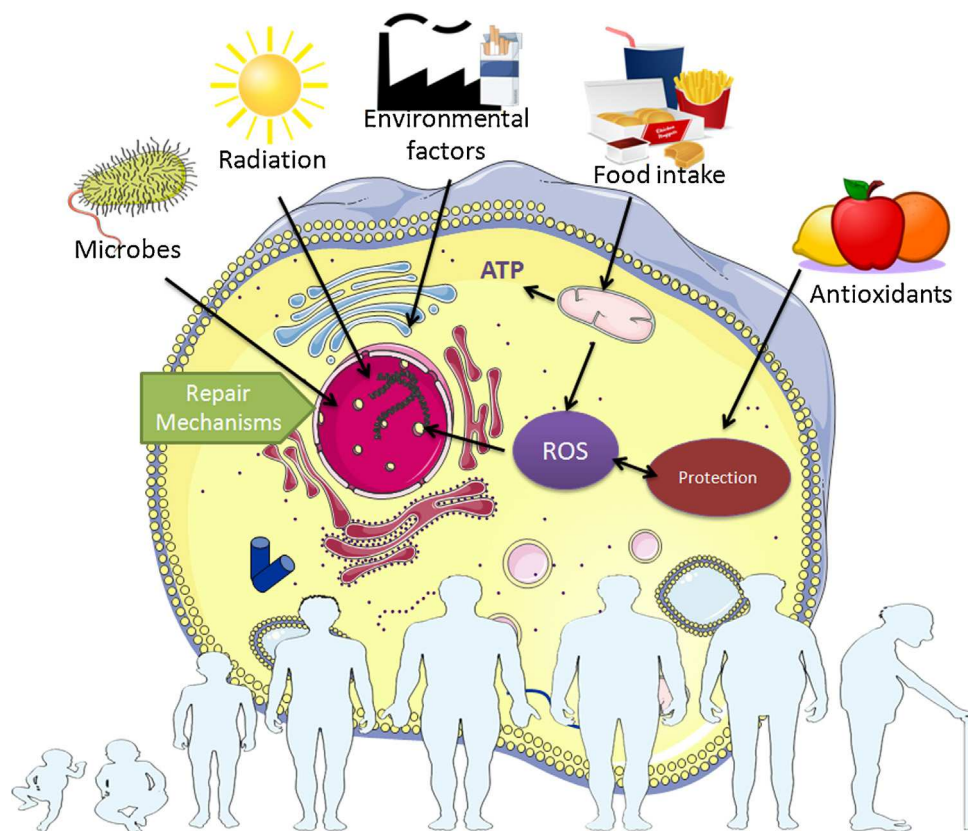
Fig. 3. The cumulative effect of ROS over time. ROS accumulation, oxidative stress and the imbalance of the normal redox state increases exponentially with age, accompanied by a marked decline of the cell repair machinery. Note that, despite only depicting the general stress response pathways, a typical Golgi pathway has yet to be described. Nonetheless, multiple stress factors may influence gene expression in the nucleus and cell homeostasis via alterations in the function of the Golgi apparatus (Kourtis and Tavernarakis, 2011). The figure was partly created using Servier medical art image bank (Servier, France).

$$O_2{}^{\bullet -} + e^- \rightarrow (O_2{}^{2-} + 2H^+) \rightarrow H_2O_2 \qquad (1.2)$$

$$OH + e^- \rightarrow + H^+ \rightarrow H_2O \qquad (1.4)$$
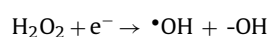
$$H_2O_2 + e^- \rightarrow {}^\bullet OH + {}^-OH \qquad (1.3)$$

2) Reactions involving H_2O_2

$$2O_2^{\bullet-} + H^+ \rightarrow H_2O_2 + O_2 \text{(dismutation)} \tag{2.1}$$

$$H_2O_2 \rightarrow 2^{\bullet}OH \text{(hemolyticscission)} \tag{2.2}$$
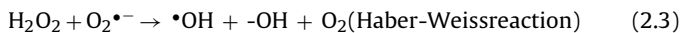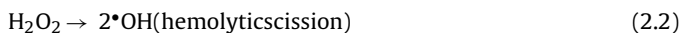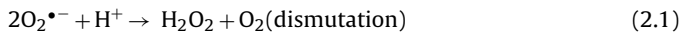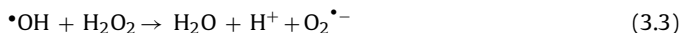
$$H_2O_2 + O_2^{\bullet-} \rightarrow {}^{\bullet}OH + {}^{-}OH + O_2 \text{(Haber-Weissreaction)} \tag{2.3}$$

3) ROS reactions in the presence of transition metal ions (Fenton's reaction)

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + {}^{-}OH \tag{3.1}$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + O2^{\bullet-} + 2H^+ \tag{3.2}$$

$${}^{\bullet}OH + H_2O_2 \rightarrow H_2O + H^+ + O_2^{\bullet-} \tag{3.3}$$

$$Fe^{3+} + O_2^{\bullet-} \rightarrow Fe^{2+} + O_2 \tag{3.4}$$

$$Fe^{2+} + {}^{\bullet}OH \rightarrow Fe^{3+} + {}^{-}OH \tag{3.5}$$
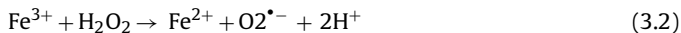
4) Carbon-centered free radicals generated through ROS

$$R\text{-}H + {}^{\bullet}OH \rightarrow \underline{R^{\bullet}} + H_2O \text{(organicradical)} \tag{4.1}$$

$$R^{\bullet} + O_2 \rightarrow \underline{ROO^{\bullet}} \text{(peroxylradical)} \tag{4.2}$$

$$ROO + R\text{-}H \rightarrow \underline{ROOH} + R^{\bullet} \text{(organicperoxide)} \tag{4.3}$$

$$ROOH + Fe^{2+} \rightarrow \underline{RO^{\bullet}} + {}^{-}OH + Fe^{3+} \text{(alkoxylradical)} \tag{4.4}$$
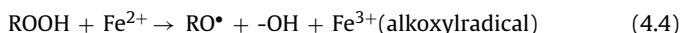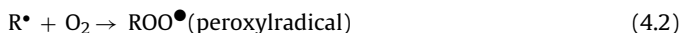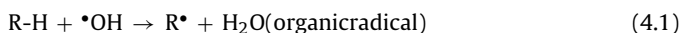
Additionally, oxidative stress is known to affect both translation and protein turnover (Vogel et al., 2011) and has been demonstrated to contribute to cell signaling in a controlled fashion (Cassina et al., 2000; Inoue et al., 2003; Sata et al., 1997). The supposition that aging may be caused by ROS has been further substantiated by studies involving transgenic animals for genes encoding antioxidants. The life-span of *Drosophila melanogaster* has been extended by overexpression of both superoxide dismutase (SOD) and catalase, both antioxidant enzymes (Orr and Sohal, 1994; Tower, 2015b), and such gene modulation can be achieved through dietary intake (Wang et al., 2015). Oppositely, mice knocked out for GPX1 (encoding glutathione peroxidase), SOD1, SOD2 or SOD3 did not display a rapid aging phenotype, either resulting in normal mice (Ho et al., 1997) or animals which expired within a short time period due to cardiac failure (Melov et al., 1998). This may be because, as demonstrated in *C. elegans*, SOD overexpression increases life span not through an enhanced removal of $O_2^{\bullet-}$, but by activating longevity-promoting transcription factors (Cabreiro et al., 2011).

Due to the fact that mitochondria are the major producer of ROS in mammalian cells, mitochondrial DNA (mtDNA) is therefore particularly susceptible to oxidative damage (Cui et al., 2011) and the main purine product of oxidative DNA base damage is 7,8-dihydro-8-oxo-2′-deoxyguanosine (8-oxodG). This, upon replication, can cause characteristic G:T transversions at a relatively low frequency (Hanes et al., 2006), which results in mutations that lead to defective Electron Transport Chain (ETC) components. The subsequent incorporation of these into the ETC causes further increase in ROS species, ultimately leading to a "vicious cycle" of ROS production and mtDNA mutations, eventually leading to cellular damage levels incompatible with life (Alexeyev, 2009). Mitochondrial maintenance is, therefore, essential to preserve cellular homeostasis and impaired mitochondrial maintenance has been described as a shared hallmark of numerous human pathologies and aging (Artal-Sanz and Tavernarakis, 2009). Though this mechanism is not fully understood, recent studies have demonstrated that mitophagy, a specific and selective type of autophagy that targets mitochondria for degradation, interact with mitochondrial biogenesis in order to regulate mitochondrial content as well as longevity in *C. elegans* (Palikaras et al., 2015).

Nonetheless, one of the most considered aspect of ROS-induced damage in DNA and aging is DNA methylation levels. These vary with age, and it is commonly considered that DNA hypomethylation is a typical aspect of the aging process (Afanas'ev, 2014). ROS are active intermediates of DNA methylation, as well as of histone modification. These reactive oxygen species may play a role in epigenetic processes (physiological phenotypic variations caused by external or environmental factors that switch genes on/off) through reactions of nucleophilic substitution at the DNA level. Consequently, it has been suggested that a better preservation of DNA methylation levels, slower cell metabolism and improved control in signal transmission through epigenetic mechanisms could be key processes involved in human longevity. In other words, ROS signaling in senescent cells probably causes DNA hypomethylation, although there are still insufficient data to sustain such hypothesis (Gentilini et al., 2013). There are, nonetheless, some evidences supporting the role of ROS in the aging process, namely, studies on the effect of ionizing radiation on living cells, dietary manipulations and works highlighting the partaking of free radicals in the pathogenesis of specific diseases (Harman, 1993).

