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REVIEW

Recent insights into the cellular and molecular determinants of aging

Linhao Ruan[1,2], Xi Zhang[1] and Rong Li[1,3,*]

ABSTRACT

Aging is the gradual decline of physiological functions and organismal fitness, which leads to age-dependent fitness loss, diseases and eventually mortality. Understanding the cause of aging constitutes one of most intriguing areas of research in biology. On both the cellular and molecular levels, it has been hypothesized that there are aging determinants to control the onset and progression of aging, including the loss of beneficial components and accumulation of detrimental factors. This Review highlights the recent advance in identifying various factors that affect the aging process, focusing on how these determinants affect the lifespan and fitness of a cell or organism. With more and more aging determinants revealed, further understanding about their functions and interconnections could enable the development of specific intervention to extend healthy lifespan and reduce the risk of age-related diseases.

KEY WORDS: Lifespan extension, Cellular aging, Organismal aging, Beneficial factor, Detrimental factor

Introduction

One thing that is certain among all the uncertainties in life is aging. Aging is the gradual and inevitable process that connects birth and death (Ocampo et al., 2016). The efforts of anti-aging have never stopped and can be traced back to as early as the Qin dynasty (260–210 BC) in ancient China (Mori and Sasakura, 2009). Although several hallmarks of aging are being actively researched (Lopez-Otin et al., 2013), such as genomic instability (Chow and Herrup, 2015), epigenetic alterations (Sen et al., 2016), cellular senescence (van Deursen, 2014), deregulated metabolism (Lopez-Otin et al., 2016), stem cell exhaustion (Goodell and Rando, 2015), loss of proteostasis (Vilchez et al., 2014) and mitochondrial dysfunction (Bratic and Larsson, 2013) (each has been summarized in detail in the cited review), much remains to be learned about whether these are the cause and/or consequence of aging.

Classic and more recent studies in budding yeast, a unicellular eukaryote, led to the hypothesis that there exist specific factors, either detrimental or beneficial, whose levels influence the degree of aging. Yeast cells undergo a finite number of asymmetric cell divisions during their lifespan (Mortimer and Johnston, 1959). During each division, a mother cell of varying replicative age produces a newborn daughter cell that has the full replicative potential. The asymmetric distribution of aging determinants between the daughter and mother cells may contribute to the difference in their fitness and residual lifespan. The first evidence that suggests there are detrimental factors that gradually accumulate during aging and dominantly determine age came from a study mating old and young yeast cells. This study found that the zygote inherits the age of the older cell, although the identities of the detrimental factors remained unclear (Muller, 1985). Several potential detrimental factors in yeast have since been explored. It has been shown that extrachromosomal rDNA circles (ERCs) (Sinclair and Guarente, 1997) and oxidatively damaged proteins (Aguilaniu et al., 2003) are asymmetrically retained in the mother cells, but it remains unclear whether and how these factors may cause cellular aging. More recently, a group of multidrug resistance (MDR) transmembrane proteins were identified as being beneficial factors whose abundance or activity may determine aging. Newly synthesized MDR proteins are preferentially deposited into the daughter cells, whereas the functions of MDR proteins in the mother cells gradually deteriorate without sufficient replenishment, which leads to loss of fitness at an advanced age (Eldakak et al., 2010). Expanding this finding, two studies that performed quantitative proteomics found a group of long-lived proteins, including Mrh1, Pma1, Sur7, Thr1, Hsp26 and nuclear pore complexes (NPCs), that may contribute to aging in a similar manner through their low turnover rates and gradual deterioration in function (Thayer et al., 2014; Toyama et al., 2013). Taken together, these studies illustrate the useful concepts of beneficial and detrimental aging determinants on the cellular level.

These general concepts are likely to be applicable in multicellular organisms, including humans. The idea that detrimental factors act in a dominant manner is supported by the observation that injection of brain lysates containing protein aggregates from old mice into young asymptomatic mice is sufficient to drive the onset and progression of neurodegenerative diseases (Meyer-Luehmann et al., 2006; Mougenot et al., 2012). Beneficial aging factors have been demonstrated in recent studies showing that introducing the blood of young mice to aged mice led to a reversal of the pre-existing decline of brain functions in aged mice, although the identity of the specific molecular factor(s) remains unknown (Villeda et al., 2014). The same group of researchers also discovered that a specific beneficial factor from human umbilical cord plasma, tissue inhibitor of metalloproteinases 2 (TIMP2), revitalized the hippocampus and improved cognitive function of aged mice (Castellano et al., 2017). These *in vivo* studies of mammals revealed that re-introducing beneficial factors that are present at high levels in young animals can reverse aging. On the other hand, mitigating the effects of detrimental factors could also delay aging. In a recent study, old mice were injected with a peptide that specifically blocks forkhead box protein O4 (FOXO4), a protein that binds to p53 to maintain cellular senescence. It was found that damaged cells were eliminated through apoptosis and many normal cellular functions were restored in the old mice (Baar et al., 2017). In two studies of

[1]Center for Cell Dynamics, Department of Cell Biology, Johns Hopkins University School of Medicine, 855 North Wolfe Street, Baltimore, MD 21205, USA. [2]Biochemistry, Cellular and Molecular Biology (BCMB) Graduate Program, Johns Hopkins University School of Medicine, 1830 E. Monument Street, Baltimore, MD 21287, USA. [3]Department of Chemical and Biomolecular Engineering, Whiting School of Engineering, Johns Hopkins University, 3400 North Charles Street, Baltimore, MD 21218, USA.

*Author for correspondence (rong@jhu.edu)

L.R., 0000-0001-9835-6563

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age-related neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS) (Taylor et al., 2016), antisense oligonucleotide (ASO) was used to reduce the expression of ataxin-2, which lessened the disease pathology and extended the lifespan of ALS mice (Becker et al., 2017; Scoles et al., 2017). The emerging evidence reinforces the existence of aging determinants and suggests that manipulating the levels of the beneficial and detrimental factors may be able to delay or even reverse aging and extend healthy lifespan.

In this Review, we aim to consolidate recent findings in model organisms that have elucidated the molecular and cellular basis of aging, especially from the perspective of changes in the levels of beneficial or detrimental factors and the intrinsic connection between them. In particular, we focus on recent advances in uncovering the identities of beneficial aging factors at the molecular and organellar levels, and the accumulation and effects of detrimental factors such as protein aggregates and senescent cells (Fig. 1).

Loss of beneficial factors during aging

Theoretically, there are two types of beneficial factors in cellular and organismal aging: (1) those factors that help to maintain normal cellular functions but whose gradual decline over the course of aging contributes to the loss of fitness; and (2) factors that are not crucial for the fitness of young cells but are particularly important in aged cells to repair or boost damaged functions. Genomics, proteomics, lipidomics, metabolomics, single-cell profiling and genome-wide association studies (GWASs) have enabled the identification of some beneficial factors (Table 1), but how these factors play beneficial roles and the dynamics of their activity and abundance over the organismal lifespan remain unclear. As such, the discussion below does not explicitly distinguish between the two classes of beneficial factors.

Molecular-level determinants

Functional studies have shown that regaining certain beneficial molecules can be sufficient to rejuvenate an aged animal. In addition to aforementioned experiments involving the transfer of young mouse blood (Villeda et al., 2014) and human umbilical cord plasma (Castellano et al., 2017), introducing gonadotropin-releasing hormone (GnRH) to old mice prevented aging-related impairment of neurogenesis and cognitive decline (Zhang et al., 2013). Daily injections of GDF11, a circulating transforming growth factor-β (TGF-β) family member, to aged mice improved the cerebral vasculature and enhanced neurogenesis (Katsimpardi et al., 2014). However, how and why these circulating and intercellular factors become limited with age and their mechanism of action to restore specific functions when introduced into aged animals remain unclear. Understanding the underlying mechanism will be essential for exploiting the therapeutic potential of these factors.