These genetic links between aging and oxidative damage have been described for animals in which most matured cells are post-mitotic. Such cells may be more susceptible to the cumulative damage of ROS due to the inability of replacing themselves. Interestingly, it has been demonstrated that such susceptibility to damage may vary greatly in mammals, even in those with identical sizes (Montgomery et al., 2011). The most vulnerable organs in these organisms are the heart, brain and the skeletal muscle. This is because these are energy-rich tissues and, in the case of the brain, susceptibility to ROS-induced damaged may stem from the abundance of redox-active compounds (de Magalhães, 2013). Although ROS are usually considered as damaging compounds, studies have confirmed that these play an important role in multiple cellular functions (Miki and Funato, 2012; Ray et al., 2012; Sena and Chandel, 2012), such as regulation of the mitogen-activated protein kinase (MAPK) signaling pathways (Cuadrado and Nebreda, 2010) and of iron regulatory proteins −1 and −2 (IRP1 and IRP2, involved in iron homeostasis) expression levels (Recalcati et al., 2010). Furthermore, a corollary of this strict view of ROS as damaging compounds is that antioxidants should curtail their effect in aging and general health (Viña et al., 2016). However, multiple studies have demonstrated that this is not always the case (Fortmann et al., 2013; Grodstein et al., 2013; Higashida et al., 2011). ROS, including in mitochondria, are not necessarily detrimental and, in fact, some health-benefits, including a positive role in life-span under stress conditions, have been reported (Lee et al., 2010). Hence, low levels of these may induce an adaptive response that ultimately leads to the general improvement of systemic defense mechanisms, a concept termed mitochondrial hormesis or mito-hormesis (Kawagishi and Finkel, 2014; Ristow, 2014).

Consequently, aging may be the result of a deregulation of the ROS signaling pathways and not of the reactive species themselves (de Magalhães, 2013). However, whether considering the available data for mitotic or postmitotic cells, evidences for a direct link between ROS and aging is still frail, at best.

While affecting DNA and lipids (Fig. 2), oxidative damage to proteins is irreversible and irreparable (Thanan et al., 2014) and must be degraded by the proteasome. The proteasome is the most important proteolytic machinery in eukaryotic cells, largely responsible for the removal of oxidized proteins and the prevention of its aggregation (Nyström, 2005). However it has been shown that the activity of proteasome is impaired during aging leading to the accumulation of oxidizing proteins, aggresome and lipofuscin, so called the age pigment.

In fact, protein aggregation is the common defining feature in age-associated neurodegenerative diseases, such as Parkinson's and Alzheimer's (David, 2012). According to this view, aging is, then, the rising collapse of protein homeostasis and is dependent

on the interplay between proteostasis network components, which have a marked consequence on the long-term health of the cell (Douglas and Dillin, 2010). This proteostasis networks are capable of buffering the constant flux of protein misfolding, which is caused by the inherently error-prone characteristics of the protein synthesis and degradation mechanisms. However, such networks also undergo deterioration over time, thus making cells more vulnerable to protein-induced toxic stress (Morimoto, 2004). Cells are capable of counteracting disease protein aggregation in their early stages of life by reducing disease protein flux via increased folding and degradation control, reduced protein synthesis and favorable protein processing (Douglas and Dillin, 2010). Nonetheless, these are highly complex mechanisms (Hetz and Glimcher, 2011) and their exact nature remains largely unknown (Jarosz et al., 2010). Attempts have been made to increase proteasome expression and activity, which lead to 15–20% increase in longevity in cellular models. However, despite some compelling evidence that there is the formation of protein aggregates in some age-related illnesses, it is unclear whether protein aggregation induces aging or vice-versa (Moronetti Mazzeo et al., 2012).

The main function of the proteolytic degradation system of damaged proteins is the prevention of the accumulation of the most damaged proteins. If the damaged protein is not recognized and degraded *via* proteosomal activity, further oxidation can take place, as well as covalent crosslinking to other protein by-products of lipid peroxidation, such as 4-hydroxy-2-*trans*-nonenal (HNE) (Friguet and Szweda, 1997) and malondialdehyde (MDA) (Voitkun and Zhitkovich, 1999), two abundant bifunctional aldehydic oxidation products. When not sufficiently rapid degraded and/or when the cell is exposed to extreme oxidative stress, there is an increased probability for the cells to reach a different stage. At this stage, the proteins are not longer degradable by the proteasome. This results in the formation of protein aggregates, hydrophobic and insoluble in nature, referred to as "aggresomes" (Amidi et al., 2007). The formation of aggresomes can be thermodynamically driven by their exposed hydrophobic residues and the by-products of lipid peroxidation (like MDA or HNE) can cause covalent cross linking (Jung et al., 2009) The proteasomal activity decreases in aging cells and it has been shown that proteasomal inhibition in young cells leads to the enhanced formation of (polyubiquinated) protein aggregates (Powell et al., 2005). Interestingly, there have been some indications that healthy centenarians exhibit levels of both proteasomal activity and of oxidatively modified proteins identical to those found in younger control groups (Chondrogianni et al., 2000). These observations have led to the idea of artificially activating the proteasomal system as an anti-aging strategy (Chondrogianni and Gonos, 2008). Despite showing markedly increased proteolysis, with higher turnover of damaged/modified proteins and enhanced recovery after externally applied oxidative stress, such strategies are still far from feasible. Only a few of the post-translational modifications of ribosomal subunits that affect proteasome activities during aging have been investigated and many areas of proteasome regulation are not entirely elucidated, including specific regulators and the transcriptional regulation of the proteasome activation pathways.

Similarly to oxidative damage, nitrosative damage – that caused by reactive nitrogen species (RNS), such as nitric oxide – has been suggested to also contribute to age-related diseases, namely, hepatic steatosis and apoptosis (Abdelmegeed et al., 2016), as well as functional and structural changes in the cardiovascular system (Novella et al., 2013; Surikow et al., 2015). Additionally, it has also been associated to impairments in sleep homeostasis (Rytkönen et al., 2010), psychological disorders (Maurya et al., 2016) and dementia (Mangialasche et al., 2009). However, the mechanisms by which RNS may interact with cellular components, such as the mitochondrion, are still unclear, particularly in vivo (Zelickson

et al., 2013). Consequently, there is the need to better understand how these species are formed and the processes through which they affect mitochondrial and cellular function.

Advanced glycation end-products (AGEs) are a complex and highly heterogeneous group of compounds capable of inducing cellular oxidative damage. They are formed when reducing sugar reacts in a non-enzymatic way with proteins, lipids or DNA (Fig. 3), dubbed the Maillard reaction (Luevano-Contreras and Chapman-Novakofski, 2010). This reaction plays a critical role in the food industry, as its products add desirable taste and coloring to foods (Rufián-Henares and Pastoriza, 2016). *In vivo*, AGEs have received increasing attention due to the fact that these have been associated with specific chronic diseases, namely, diabetes (Forbes et al., 2004), cardiovascular pathologies (Bucala et al., 1994) and, more recently, to cognitive impairment (West et al., 2014; Yaffe et al., 2011). Their biological deleterious effects can be attributed to their pro-oxidative, inflammatory and chemical actions (Ahmed, 2005), which are exerted by two distinct mechanisms. One is independent of the receptor, while the other involves the receptor for AGEs (RAGE) (Luevano-Contreras and Chapman-Novakofski, 2010) (Fig. 4). The interaction of RAGE and AGEs ultimately leads to a positive feedback cycle (Ishibashi et al., 2014; Lohwasser et al., 2006; Nakamura et al., 2009; Tanaka et al., 2000), increasing RAGE expression. Additionally, the AGEs-RAGE interaction activates NADPH oxidase, which is upregulated, thus increasing intracellular oxidative stress (Luevano-Contreras and Chapman-Novakofski, 2010). Despite some interesting works describing the accumulation of AGEs in age-related illnesses (Srikanth et al., 2011; West et al., 2014), and in the elderly (Peppa et al., 2008; Uribarri et al., 2007; Vlassara et al., 2009), there is still no established direct link between these and senescence.

Another prevailing damage theory as the cause for aging is that of genome instability, thoroughly reviewed elsewhere (Lopez-Otin et al., 2013). Both stability and integrity of DNA are challenged on a continuous basis by numerous endogenous and exogenous factors, including DNA replication errors and physical, chemical and biological agents (Lopez-Otin et al., 2013). Organisms have evolved a complex system of DNA repair mechanisms which, in most cases, deal effectively with these damages inflicted to DNA. However, if defective, these mechanisms can result in genome instability and yield premature aging syndromes. DNA helicases play an essential role in the maintenance of genomic stability and, in fact, a number of mutations in human helicase genes have been linked to chromosomal instability diseases characterized by age-related ailments (Suhasini and Brosh, 2013), including *Xeroderma Pigmentosum* (XP), *Cockayne Syndrome* (CS), and *Werner Syndrome* (WS) (Brosh, 2013; Fang Evandro et al., 2014). Nuclear events, including transcription coupled repair (TCR), nucleotide excision repair (NER) and, perhaps more familiar to the wider audience, telomere maintenance, are thought to be individually affected by CS-A/CS-B, XP-B/XP-D and WRN helicases, respectively (Uchiumi et al., 2015).