Several intracellular beneficial molecules have also been uncovered. Repressor element 1-silencing transcription factor (REST) represses genes that promote neuronal cell death and Alzheimer's disease (AD) pathology, while it upregulates stress-response genes. The expression level of REST correlates with cognitive preservation and longevity during aging (Lu et al., 2014). Whether the protective roles of REST are specific to AD or applicable to other age-related neurodegenerative diseases remains unknown. In addition to transcriptional regulation, post-translational modifications are also linked to longevity. Regulated NAD-dependent protein deacetylase sirtuin-3 (SIRT3) controls the activity of some mitochondrial proteins by deacetylating their key lysine residues. Elevating SIRT3 activity improves the survival and extends the lifespan of neuronal cells (Cheng et al., 2016; van de Ven et al., 2017). Another study found that SIRT3 upregulation in aged hematopoietic stem cells (HSCs) restored their regenerative

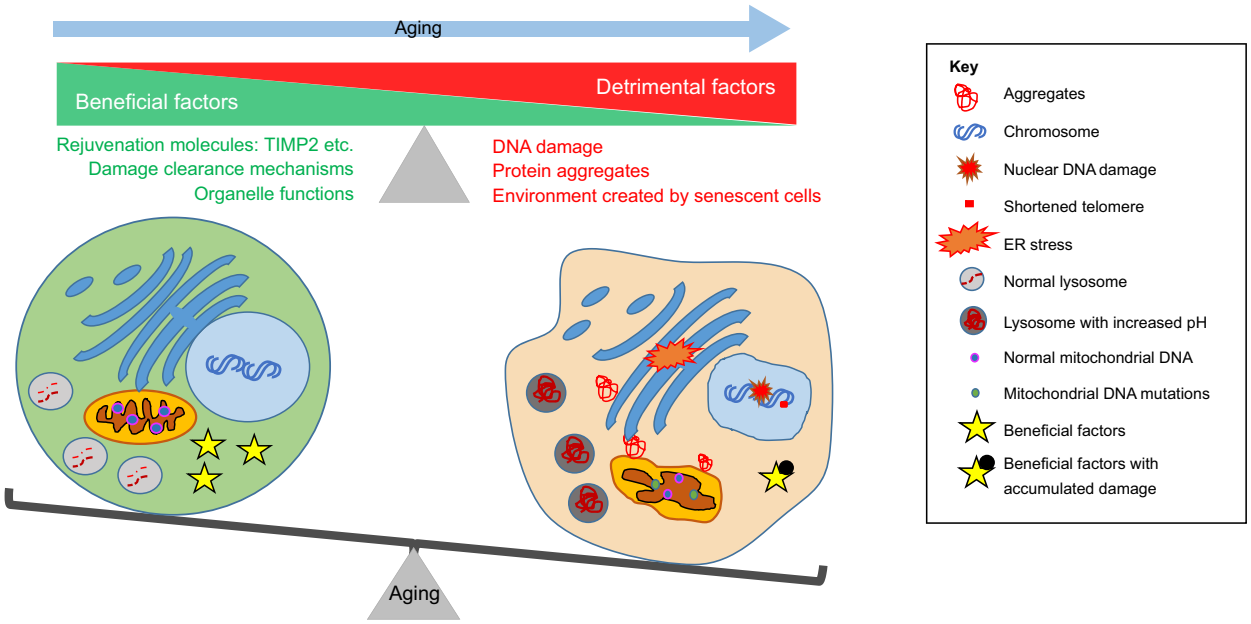

Fig. 1. Balance between beneficial and detrimental factors during aging. Declining levels or activities of beneficial factors and accumulation of detrimental factors during aging lead to fitness collapse at the cellular level. The scheme enumerates several beneficial and detrimental factors during lifespan as described in this Review. Beneficial factors include rejuvenation molecules such as TIMP2 (others in Table 1), damage clearance mechanisms and organelle functions, whereas detrimental factors include DNA damage, protein aggregates and the micro-environment created by senescent cells. Illustrated below are the typical cellular changes during aging that are mediated by the decreased level or activity of beneficial molecules. For instance, mitochondrial functions decline, including the accumulation of mutations in mitochondrial DNA. In addition, the lysosomal pH increases with a resulting decrease in its degradation capacity (dark gray circles in aged cell). Furthermore, there is also a loss of nuclear integrity and genome stability, as well as a shortening of telomeres and an accumulation of protein aggregates.

Table 1. Overview of known beneficial factors during aging

Beneficial molecules	Source	Effect	Reference
Blood from young mice	Heterochronic parabiosis of young and old mice	Reverse brain aging, improve learning and memory in aged mice	Villeda et al., 2014
TIMP2	Injection of human umbilical cord plasma to old mice	Rejuvenate the hippocampus and improve cognitive function in aged mice	Castellano et al., 2017
GnRH	Injection of GnRH to old mice	Promote adult neurogenesis at hypothalamus and hippocampus	Zhang et al., 2013
GDF11	Daily injection of GDF11 to old mic	Enhance neurogenesis	Katsimpardi et al., 2014
REST	Conditional knockout in the mouse brain	Repress genes that promote cell death and Alzheimer's disease pathology	Lu et al., 2014
SIRT3	Overexpression via lentiviral transduction in old mice cells	Reduce oxidative stress and rejuvenate aged HSCs	Brown et al., 2013
SIRT1	Brain specific overexpression in transgenic mice	Increase lifespan	Satoh et al., 2013
RanGAP	Overexpression of RanGAP in the eyes of a fly model that expresses C9orf72 repeats	Reverse age-dependent degeneration of fly eyes	Zhang et al., 2015
BubR1	Overexpression in transgenic mice	Maintain genomic integrity and extend lifespan	Baker et al., 2013
ABT263	Oral administration of ABT263 that targets senescent cells	Eliminate senescent cells in aged mice and rejuvenate the stem cells	Chang et al., 2016

capacity and reversed aging-associated degeneration (Brown et al., 2013). Beneficial factors can also be intracellular molecules other than proteins. For instance, there is a positive correlation between sphingomyelin levels in the brain and longevity (Ma et al., 2015).

While the above studies demonstrated the presence of beneficial aging factors, studies in animal models do not always recapitulate human aging and age-related diseases. Some human diseases have provided valuable opportunities for identifying genes that regulate the aging process in humans. For example, loss of function of some genes can cause human premature aging syndromes. For example, a mutation in *LMNA*, encoding a component of nuclear lamina, causes Hutchinson–Gilford progeria syndrome (HGPS), which is characterized by accelerated growth cessation, cardiovascular dysfunction and early mortality (Hennekam, 2006). Mutations in *BANF1*, a gene involved in nuclear assembly and chromatin organization, cause Nestor–Guillermo progeria syndrome with similar pathologies (Puente et al., 2011). Strikingly, reprogramming fibroblasts from HGPS patients to induced pluripotent stem cells (iPSCs) reversed the abnormal nuclear envelope morphology and age-associated defects, even when the *LMNA* mutation was still present (Liu et al., 2011). The premature aging phenotype could also be restored after inducing iPSCs to differentiate into vascular smooth muscle cells. This suggests that abnormalities from premature aging can be remodeled or reversed by reprogramming toward an embryonic state.

In addition, large scale GWASs have identified several loci that correlate with exceptional longevity, such as *TOMM40-APOE-APOC1*, *FOXO3*, *CDKN2B/ANRIL*, *ABO*, *SH2B3-ATXN2* (Broer et al., 2015; Broer and van Duijn, 2015; Fortney et al., 2015; Shadyab and LaCroix, 2015). However, the cellular mechanisms of how these gene products contribute to longevity remain unclear. Future GWASs combined with molecular and cellular studies might be useful in uncovering additional genes that are beneficial during aging.