Most supporters of the genomic instability theory of aging refer to telomere shortening (Kruk et al., 1995) (Fig. 4). Telomeres are the repeated DNA sequences at the ends of linear chromosomes, which are unable to be fully replicated by DNA polymerases (Johnson et al., 1999). Consequently, telomeres shorten with each cell division, unless maintained by telomerase, a ribonucleoprotein enzyme (Fig. 5). Nonetheless, most mammals lack this enzyme and telomere exhaustion is, in fact, the root of the so-called Hayflick limit, the maximum proliferative capacity of some types of *in vitro*-cultured cells (Hayflick and Moorhead, 1961). This happens due to the presence of shelterin, a multiprotein complex that bounds telomeres, functioning as a barrier against DNA repair proteins. Furthermore, the introduction of telomerase in normal human cells as yielded immortal cell lines (Bodnar et al., 1998; Stampfer and Garbe, 2015).
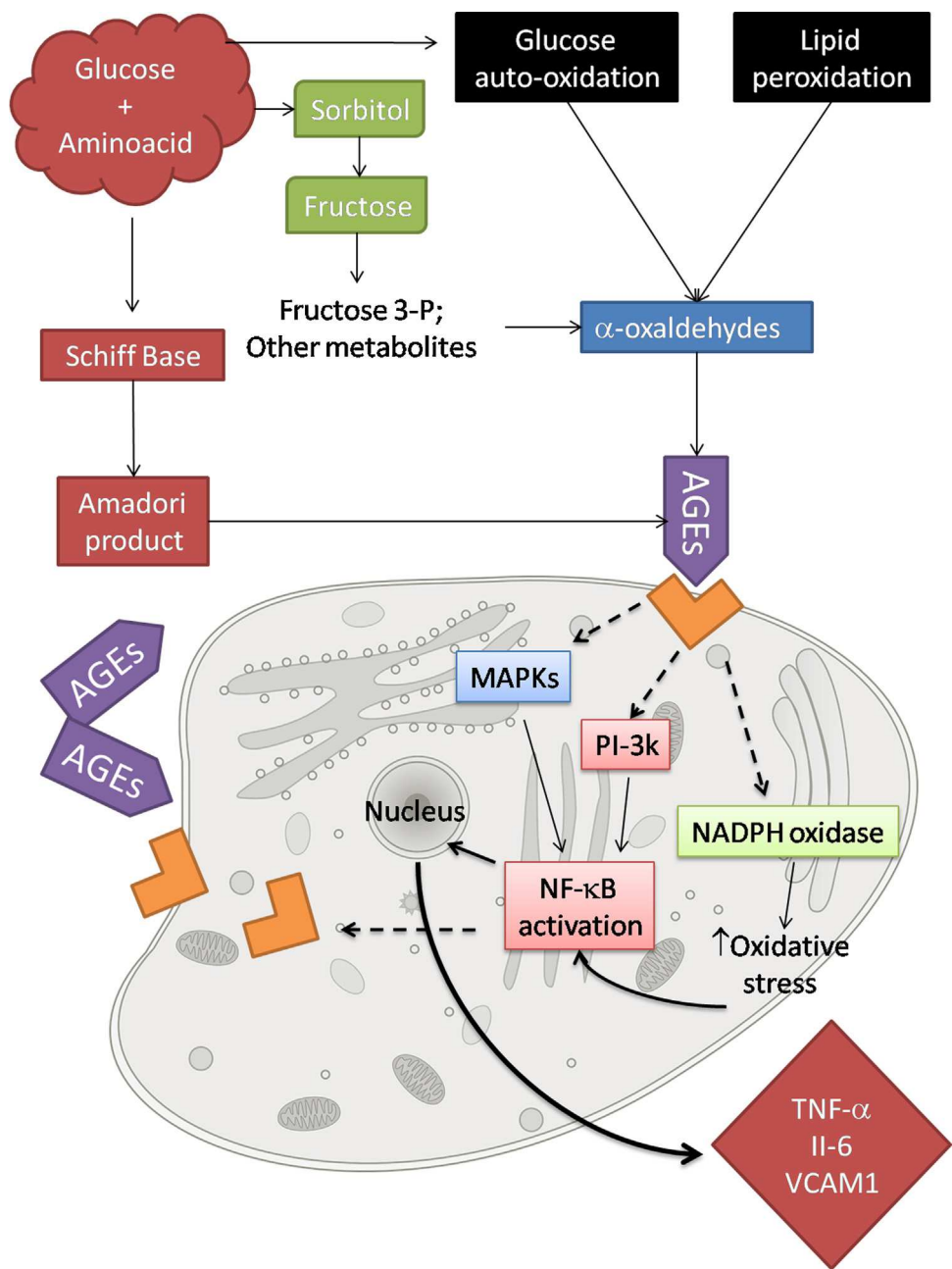
Fig. 4. The formation of AGEs and their mechanism of action at a cellular level. The positive feedback loop of NF-κB activation with subsequent RAGE expression is highlighted.
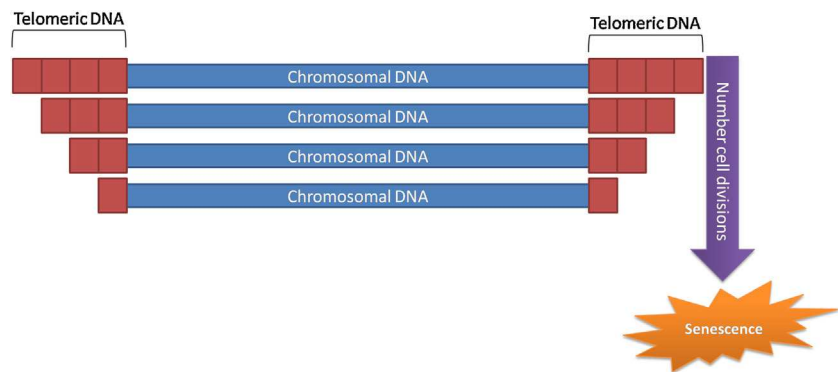
Fig. 5. Model of telomere shortening on aging.

Although the presence of shelterin may, at first glance, seem detrimental to the cell, such mechanisms prevent chromosome fusions (Meena et al., 2015). Despite all this apparently overwhelming evidence for telomere shortening as a major driver of aging, many works have casted a shadow of doubt over this assertion. Studies of both cross-sectional and longitudinal samples have revealed that, if donor health status and biopsy conditions are controlled, no significant correlation between the age of the donor and replicative life-span of the culture cells can be determined (Holliday, 2014). Additionally, the premature aging process of progeroid fibroblasts (enhanced aging cells) have been demonstrated to share only part of the *in vitro* aging process of normal fibroblasts (Toda et al., 1998), though recent experimental work showed that telomerase confers protection of accelerated aging in Werner syndrome lineage-specific stem cells (Cheung et al., 2014). Finally, contrary to what expected, CD28− T cells, which exhibit shortened telomeres and markedly decreased proliferative capacity in culture, accumulate with age (Effros, 1998). Hence, telomere shortening may be involved in aging, but surely is not the sole cause of senescence and its action mechanism, though seemingly simple in principle, remains to be fully understood.

Mutations and deletions in mitochondrial DNA (mtDNA) may also contribute to aging. This type of DNA is extremely gene dense and encodes numerous factors that are critical for oxidative phosphorylation. Hence, mutations in mtDNA – which are believed to be ten times higher than that of nuclear DNA (Jeppesen et al., 2011) – cause a wide range of human mitochondrial diseases and have been implicated in age-related diseases and aging (Park and Larsson, 2011), which is further enhanced by the oxidative microenvironment of the mitochondria and by the lack of protective histones, involved in the repair mechanisms of nuclear DNA (Pinto and Moraes, 2015). Causative evidences of the role of mtDNA damage in aging come from studies on mice that are deficient in mitochondrial DNA polymerase γ. These mutants exhibit aspects of premature aging and reduced life-span (Vermulst et al., 2008). In humans, deletions taking place through clonal expansion of single mutation events in aged brains have been described (Williams et al., 2013), interestingly, in regions of the brain highly susceptible to oxidative damage (Pickrell et al., 2011). However, the implication of mtDNA mutations in aging is controversial, due to the multiplicity of mitochondrial genomes (Lopez-Otin et al., 2013). This means that mutant and wild-type genomes can coexist within the same cell, a phenomenon called "heteroplasmy", and, recently, the degree of heteroplasmy has been suggested as a simple and noninvasive predictor of age-related neurologic and movement impairments (Tranah et al., 2015). Despite this possible coexistence of mtDNA's and of a globally low level of mtDNA mutations, single-cell analyses have revealed that the load of individual aging cells becomes significant (Khrapko et al., 1999) and may ultimately reach a state of homoplasmy, in which one mutant genome dominates (Lopez-Otin et al., 2013). Though the mechanisms by which mitochondrial dysfunction lead to diseases have been described (Ylikallio and Suomalainen, 2012), how mtDNA mutations may induce aging is not completely clarified (Pinto and Moraes, 2015). One key limitation of this theory is that how a focal impairment of mitochondrial function can spread throughout the tissue remains unexplained. Thus, it is clear that further studies are required to better elucidate how mtDNA mutations eventually lead to aging.

(3) Combined theories

One of the first efforts at developing a unified theory for aging was carried out by Strehler (1976) in 1976. He formulated four postulates: (1) aging is universal, and, as such, a phenomenon associated with aging must occur in all individuals of a species, albeit in different degrees; (2) aging must be intrinsic: the causes must be endogenous and they do not depend on extrinsic factors; (3) aging is progressive and must occur incrementally throughout the life-span and; (4) aging must be deleterious, i.e., a phenomenon associated with aging will only be considered a part of the aging process if it is holds no advantages for the individual.

Soon thereafter, stemming from these postulates, a membrane hypothesis of aging was developed (Zs.-Nagy, 1978), based on the fact that cell membranes become more rigid during aging and that a decrease of intracellular potassium content could lead to a sort of "rejuvenation". In other words, aging was related to changes in the cells' ability to transfer chemicals, heat and electrical processes.

In the early 1980s, Cutler put forth the dysdifferentiative hypothesis of mammalian aging and longevity (Cutler, 1982), based on the notion that the underlying cause for most of the vast complexities of the aging process was the drifting away of cells from their proper state of differentiation, as dysdifferentiated cells are responsible for the initiation of a cascade of changes in the entire organism and that the sum of these is aging (Taylor and Johnson, 2008). Though some studies were carried with the dysdifferentiative hypothesis of aging as their underlying premise (Kator et al., 1985; Ono et al., 1985), this idea has been largely abandoned in favor of some of the previously described views in the process of senescence.

More recently, a new integrative theory has been proposed, based on the notion that aging is not fundamentally a chemical process, but rather a biophysical, electrical in nature, mechanism. The fading electricity theory of aging (De Loof et al., 2013) postulates that, as cells gradually lose their ability to produce their own electricity, the biochemical processes that have been suggested as the drivers of aging come into play, eventually leading to death by senescence. Though certainly plausible, this theory lacks data supporting this hypothesis. It does raise, nonetheless, an interesting aspect in aging research: scientists should not limit themselves to the biochemical and genetic causes of aging. All biophysical activities of the living cell should be taken into consideration, namely, the bioelectrical one, as a possible cause for senescence.

While multiple theories of aging have been proposed, there is no consensus on the matter to date. In fact, many of the suggested mechanisms seem to, in one way or another, interact with each other (Jin, 2010). Hence, an integrative analysis of the quantitative available evidence at the different levels of biological hierarchy is necessary to fundamentally understand how the aging process takes place. Multiple attempts at finding synergies and combining diverse views and theories of aging have been made (Barja, 2013; Bengtson et al., 1999; Gems, 2000; Miquel, 1991; Weinert and Timiras, 2003), though none has established itself as a prevailing detailed and comprehensive view of what is, and, most importantly, how aging occurs. Nonetheless, combined theories view aging as a highly networked process on a systems level, regulated through feedback loops between levels of biological organization (Kriete et al., 2006) (Fig. 6).

3. The chemical interplay

Aging has been dubbed as a war raged between chemical and biochemical processes (Clarke, 2003), though a more accurate description might be that of a complex and rather interconnected gear mechanism. However, on the basis of this perspective, aging is fundamentally the end-result of unwanted chemical processes, which yields spontaneous side products of normal metabolism, including mutated, less active, and potentially toxic species of lipids, proteins, RNA, DNA and small molecules (Clarke, 2003). Hence, organisms endure to the extent that they can minimize the accumulation of these modified biomolecules (Yin and Chen, 2005). Such minimization processes rely on enzyme-mediated reactions, which are the backbone of the metabolic pathways involved in energy generation, biosynthesis, and signal transduction (Vogel
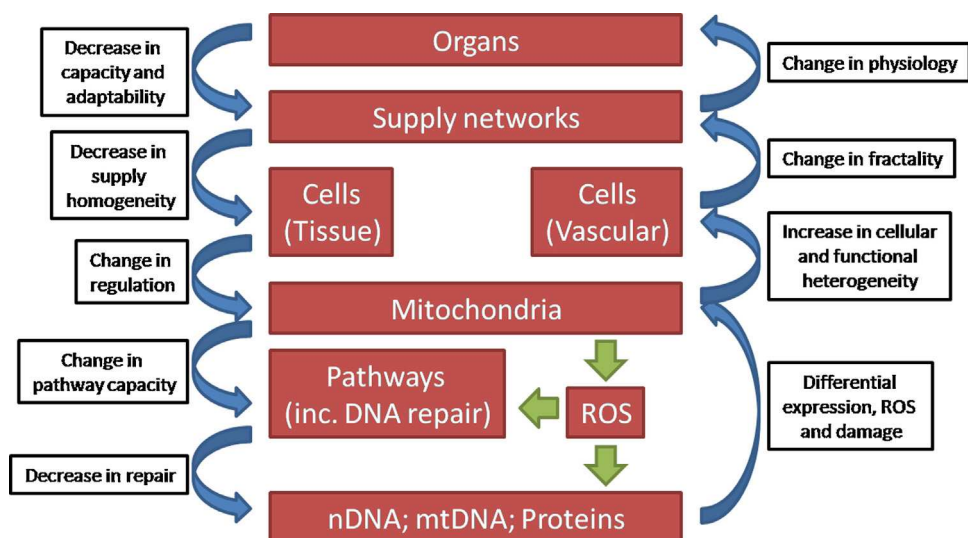
Fig. 6. An integrated physiological view of the functional and structural changes observed during aging. Multiple feedback loops exist at both the genomic and the organ levels, suggesting that aging is an accelerated process, countered only by the robustness of each level. Adapted from (Kriete et al., 2006).
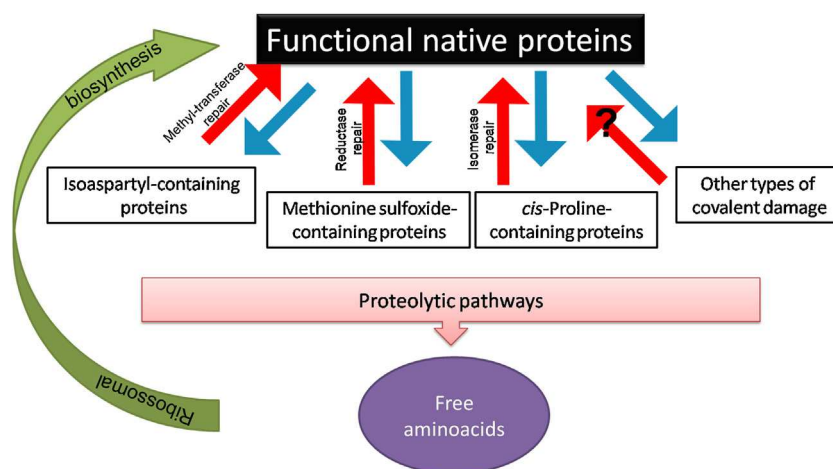
Fig. 7. Pathways of non-enzymatic degradation, repair, and replacement of aged proteins. Functional proteins can be covalently altered by a number of pathways (blue arrows). Enzymatic mechanisms exist that are capable of directly repairing, at least partially, this damage (red arrows), though, so far, no repair mechanisms have been described for many other types of damage. Altered proteins can be proteolytically digested to free amino acids and these can be used for synthesizing new functional proteins (green arrow). Adapted from (Clarke, 2003).

et al., 2004). Therefore, an optimization of these processes could, in theory, make life indefinite. What seems to work against biochemistry is chemistry itself. While enzymes may act as catalysts to speed up these reactions, it becomes difficult to slow them down, and, consequently, side reactions continue, leading to the build-up of undesirable side products (Clarke, 2003). These products are not limited to small molecules and include complex biomolecules, such as proteins and nucleic acids. Because nearly all biomolecules are thermodynamically unstable (Ross and Subramanian, 1981), they are susceptible of undergoing non-enzymatic conversion. These conversions can impact orderly biochemical process, which is at the heart of the damage-based theories of aging. These modified molecules can sometimes be repaired, though such mechanisms are rarely 100% effective (Yin and Chen, 2005). Fig. 7 illustrates how the pathways of spontaneous degradation, repair, and replacement described for aged proteins (Clarke, 2003; Grimaud et al., 2001; Ruan et al., 2002; Schiene and Fischer, 2000). In Fig. 8, the pathways of spontaneous chemical degradation of the aspartyl and asparaginyl residues in proteins, as well as of the

methyltransferase-mediated repair mechanism, are described as an example.

While the understanding of aging mechanisms has become much more complicated than ever before, it seems clear that phenomena such as oxidative stress and associated damages are neither parallel with alterations observed during aging nor correlated with maximum life span, due to the existence of these repair and defense mechanisms (Sohal et al., 2002). It is the remaining alterations after repairing which may define the extension of the age-related changes, namely, the interactions of these with other biomolecules. This results in rather complex, interconnected processes, as highlighted in Fig. 9, which describes the predicted and known interactions of some of the molecules whose expression changes more dramatically during aging (see Table 1) and the more common reactive oxygen species.

4. Models of senescence—what changes?

Aging is intrinsically complex and is characterized by numerous changes that take place at different levels of the biological
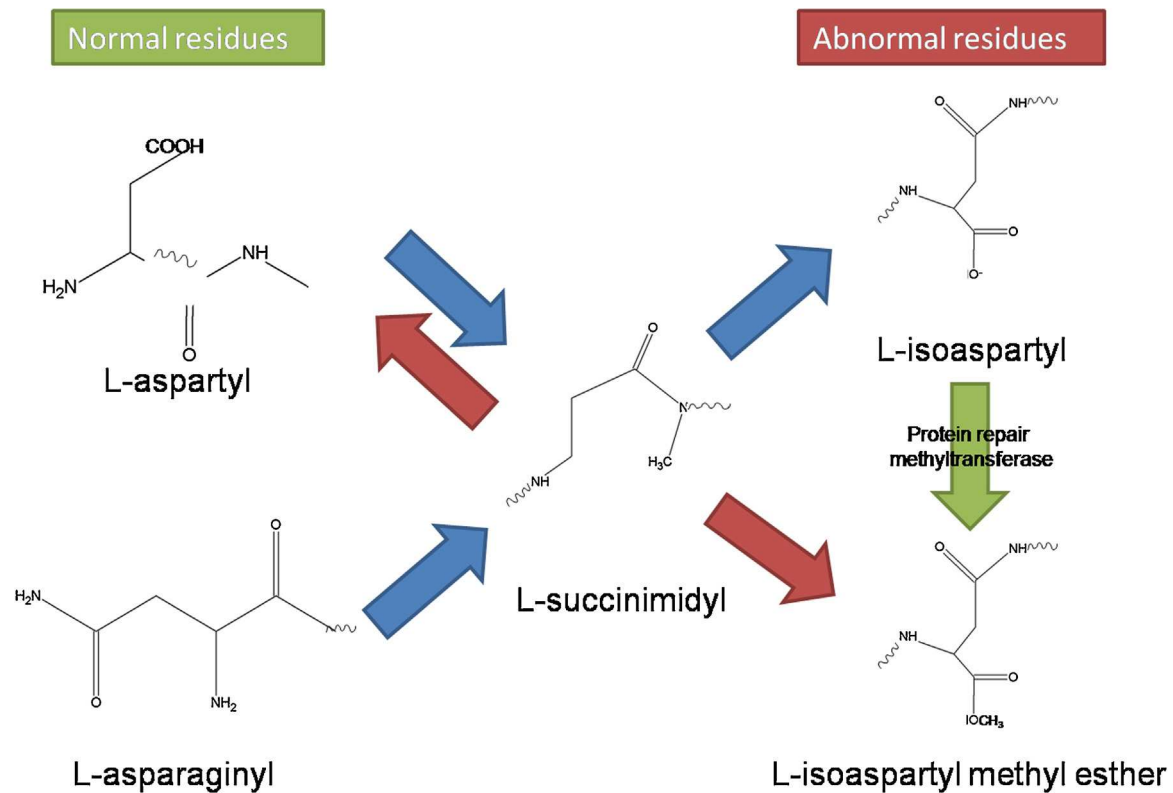
Fig. 8. Pathways of non-enzymatic chemical degradation of aspartyl and asparaginyl residues in proteins and of the methyltransferase-mediated repair mechanism. Spontaneous degradation of normal l-aspartyl and l-asparaginyl residues lead to the formation of a ring succinimidyl intermediate. This can spontaneously hydrolyze to either the l-aspartyl residue or the abnormal l-isoaspartyl residue. The l-isoaspartyl residue is specifically recognized by the protein l-isoaspartate (d-aspartate) O-methyltransferase. The result is the formation of an unstable methyl ester that is converted back to l-succinimidyl. Net repair occurs when the l-succinimidyl residue is hydrolyzed to the l-aspartyl form. With the exception of the repair methyltransferase step, all the reactions are non-enzymatic. Adapted from (Clarke, 2003).