Organelle-associated determinants

Mitochondrial functions are known to decline with age in humans (Lopez-Otin et al., 2013) (Fig. 1). Apart from their roles in ATP production, the tricarboxylic acid (TCA) cycle and apoptosis (Friedman and Nunnari, 2014), mitochondria have been increasingly recognized as a versatile organelle that regulates innate immunity (Pellegrino et al., 2014; West et al., 2015), maintains stem cell proliferative potential (Katajisto et al., 2015) and assists cytosolic proteostasis (Ruan et al., 2017). Consequently, the decline of these beneficial functions of mitochondria may accelerate aging. The accumulation of mutations in mitochondrial DNA (mtDNA) have been proposed to be a cause of aging (Linnane et al., 1989). However, since there are hundreds to thousands of copies of mtDNA in each cell, the level of mtDNA mutations would have to reach a certain threshold in order to cause a decline in functions encoded by the mitochondrial genome. A study using mtDNA mutator mice, which express proof-reading-deficient mtDNA polymerase, found that these mice have reduced lifespan and premature onset of age-related phenotypes (Trifunovic et al., 2004). This provides the first causative link between abnormal accumulation of mtDNA mutations and premature aging, but it does not necessarily indicate that mtDNA mutations are the limiting factor of aging under normal conditions because the mtDNA mutation rates in the mutator mice were three- to five-fold higher than in normal mice. Another theory of mitochondria-mediated aging focuses on the role of reactive oxygen species (ROS). During aging, reduced mitochondrial function or structural integrity can lead to electron leakage and increased ROS (Liochev, 2013). However, there is no direct evidence to support that mitochondrial ROS cause aging (Kauppila et al., 2017).

The beneficial effect of elevated mitochondrial activity can be achieved through increased mitochondrial biogenesis as a result of exercise. One study showed that endurance exercise induced systemic mitochondrial biogenesis and prevented the premature aging phenotype of mtDNA mutator mouse (Safdar et al., 2011). Endurance exercise also restored mitochondrial morphology and functions (Safdar et al., 2011). Clinical evidence supports the idea that endurance exercise improves age-related decline of muscle and cognitive functions possibly due to increased mitochondria biogenesis (Rowe et al., 2014), which is regulated by peroxisome proliferator-activated receptor γ coactivator 1-α (PGC-1α; also known as PPARGC1A) (Arany et al., 2005). Although there are positive correlations between upregulation of mitochondrial biogenesis and alleviated aging phenotypes, the underlying mechanisms remain unclear as mitochondria are multifunctional organelles and interact closely with other cellular components, and endurance exercise may have other physiological targets that affect organ fitness and function.

Lysosomes, the major digestive organelles of the cell, are also linked with aging and lifespan determination (Fig. 1). Autophagy can be broadly categorized into macroautophagy, microautophagy and chaperone-medicated autophagy (CMA) processes, and is the process whereby cytosolic substrates are delivered to lysosomes for degradation and recycling (Ohsumi, 2014). Increased autophagy has been associated with longevity, whereas compromised autophagy has been linked to an acceleration of aging and age-related diseases, such as neurodegenerative diseases and cancer (Madeo et al., 2010). During yeast replicative aging, the acidity of the vacuole (the yeast equivalent of the mammalian lysosome) gradually declines, which leads to mitochondrial dysfunction, although the mechanism of this link remains unclear (Hughes and Gottschling, 2012). A screen in yeast that aimed to identify the genes associated with longevity found ten autophagy-related genes, mutations in which led to shortened lifespan (Matecic et al., 2010). Similar phenotypes associated with autophagy mutations have been observed in *Caenorhabditis elegans* (Tóth et al., 2008) and *Drosophila* (Lee et al., 2010). In humans, mutations in *SQSTM1*, encoding the autophagy receptor p62, have been identified as causing familial ALS (Fecto et al., 2011). Similarly, in Parkinson's disease, mutations in *ATP13A2*, which encodes a lysosomal ATPase, result in unstable pH in the lysosome and decreased degradation capacity (Jinn et al., 2017). Autophagy also plays important roles in maintaining the proliferative potential of stem cells. In HSCs of aged mice, active autophagy is required to remove healthy mitochondria that are highly metabolically active in order to keep the stem cells in the quiescent state, whereas loss of autophagy in HSCs drives differentiation and reduces regenerative potential (Ho et al., 2017). In muscle stem cells, impaired autophagy leads to the senescence of quiescent stem cells, whereas re-establishment of autophagy reverses senescence and restores regenerative capacity (Garcia-Prat et al., 2016).