Table 1
Molecular age-related changes. The level of variation is also indicated, as well as the statistical significance. Data compiled based on the information available at the Aging Digital Atlas (Craig et al., 2015).

Measured variable	Variation (%) P value	Observations	Ref.
Calcium binding protein A8	↑1228.2% $p < 0.01$	Expression in the parietal lobe from elderly (aged 69–99 years old) compared to cells from younger persons (aged 20–52 years old)	Cribbs et al. (2012)
Major histocompatibility complex, class II, DQ alpha 1	↑496.7% $p < 0.01$		
CD163 molecule	↑515.7% $p < 0.01$		
miR-320b	↑1049.9% $p = 1.33 \times 10^{-8}$	Expression from blood samples of German elderly (mean age: 98.9 years) compared to younger controls (mean: 43.8 years)	ElSharawy et al. (2012)
miR-320d	↑529.9% $p = 6.93 \times 10^{-7}$		
miR-106a	↓98.7% $p = 1.20 \times 10^{-10}$		
miR-668	↑251.4% $p = 5.67 \times 10^{-12}$	Significantly increased expression in foreskin cells from elderly when compared with younger controls.	Hackl et al. (2010)
miR-144	↑390.2% $p = 1.00 \times 10^{-25}$		
miR-100	↓49.1% $p = 1.28 \times 10^{-16}$		
transmembrane protein 33	↓63.9% $p = 8.62 \times 10^{-3}$	Decrease in the expression of TMEM33 in oocytes donated from women aged 37–39 years) compared to those from women aged 25–35 years	Grondahl et al. (2010)
zinc finger RNA binding protein	↑150.0% $p = 5.68 \times 10^{-3}$		
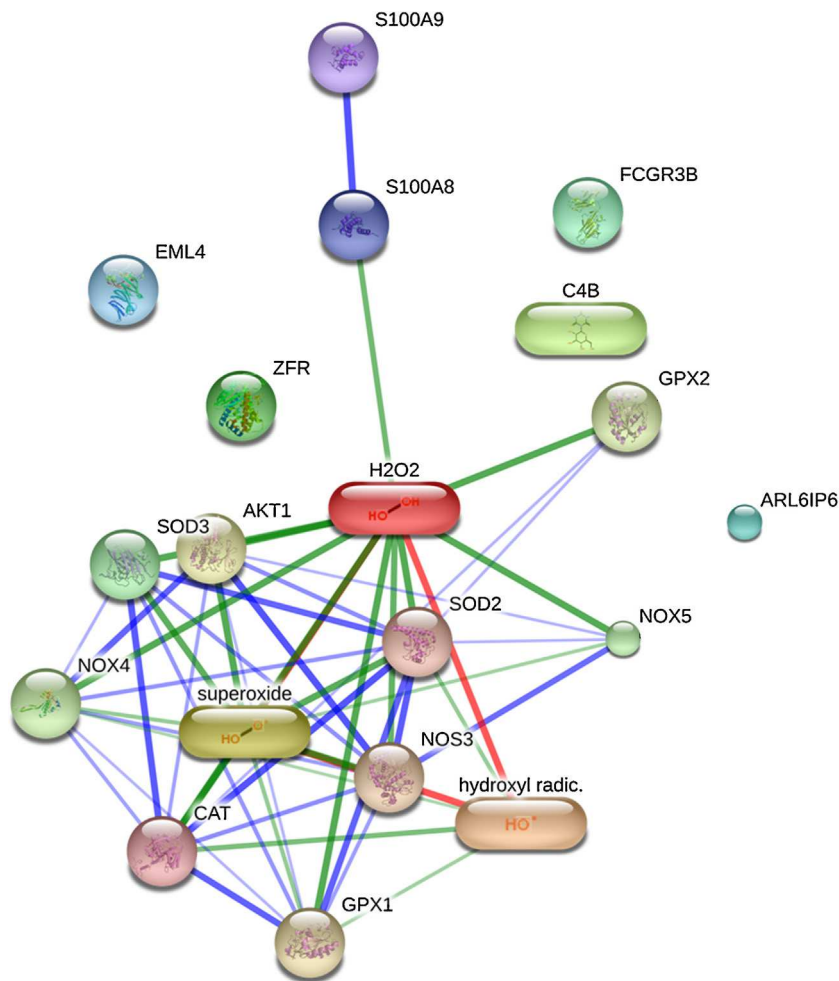
Fig. 9. Confidence view of the predicted and know protein and chemical interactions. Stronger associations are represented by thicker lines. Protein-protein interactions are shown in blue, chemical-protein interactions in green and interactions between chemicals in red. ZFR – zinc finger RNA binding protein. FCGR3B – Fc fragment of IgG, low affinity IIIb, receptor (CD16b). ARL6IP6 – ADP-ribosylation-like factor 6 interacting protein 6. EML4 – echinoderm microtubule associated protein like 4. S100A8 – S100 calcium binding protein A8. C4B – complement component 4B. Predicted functional partners include catalase, SOD2 – superoxide dismutase 2; SOD3 – superoxide dismutase 3; NOS3 – nitric oxide synthase 3; GPX1 – glutathione peroxidase 1; AKT1 – v-akt murine thymoma viral oncogene homolog 1; GPX2 – glutathione peroxidase 2; NOX4 – NADPH oxidase 4; NOX5 – NADPH oxidase, EF-hand calcium binding domain 5; TYRP1 – tyrosinase-related protein 1. A more comprehensive and in-depth study of all the modified proteins during aging may yield strong known and/or predicted protein and chemical interactions. Data obtained using STITCH (Kuhn et al., 2014).

hierarchy. There is no clear evidence which molecular, cellular or physiological changes are the most important drivers of the aging process and/or how they influence one another. Each mechanism tends to be – at least in part – supported by data indicating that it may play a role in the overall process. Nonetheless, the magnitude of an isolated mechanism is usually modest (Kirkwood, 2011). Consequently, such confined approaches may hinder a full appreciation of how different molecular, cellular and physiological components interact with each other. An important effort to circumvent this limitation has been the development of the Digital Aging Atlas (http://ageing-map.org), which aims at integrating the multiplicity of reported age-related changes into a unified, freely accessible resource (Craig et al., 2015). Ultimately, the goal of an integrative approach will be the compilation of the acquired knowledge into a single depiction of how the aging process takes place, ideally capable of characterizing the phenotype at a systemic/organism level (Cevenini et al., 2010). Such approaches will inevitably rely on the identification of pivotal genes, biochemical pathways and interactions involved in the aging process, as well as on the study of hereditable genetic diseases that result in premature aging and physiological experiments targeted at correlating caloric intake and "speed" of aging. Cell and molecular biology will play a key

role in unveiling the basis of the changes that organisms undergo during senescence and the multitude of available data, especially from high-throughput studies (de Magalhães et al., 2009), will be managed through systems biology approaches, where computational and mathematical modeling could decisively contribute to the understanding of the old problem of aging (Hou et al., 2012).

In the following sections, we will explore some of the most notorious molecular, physiological and pathological changes associated with aging, though many more exist and such description could not but be invidious.

(1) Molecular changes

Approximately 25–32% of the overall variation in adult life-span may be attributed to genetic variation, making it a particularly important feature for survival at advanced age (Hjelmborg et al., 2006). As such, there has been a remarkable effort into the elucidation of the molecular mechanisms of senescence, searching for "signatures" that can be definitively associated to the aging process and many gene-centric studies have identified genes whose expression is altered in senescent cells (Zhang, 2007). Table 1 highlights some of the most expressive findings in age-related changes at the molecular level. However, what these studies fail to unequivocally demonstrate is whether such alterations in gene expression

are unique and causal to senescence or if they are a mere non-specific consequence of cessation of cell proliferation. Adding to this complexity, studies carried out in animal models may contribute in a very limited way in our understanding of aging in humans, as senescence pathways vary significantly among cells from different species. For example, mouse fibroblasts express telomerase and exhibit very long telomeres, unlike human fibroblasts (Greenberg et al., 1998; Kipling and Cooke, 1990). In culture, mouse fibroblasts undergo senescence, which is independent of telomere shortening (Banito and Lowe Scott, 2013; Sherr and DePinho, 2000). Within the same species, cells can vary significantly in their senescence pathways (Zhang, 2007). For example, human fibroblasts undergo senescence after a finite number of divisions and telomerase expression has been proven to avoid this arrest (Yamashita et al., 2012). However, human mammary epithelial cells reach a growth arrest state unrelated to telomere shortening, but mediated by the tumor suppressing protein p16 (Stampfer et al., 2013b). The importance of p16 in growth arrested was demonstrated by the immortalization of these cells by short hairpin RNA (shRNA) targeting p16 (Stampfer et al., 2013a). Thus, these data strongly suggest that there are multiple pathways to senescence (Zhang, 2007). In this post-genome era, where −omics approaches allow for the detailed and comprehensive characterization of molecular changes during aging (da Costa et al., 2016), it will be possible to link such changes to cellular and physiological processes (Craig et al., 2015). However, it should be noted that different platforms and/or methodologies by which these molecular changes are evaluated often yield disparate results, and even distinct nomenclatures can have an impact on the final conclusions (da Costa et al., 2016). Consequently, it is of the utmost importance to create standardized methods for data acquisition and analysis, and, despite many attempts (Kohl et al., 2014; Sun et al., 2014; Weis, 2005; Zheng et al., 2015), these have so far failed to be universally implemented. The recent technological advances observed in −omics research allow for the simultaneous measurement of millions of biochemical entities (Zierer et al., 2015). Reductionist association studies have shown a high degree of correlation between −omics data with aging and age-related diseases, it is becoming increasingly evident that integrated network and -omics analyses targeting the aging process at a systems level could provide information previously unattainable, namely, pathways involved and interactions with key internal and external factors and variables (Valdes et al., 2013; Van Assche et al., 2015; Zierer et al., 2015).

Another key aspect of −omics research is that software packages and databases are continuously updated, and, therefore, studies should not end in a stable list of proteins and genes, but, rather, should periodically be revisited (Zhou et al., 2016). Not only could the initial studies benefit from new annotated information, but the original raw data could enclose previously unreported and valuable results, as evident from studies disclosing new findings from earlier published data (Mann and Edsinger, 2014; Matic et al., 2012). Nonetheless, no standard guidelines for such comprehensive re-analysis exist, although some efforts have been developed towards that goal (Zhou et al., 2016) and a successful implementation of such strategies could hold potential key discoveries towards the understanding of the aging mechanism(s).

(2) Physiological changes

Physiological changes occur with aging in all organ systems. Cardiac output decreases and blood pressure increases, often leading to arteriosclerosis. Degenerative changes occur in multiple joints and, combined with loss of muscle mass, movement becomes impaired in elderly (Boss and Seegmiller, 1981). Consequently, numerous studies have focused on the physiological changes that occur with age and, while listing all would be a herculean endeavor, some of the most prominent works are briefly listed in Table 2.

Nonetheless, one must consider such findings with a grain of salt. For example, though it has been described that there is a positive correlation between the atrophy of the hypodermal layer with age (Arking, 2006), it should be underlined that this is a regional change and usually affects the face and back of hands, but not the waist and/or thighs, which may be related to exposure. Another example is that of the somewhat widespread belief that there is a global neuron loss with age. In fact, the difference in total neuron number over the age range of 20–90 years is less than 10% (Pakkenberg et al., 2003; Pannese, 2011), though some morphological alterations do take place, such as significant decrease loss of synapses (Mostany et al., 2013), axon demyelination (Adamo, 2014) or loss of dendritic spines (Dickstein et al., 2013).

(3) Pathological changes

What's in a name? Contrary to what one might expect, this question is more closely related to pathological age-related changes than expected. In fact, pathological changes are not always readily and easily identified and what distinguishes them from "normal" age-related changes is, hence, somewhat elusive. For example, mild changes in neurologic functions occur with aging, though these do not substantially interfere with everyday activities, unless disease intervenes (Morris and McManus, 1991). However, there are macroscopic changes in the aging brain that are almost universally seen, such as the thickening of the arachnoid, increased ventricular volume and variable degrees of cortical and white matter atrophy have also been reported (Donahue, 2012).

Additionally, some of the data reported should be critically appraised. For example, Banks et al. (2009) reported a positive correlation of hip fracture incidence with age among postmenopausal women, though this is – in our view – not surprising, considering that movement, coordination and visual impairment significantly increase with age, inevitably leading to more falls and collisions, which, in turn, ultimately lead to hip fractures (as well as other fractures).

Consequently, most of the age-related pathological changes are not a result of single-measurements, but rather of continuous observation and serial incidence reports. In Table 3, some of these incidences of pathological age-related changes are listed.

(4) Psychological changes

Discussing the psychology of aging inevitably leads, albeit ever so slightly, to sociological considerations (Tischler, 2013). Although concrete analyses can be performed, such as measurements of cognitive deficits and alterations in sleep patterns, psychological age-related changes are intimately interweaved with the dynamics of stress and coping mechanisms during aging. In other words, as eloquently put by a personal connection, "elderly must learn to age". Western societies tend to show a mixed feeling towards the elderly. While generally appreciated, there is a pop culture that is youth-oriented, we look to preserve our younger self, resorting to a wide variety of hyped age-delaying crèmes, and, when picturing older people, we often think of people who are physically and/or mentally slower and TV programs do little to contradict such stereotypes (Lee et al., 2007). Yet, there are definite alterations that we can evaluate, such as those observed in the functional neuroanatomy that induce alterations in overt speech production (Soros et al., 2011). In Table 4, some of these quantifiable variations are listed.

5. Aging therapies—cure aging or die trying?

Is aging a disease? Aging is a process characterized by numerous pathologies, the sum of which inevitably leads to death and its biology by loss of homeostasis and the accumulation of molecular damage (Vijg and de Grey, 2014). Yet, if disease is defined as a disorder or abnormality of structure or function (Scully, 2004),

Table 2
Physiological age-related changes. The variables measured, as well as the affected tissue(s)/organ(s) are also indicated.

Measured variable	Tissue/Organ	Observations	Refs.
Function of epithelial barriers	Lung; oral cavity; pharynx; esophagus; stomach; intestine; epidermis	Decreased epithelial barrier function associated with increased pathogenic invasion of mucosal tissues	Weiskopf et al. (2009)
Expiratory volume	Lung	Lung forced expiratory volume decreases with age.	Klocke (1977)
Modifications to proteins and membrane components	Lenses	Increased incidence of presbyopia and age-related nuclear cataract	Truscott and Zhu (2010)
Rates of neuronal and astroglial tricarboxylic acid cycles and neuroglial glutamate–glutamine cycling	Brain (mitochondria)	Neuronal mitochondrial metabolism and glutamate–glutamine cycle decreased in elderly (~30%)	Boumezbeur et al. (2010)
Protein level	Arterial intima	Collagen content of human arterial intima shows an average increase of 100%. Accompanied by a large increase in intimal embrittlement.	Johnson et al. (1986)
Cell proliferation		Increase in intima cell proliferation.	Chisolm and Steinberg (2000)
Prevalence of arteriosclerosis	Artery	Arteriosclerosis incidence increases with age	Wilkinson and McEniery (2012)
Cholesterol level	Plasma	Incidence increases in age brackets 45–64 and 65–74	CDC, (2015)
Hematopoietic bone marrow volume	Bone marrow	Volume of hematopoietic bone marrow decreases with age	Sharma et al. (2009)
Clonal mosaic abnormalities	Blood; oral cavity	Detectable clonal mosaic events increased with age	Jacobs et al. (2012)
White matter volume	White matter	White matter volume decreases in the individuals aged 59–85	Resnick et al. (2007)
Atrophy of hypodermal layer	Skin (subcutaneous)	Hypodermal layer suffers atrophy with age	Arking (2006)

Table 3
Pathological age-related changes.

Measured variable	Tissue/Organ	Observations	Refs.
Cancer incidence	Multiple	Morbidity per 100,000 was >370 times in 85 years old than individuals aged 18–24.	CDC (2006)
Incidence of acute rheumatic fever and chronic rheumatic heart diseases	Heart; skin; brain	Morbidity per 100,000 was 165 times higher in 85 years old than individuals aged 25–44.	
Coronary artery disease incidence	Heart; artery	Prevalence of coronary artery disease increases markedly with age	CDC (2006), Odden et al. (2011)
Chronic obstructive pulmonary disease (COPD) and small airway obstruction incidence	Lung	COPD increases with age, as well as small airway obstruction.	Sharma et al. (2009)
Incidence of prebyscusis (hearing loss)	Cochlea (inner ear)	Positive correlation of prebyscusis with age	Albert and Knoefel (2011)
Renal arteriosclerosis incidence	Kidney	Renal arteriosclerosis increase with age	Bolignano et al. (2014), Glassock and Rule (2012)
Gastroesophageal reflux disease incidence	Esophagus; stomach	Incidence and severity of gastroesophageal reflux disease increases with age, particularly after 50	Becher and Dent (2011)
Asthma incidence	Lung	Morbidity per 100,000 was >40 times higher in 85 years old than individuals aged 18–24	CDC (2006)
Clinical presentation and pathological staging in colorectal cancer	Colon	Older patients exhibited lower frequency of abdominal pain; time from onset to diagnosis and pathological staging were similar	Paganini Piazzolla et al. (2015)

Table 4
Psychological age-related changes.

Measured variable	Observations	Refs.
Speech production	Speech production problems and reduced speech rate increased with aging	Soros et al. (2011)
Alterations in sleep patterns	Older individuals reported higher number of awakenings and modifications in sleep duration.	Crowley (2011), Feinberg et al. (1967)
Long-term depression	Individuals age 65+ showed increased incidence of depression	Roblin (2015)
Cognitive decline	Cognitive decline was found to be almost universal in the general elderly population and increases with age.	Park et al. (2003), Schönknecht et al. (2005)
Cognitive processing speed	Processing speed decreases with age	Eckert (2011)
Cognitive executive functions	Executive functions (e.g., planning), decreased with age	Glisky (2007)
Subjective memory	Normal aging found to be accompanied by memory impairment	Gazzaley et al. (2005)
Visual memory	Interaction of deficits in inhibition and processing speed was found to contribute to age-related cognitive impairment	Gazzaley et al. (2008)
Verbal memory	Age-related differences were found in 8 verbal span tasks	Bopp and Verhaeghen (2005)
Long-term potentiation	Greater and longer stimulation was necessary for long-term potentiation in older subjects	Kumar (2011)

than certainly aging is not a disease, as everyone suffers from it, though aging and disease often overlap. Hence, the question shifts towards *should* we cure aging? Opinions diverge (e.g., (Aledo and Blanco, 2015; Anton et al., 2005; Baars, 2012; Caplan, 2005; de Magalhaes, 2014; de Magalhães, 2013; Vijg and de Grey, 2014)), and commonly described fears include concerns about overpopulation and inequality, economic collapse due to healthcare and the idea that aging is natural and should not be tampered with (de Magalhães, 2014; de Magalhães, 2013). Advocates of life-extension research state that curing aging is not scientifically implausible and we may soon reach the "longevity escape velocity" (de Grey, 2004), a stage of medical progress that will result in delaying aging-related degeneration and death to such an extent that there is time to carry out research seeking more effective therapies later on (Vijg and de Grey, 2014) and dispute the alarms raised by others by noting the failed predictions of Malthus regarding the disasters due to over-population (Sethe and de Magalhães, 2013; Trewavas, 2002). No matter where one stands in respect to the pursuit of an increasingly longer life, there is no disagreement about the necessity of fighting age-related illnesses and comorbidities (Longo et al., 2015). Nonetheless, ultimately, finding a "cure" for aging is certainly a matter of personal belief.