Overexpression of sirtuin-1 (SIRT1) activates autophagy and extends lifespan in different model organisms (Morselli et al., 2010; Satoh et al., 2013), but this beneficial effect is absent in autophagy-deficient models (Madeo et al., 2015). How SIRT1 activates autophagy remains unclear. Calorific restriction (CR) by reducing food intake without malnutrition is one of the most conserved and effective methods in extending the lifespan of model organisms and humans (Rubinsztein et al., 2011). CR activates autophagy mainly through the effects of AMP-activated protein kinase (AMPK) (Egan et al., 2011) and SIRT1 (Wang, 2014), and the lifespan-extending effects of CR are also abolished in autophagy-deficient *C. elegans* mutants (Jia and Levine, 2007). However, it is still controversial whether SIRT1 is dispensable in CR-induced longevity. There is evidence suggesting that SIRT1 and Sir2 (the yeast homolog of SIRT1) are not required for CR response and lifespan extension in mammals and yeast, respectively (Chen et al., 2008; Kaeberlein et al., 2004; Lamming et al., 2005). This may be explained by compensatory effects of AMPK. One beneficial effect of autophagy and/or the lysosome may be to clear damaged proteins, thus preventing the accumulation and propagation of protein aggregates (Heras-Sandoval et al., 2014; Wei et al., 2014). Another beneficial effect may be linked to a signaling function of the lysosome. The lysosomal acid lipase LIPL-4 has been shown to increase the longevity of *C. elegans* by inducing the translocation of the lysosomal lipid chaperone LBP-8 to the nucleus to activate the nuclear hormone receptors NHR-48 and NHR-80 (Folick et al., 2015). The lysosome is also involved in the turnover of dysfunctional mitochondria, a process termed mitophagy (Ashford and Porter, 1962; Lemasters, 2005). Loss-of-function mutations in the components of the mitophagy machinery, such as the mitochondrial serine/threonine-protein kinase PTEN-induced putative kinase 1 (PINK1) and the E3 ubiquitin ligase Parkin, cause early-onset Parkinson's disease in human (Lücking et al., 2000; Valente et al., 2004).

Emerging evidence suggests that impairing the translation machinery post development, but not during development, can extend lifespan (Hansen et al., 2007; Kaeberlein et al., 2005; Kapahi et al., 2004; Steffen and Dillin, 2016; Syntichaki et al., 2007). The ribosomes and endoplasmic reticulum (ER) are the major factories of proteins that are essential for building the cell, but the over-production and accumulation of unstable proteins could lead to loss of protein homeostasis (proteostasis) and protein aggregation (Lopez-Otin et al., 2013). Interestingly, inhibiting translation elongation via cycloheximide in yeast blocked the formation of protein aggregates that are caused by a variety of proteotoxic stressors (Zhou et al., 2014). The mechanism underlying the beneficial effects of impairing translation during aging remains unclear, but a simple explanation could be the reduced burden of managing newly translated (Medicherla and Goldberg, 2008), unfolded proteins, which are inherently aggregation prone. Furthermore, reducing protein synthesis could free up chaperone proteins that are required to maintain the integrity and functionality of the existing proteome to refold misfolded proteins, as well as degradation machineries to timely remove damaged proteins.