As detailed in the following paragraphs, the technological advances aiming at the explicit purpose of curing aging, much as we would cure a disease, is essentially non-existent, though great endeavors are being undertaken to prolong an healthy life, whether we agree with it or not. However, the research is very much in its infancy and the road to longevity is still long. Moreover, considering the multifactorial nature of the aging process, it is not likely that there will be a silver bullet for aging.

(1) Caloric restriction

Contrary to what pharmaceutical companies would have you believe, there is still no way to delay aging, even faintly, and the long searched Fountain of Youth (Grene, 2010) remains elusive to this day. Yet, some of the *effects* of aging can be delayed. For example, skin aging can be minimized by reducing exposure to the sun (Kimlin and Guo, 2012) and it has been known since the 1930s that restricting calories (caloric restriction, CR) can extend life-span in laboratory animals (McCay, 1935). Some have postulated that this is due to an increased formation of free radicals within the mitochondria, which causes a secondary induction of increased antioxidant defense capacity (Shimokawa and Trindade, 2010), while others suggest that the limited availability of nutrients forces the metabolism to undergo optimization (de Magalhães, 2013). Considering observations made in mice, others believe that the genetic program may be "slowed down", thus indirectly affecting aging (de Magalhaes and Church, 2005). Additionally, because CR also induces various alterations, both at the hormone (Kim et al., 2015; Masoro et al., 1992) and at the proteome level (Baumeier et al., 2015), caloric restriction is recognized as the sole therapy capable of potentially delaying aging. In Fig. 10, simplified views of the complex metabolic pathways that regulate mammalian longevity are highlighted.

(2) Stem cell therapies

There has been a continuous and widespread *buzz* over stem cells in the general public and this notoriety is thoroughly deserved. These cells have been demonstrated to be a viable solution to health issues ranging from blindness (Nazari et al., 2015) and nerve regeneration (Faroni et al., 2013) to liver restoration (Christ et al., 2015), as well as potential therapies in movement disorders (Mochizuki et al., 2014) and other age-related illnesses, namely, muscular dystrophies (Bose and Shenoy, 2016) and skin deterioration (Peng et al., 2015). It is then not surprising that stem cells have been touted as potential treatments for the diseases of aging and for rejuvenation. Recently, Liu and co-workers reported the use of platelet rich plasma for the recovery of stem cell senescence in SAMP8 mice

(Liu et al., 2014) and postulated that rejuvenation of lineage could be achieved through the transplantation of restored stem cells in aged individuals, which could be applied in the treatment of age-related ailments. Experimental studies have also suggested that CR exerts its effect over stem cell dynamics and viability, by enhancing the preservation of a more durable population in the diverse stem cell niches of body tissues (reviewed elsewhere (Mazzoccoli et al., 2014)). Nonetheless, there is no direct evidence that stem cell-based anti-aging therapies will work, and, before such treatments are available, it is necessary to fully understand the mechanisms of action. Though depletion of stem cells are considered to play a role in aging, it remains largely unknown whether decline in stem cell function during aging influences longevity (Signer Robert and Morrison Sean, 2013) and the exact comprehension of the mechanisms are still vague (Oh et al., 2014), though, in somatic stem cells, it has been suggested that mitochondrial metabolism is an important regulator in aging (Ahlqvist et al., 2015). Additionally, numerous technical challenges remain. Harvesting and/or preparing stem cells remains an uncertain and laborious process (de Magalhães, 2013) and, in the case of induced pluripotent stem cells, there is the need to take pause and ascertain whether subtle differences between these and embryonic stem cells may affect both their research applications and therapeutic potential (Robinton and Daley, 2012). Stem cells applications are very much in their infancy and there is the need to investigate further, namely, at the tissue-specific level, where variations in mechanisms and signaling pathways may yield significant exceptions in delaying the aging process.

(3) Breaking AGEs

Intervention studies have clearly demonstrated that high intake of AGEs positively correlates with tissue damage and that it can be prevented by dietary AGEs restriction (Feng et al., 2007; Poulsen et al., 2013; Van Puyvelde et al., 2014). This is further evidenced by the low-calorie intake described in numerous studies of centenarians (Martin et al., 2013; Redman and Ravussin, 2011; Weiss and Fontana, 2011). Whether the low-calorie diet itself or the AGEs content could affect aging has also been studied and, in animal models, the high levels of AGEs in the CR-high diet were shown to compete with the benefits of CR, though the mechanism remained uncertain (Cai et al., 2008).

Numerous pharmacological agents have also been studied as blockers of the crosslinking reactions leading to AGEs, or as blockers of their actions, such as aminoguanidine (Thornalley, 2003), benfotiamine (Stirban et al., 2006), aspirin (Urios et al., 2007), metformin (Ishibashi et al., 2012) and inhibitors of the renin-angiotensin system (Zhenda et al., 2014). Among these compounds, ALT-711 has received much of the public attention as the next-generation anti-aging product. It acts by catalytically breaking AGE crosslinks and research has highlighted its potential in alleviating numerous age-related conditions, such as heart failure (Little et al., 2005), diabetic nephropathy (Thallas-Bonke et al., 2004), type II diabetes (Freidja et al., 2012) and age-associated ventricular and vascular stiffness (Steppan et al., 2012), among others.

Nonetheless, despite the extensive research carried out, and although some of these agents are in preclinical trials, the full effects and side-effects of these drugs are still unknown and it could be a long time before any of these compounds emerge as safe and efficient agents with therapeutic actions against AGES and/or their effects (Luevano-Contreras and Chapman-Novakofski, 2010).

More recently, exercise has been described as a promising venue for the amelioration of the effects of AGEs. These reports, however, are sparse, and the direction of causality between exercise tolerance and AGEs is not always clear. For example, Hartog et al. (2011) describe that breaking AGEs yields positive effects over exercise tolerance and cardiac function, but Delbin et al. (2012) postulate that the exercise itself can lead to a decrease in AGEs and, conse-
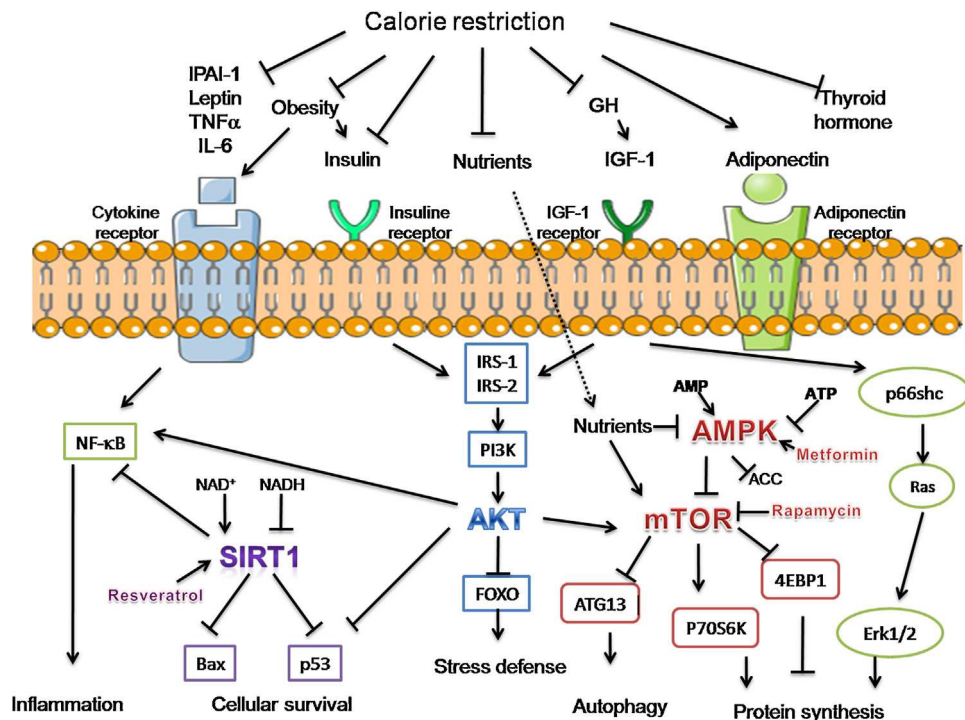
Fig. 10. Main metabolic pathways involved in the regulation of mammalian longevity and affected by CR. These include reduced cytokine levels, adiposity, thyroid hormone levels, IIS signaling and increased adiponectin. CR engages multiple downstream cellular pathways, including SIRT1 activation, insulin/IGF-1/phosphatidylinositol 3-kinase (PI3 K)/Akt signaling, as well as AMPK/mTOR and extracellular signal-regulated kinase 1/2 (Erk1/2) signaling. Ultimately, the collective response is believed to lead to the promotion of longevity through activation of stress defense mechanisms, autophagy and survival pathways, with concomitant attenuation of pro-inflammation mediators and cell growth. Pharmacologic approaches, such as those involving the use of rapamycin, metformin or resveratrol are believed to exert an analogous effect via the mechanisms shown. Arrows indicate the directional stimulatory effect and blunt-ended line an inhibitory one. IL-6 stands for interleukin-6, TNFα for tumor necrosis factor-α, NF-κB for nuclear factor-κB, IRS-1 for insulin receptor substrate-1 and PAI-1 for plasminogen activator inhibitor 1. Adapted from (Barzilai et al., 2012).

quently, improve vascular responsiveness. As such, the interaction mechanism remains unclear, though it certainly exists and further research is required.