Loss of nuclear integrity and genome stability have also been implicated in aging (Fig. 1). As mentioned above, progeria syndromes are caused by the disruption of nuclear integrity and organization (Hennekam, 2006; Puente et al., 2011). Mutations in nuclear lamins, the main proteins of the nuclear lamina that associate with the inner nuclear membrane, lead to the accelerated aging disease HGPS (De Sandre-Giovannoli et al., 2003; Eriksson et al., 2003). Lamins are long-lived proteins (Toyama et al., 2013) and thus may accumulate damage and gradually lose their functions during aging. Indeed, in normal aged humans, HGPS-like lamin A-dependent nuclear defects have been observed (Scaffidi and Misteli, 2006). These findings suggest that lamins fit into the category of beneficial factors that gradually lose their functionality during aging. Moreover, epigenetic and chromosomal regulations also have been implicated in affecting lifespan. Reduced trimethylation of histone H3 at lysine 4 (H3K4) in the *C. elegans* germline increases the lifespan of their descendants (Gong et al., 2015; Greer et al., 2011). In the mouse, it also has been shown that histone methylations in the brain increase with age (Gong et al., 2015). Although the mechanism is still unclear, this may affect the lifespan indirectly through changing the expression profiles of certain genes, or might even induce large-scale changes of the landscape of chromosomal organization.

Most nuclear proteins are translated in the cytoplasm and imported into the nucleus. The *C9orf72* repeat expansion causes ALS through the disruption of nucleocytoplasmic transport (Freibaum et al., 2015). This repeat expansion disrupts NPCs and nuclear protein import machinery (Freibaum et al., 2015). Overexpression of RanGAP, a key regulator of nucleocytoplasmic transport, greatly reduced the neurodegeneration phenotype in an ALS fly model (Zhang et al., 2015). Similar observations of disrupted nuclear protein import have been made in other neurodegenerative disease-related mutations, such as those in huntingtin and TAR DNA binding protein-43 (TDP-43) (Woerner et al., 2016). These studies suggest that disruption of nucleocytoplasmic transport compromises homeostasis and leads to age-associated neurodegenerative diseases. Targeted intervention

to restore normal nucleocytoplasmic transport may have therapeutic potential.

Apart from nuclear architecture and organization, increased DNA lesions and genome instability also contribute to aging. Disruption of DNA repair mechanisms or the spindle assembly checkpoint (SAC), which ensures chromosome transmission fidelity, results in accelerated aging (Chen et al., 2011; Gregg et al., 2012; Krishnan et al., 2011; Murga et al., 2009). Accordingly, enhancing SAC activity through overexpression of BubR1, a key component of the checkpoint, prevented aneuploidy in aged mice and extended the lifespan of the animal (Baker et al., 2013; Weaver et al., 2016).

Apart from changes in chromosome stoichiometry, the end region of chromosomes, called a telomere, is gradually shortened during aging (reviewed in Blackburn et al., 2006) (Fig. 1). In fact, the length of telomeres in early life can be used to predict lifespan (Heidinger et al., 2012). During DNA replication, DNA polymerase is unable to synthesize the end region when the terminal RNA primers are removed, known as 'the end-replication problem' (Lundblad, 1997). This is overcome by telomerase, which is capable of adding telomere repeat sequences onto the ends of chromosomes using RNA as the template. Overexpression of telomerase in adult and old mice is sufficient to delay aging (Bernardes de Jesus et al., 2012; Tomas-Loba et al., 2008), whereas mutations in telomerase lead to premature aging syndromes (Anchelin et al., 2013; Vulliamy et al., 2008). However, a recent study argues that the lack of telomerase activity accelerated aging independently of telomere length in budding yeast (Xie et al., 2015). Collectively, the existing evidence nevertheless suggests that maintaining genome and nuclear integrity is important for delaying aging.