(4) Hormonal therapies

On the heels of the realization that patients with GH and IGF-1 deficiencies exhibit signs of early aging (Anisimov and Bartke, 2013; Vanhooren and Libert, 2013), growth hormone began being used as an anti-aging treatment and there are some evidences suggesting that human GH has beneficial effects in the elderly (Taub et al., 2010) and hGH supplements have been implicated in muscle mass and libido increase, as well as strengthening of the immune system (de Magalhães, 2013). Alas, similarly to many other anti-aging products, it failed to live up to the expectations, partially due to its negative side-effects, such as alterations in body composition and metabolism (Carroll et al., 1998), high blood and intracranial pressure (Malozowski et al., 1993) and diabetes (Lewis et al., 2013). There are also concerns as to whether hGH could stimulate cancer, particularly in patients with existing malignant or pre-malignant tumors (Clayton et al., 2011). As such, the general consensus is that its use as an anti-aging therapeutic agent is imprudent (Liu et al., 2007). More research is needed to evaluate any possible deleterious effects and ensure its safe use as a therapeutic agent.

(5) Antioxidants

In order to fight ROS Eqs. (1)–(4) and their effects over lipids (Sharma et al., 2012), proteins (Youle and Van Der Bliek, 2012) and nucleic acids (Ray et al., 2012), cells exhibit an array of endogenous antioxidant systems, further amplified by an input from co-factors and by the ingestion of exogenous antioxidants (Rahman, 2007). Many of these can either be synthesized or extracted and subsequently purified and then sold (de Magalhães, 2013). The most common antioxidants include vitamins A, C and E, as well as the co-enzyme Q_{10}, the latter extensively advertised in face creams (Prahl

et al., 2008), but also found to be effective in preserving mitochondrial respiratory function in aged rat skeletal (Sugiyama et al., 1995) and cardiac muscles (Park and Prolla, 2005). However, some studies have revealed that antioxidants do not delay the aging process *per se*, but rather contribute to increase longevity (Holloszy, 1998). Vitamin C, for example, has proven to be ineffective at prolonging life-span in mice, partly because any positive benefits were offset by compensatory reductions in endogenous protection mechanisms, ultimately resulting in no net reduction of the accumulated oxidative damage (Selman et al., 2006). Despite these data, antioxidants are repeatedly hailed as miracle cures against aging, and are often found in dietary supplements (Bailey et al., 2013; Wolfe and Liu, 2007). The increased commercialization of these products should be worrisome, as not only large cohort studies have shown that dietary supplements do not affect mortality either positively or negatively (Park et al., 2011), but have also been proven to be involved in the accelerated cancer development in mice (Sayin et al., 2014). Moreover, high-dose antioxidant supplements may in fact do more harm than good (Bjelakovic et al., 2004, 2008; Combet and Buckton, 2014), partly due to the fact that, as previously mentioned, low levels of ROS may be beneficial and may have a positive role in life-span (Lee et al., 2010). Therefore, although low-dose mixtures of antioxidants can sometimes have a beneficial effect (Gutteridge and Halliwell, 2010), it reflects mostly (if not only) in those members of populations whose diet and lifestyle result in micronutrients deficiencies (Shenkin, 2013).

Overall, there is little evidence that antioxidants have the power to delay aging and these are perhaps more suited to be used in alternative applications, such as functional ingredients in food systems to reduce oxidative changes (Samaranayaka and Li-Chan, 2011) and "cosmoceuticals" (Bogdan Allemann and Baumann, 2008). The intake of antioxidants should, hence, occur when, and only when,

supplemented in our diet, not tablets or pills (Bjelakovic et al., 2014).

(6) Telomere-based therapies

If telomere extension can increase cell proliferative capacity *in vitro* (Ramunas et al., 2015) and account for the reversal of tissue degeneration in mice (Jaskelioff et al., 2011), than there is the possibility of being used to attenuate the rate of aging. That is certainly the core concept behind the commercialization, by some companies, of telomere measurement kits (Wolinsky, 2011), aimed at estimating the biological age of individuals and, to some extent, the risk of developing telomere shortening-associated diseases, such as atherosclerosis (Samani et al., 2001), coronary heart diseases (Ogami et al., 2004) and liver cirrhosis (Wiemann et al., 2002). Nonetheless, despite the media hype (Geddes and Macrae, 2015; Knight, 2015; Pollack, 2011), you might be better off looking at a calendar, as there is little evidence to support the claim that telomere measurement provides a better estimate of biological age than chronological age (de Magalhães, 2013). Pharmaceutical companies are, however, making efforts in developing telomerase-based therapies. One natural telomerase activator product, TA-65®, is already available (Harley et al., 2011) and, although it has failed to prolong life-span, it has yielded apparent positive immune remodeling and beneficial effects over metabolic, bone, and cardiovascular health (Harley et al., 2013).

However, conflicting evidence (Cheung et al., 2014; Effros, 1998; Holliday, 2014; Toda et al., 1998) and the realization that mice over-expressing telomerase do not live longer (de Magalhães and Toussaint, 2004) are powerful reasons to take pause regarding such therapies. Moreover, telomerase expression has long been linked to promotion of tumor growth and cell proliferation (Peterson et al., 2015), and, therefore, there is the justified fear that the use of telomerase activators may increase cancer development risk.

(7) Therapies to come

There are various approaches that have yielded promising initial results in delaying aging.

The use of rapamycin is one such approach. This is an immunosuppressant, commonly used to prevent organ rejection (Dumont and Su, 1996). Rapamycin has been demonstrated to extend maximal life-span in mammalian species, but it is not clear whether the drug slows mammalian aging or if it has isolated effects on longevity by suppressing cancers, which is the main cause of death in mouse strains (Ehninger et al., 2014) and it has been shown to extend murine life-span, tough exhibiting limited effects on aging (Neff et al., 2013). Rapamycin works by inhibiting a complex pathway called the target of rapamycin (TOR), and, more specifically, over the mammalian target of rapamycin (mTOR), a kinase at a key signaling node that aggregates and integrates information regarding extracellular growth factor stimulation, nutrient availability and energy supplies (Ehninger et al., 2014) (Fig. 10). This compound shows, nonetheless, serious side effects, such as nephrotoxicity (Murgia et al., 1996), thrombocytopenia (decrease of platelets) (Sacks, 1999) and hyperdyslipidemia (elevated levels of lipids) (Stallone et al., 2009). Consequently, different laboratories and companies are currently targeting more specific downstream nodes of this pathway, in order to develop anti-aging drugs without the side-effects of rapamycin (de Magalhaes et al., 2012).

The *klotho* gene, which codes for one membrane protein and one secreted transcript that acts as a circulating hormone, appears to influence aging, as mutations in this gene have resulted in accelerated aging in mice, as well as low-level expression (Kuro-o et al., 1997). Overexpression of *klotho*, in turn, extended life-span by about 30% (Kurosu et al., 2005). The action mechanism of this gene remains largely unknown, but evidences point to the insulin/IGF-1 signaling pathways and may also be involved in calcium metabolism and in a vitamin D endocrine system (Tsujikawa et al., 2003). Additionally, resveratrol has also been described as an

inducer of *klotho* expression (Hsu et al., 2014). These data make the involvement of the *klotho* gene in aging rather plausible, though more work is necessary to confirm this claim and elucidate the mechanisms involved in this process, for this and other genes which have been implicated in the aging process (ElSharawy et al., 2012; Hackl et al., 2010; Klement et al., 2012; Zhong et al., 2015). Such extensive knowledge would allow for effective gene therapies, based on the modulation of these aging-related genes and, hence, extend life-span.

Supplementation with precursors of the oxidized form of cellular nicotinamide adenine dinucleotide (NAD$^+$) has also been demonstrated to extend life-span and to rescue premature aging phenotypes in both nematodes (Fang Evandro et al., 2014) and mice (Scheibye-Knudsen et al., 2014; Zhang et al., 2016). Hence, strategies aiming at the conservation of cellular NAD$^+$ may result in improvements in mammalian life-span, though it remains to be seen whether NAD$^+$ precursor supplementation will, in effect, yield overall health benefits in aging human populations.

Perhaps the most futuristic anti-aging therapy – at least, in our collective imagination – is nanotechnology, which may be due, in part, to the book in which the term was coined, Engines of Creation (Drexler, 1996), immediately evoking images of tiny, highly complex nano-machines, or nanobots, sometimes also referred to as nanites. Nanotechnology holds many promises and expectations in a wide range of applications. Nonetheless, thus far, the biomedical applications, including the nanotech fight against aging, entail a level of technological advancements that are certainly within our reach, though not yet available. The first steps into this brave new world have been taken and, recently, an intelligent system has been devised that lays the foundations for the future development of new therapies against aging. This nanodevice consists of capped silica nanoparticles that can selectively release drugs in aged human cells (Agostini et al., 2012) and has enormous potential in the treatment of a myriad of diseases, namely, cancer or Alzheimer's. Hence, there is a promise that nanostructures of the like will be able to drive chemical reactions that are capable of slowing down or even reverse senescence, by reversing the chemical reactions and damage that take place with aging. Soon.

6. Conclusions

(1) Biological aging, termed senescence, is one of the most complex biological processes. Theories of aging are generally classified as either program theories or damage theories. More recently, combined theories, in which the aging process is considered at a more comprehensive and global degree, have emerged, but definitive evidences are still elusive.

(2) The complexity of the aging process has led to the realization that an integrative approach is necessary to better understand the mechanisms of aging. In this regard, *omics* – genomics, transcriptomics, proteomics, lipidomics and metabolomics – can play a pivotal role in the elucidation of the complex, interconnected changes that take place at the different levels of the biological hierarchy during aging, though the current knowledge of these molecular interactions is still very limited.

(3) Senescence is not the inevitable fate of all organisms and it can be delayed. In the last few decades, there has been an increase of in evidences showing that aging is not an irreversible process. Additionally, we are now privy to a multitude of mechanisms that allow considerable life-span extensions.

(4) Most of the reported life-extension mechanisms have been observed in simpler organisms and these have still to be demonstrated as viable anti-aging therapies in humans. Additionally, these do not curtail one of the hallmarks of aging, cognitive impairment.

(5) Research into aging is blooming but biogerontologists must be aware of this interconnectivity that, not seldom, obfuscates the primary cause(s) of aging and greatly limits the ability to reach valid and definitive conclusions.

Conflict of interest

The authors declare that they have no conflict of interest.

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