Accumulation of detrimental factors

Detrimental factors in cellular and organismal aging refer to the components that negatively affect fitness and lifespan. These factors gradually increase their abundance or activities during aging, which may reflect either an increased production, or decreased ability of other components that counteract their effects (such as beneficial factors). Therefore, lowering the levels of those detrimental factors in the aged population could extend lifespan, and vice versa.

Protein aggregates

Formation of protein aggregates has been recognized as a general marker of aging and reflects the gradual loss of proteostasis during aging (Lopez-Otin et al., 2013). Protein aggregation itself may be beneficial upon acute stresses because it helps to consolidate structurally unstable proteins and prevent them from impairing the functions of other normal proteins (Pastore and Temussi, 2012). Moreover, recent evidence suggests that protein aggregation may also have important roles in development (Boke et al., 2016), the innate immune response (Hou et al., 2011) and memory (Si et al., 2003). Once the stress is removed, cells can dissolve the aggregates and restore proteostasis through an array of quality control mechanisms, including chaperone-assisted folding and structural maintenance of the proteome (Balchin et al., 2016), organelle-based aggregate retention by the mitochondria and ER (Coelho and Tolic, 2015; Pina and Niwa, 2015; Zhou et al., 2014), proteolytic degradation through the ubiquitin-proteasome system (UPS) (Maupin-Furlow, 2011) and the autophagy–lysosome system discussed above (Ohsumi, 2014), as well as mitochondria-mediated import and degradation of misfolded proteins (Ruan et al., 2017) (Fig. 2). However, the aggregates that fail to be removed during aging will accumulate and may be detrimental. Aggregates found in the brain of patients with neurodegenerative diseases have been historically proposed to cause debilitating diseases, such as intercellular amyloid-β deposits and intracellular tau neurofibrillary tangles in Alzheimer's disease, α-synuclein aggregates enriched in the Lewy body in Parkinson's disease, TDP43- or FUS-containing inclusions in the motor neurons in ALS, and Huntingtin protein inclusions in Huntington's disease (Jucker and Walker, 2013). These diseases are therefore viewed as accelerated aging (Douglas and Dillin, 2010). However, in older humans without any signs of neurodegeneration, aggregates still exist in a form that is similar to the pathological inclusions mentioned above and their abundance also increases with age (Elobeid et al., 2016; Morris et al., 2010). These observations suggest that aggregate accumulation is a general hallmark of aging. The level of aggregates in these aged brains without neurodegenerative symptoms may have not reached the critical threshold to induce systematic neuronal death and cognitive decline, or are counterbalanced by protective cellular functions in these brains. Conditions that prevent aggregate formation by enhancing proteostasis delay aging and increase fitness (Kumar and Atamna, 2011; Zhang and Cuervo, 2008), whereas those promoting aggregate formation accelerate aging (Erjavec et al., 2007).

The detrimental effects of aggregates may be due to either loss of the normal functions of the respective protein or the gain of toxicity of the aggregates. The cellular components that are sequestrated in such aggregates include not only misfolded proteins but possibly also normal proteins that otherwise would not aggregate and that therefore lose their normal activities. For example, TDP43 polypeptides are trapped in the cytoplasmic inclusions in the motor neurons of ALS patients (Neumann et al., 2006); therefore, their mRNA binding and splicing functions needed in the nucleus are lost (Buratti and Baralle, 2001). Normal proteins that bind ubiquitin (Ub), such as ataxin-3 and A1Up (also known as UBQLN4) that both are involved in regulating protein degradation, are recruited to polyQ aggregates, which are involved in Huntington's disease, through their Ub-binding motifs (Donaldson et al., 2003).

The detrimental effects of aggregates may also be mediated by a gain in toxic functions that lead to cellular mortality. One possibility is the emergence of new activity of a misfolded conformation. For instance, mutants of α-synuclein and amyloid beta (Aβ) form oligomeric fibrillization intermediates (protofibrils), which can be mal-inserted into the cell membrane and so result in pore formation and permeabilization of the cell membrane (Lashuel et al., 2002). α-Synuclein aggregates also lead to defects in ER-to-Golgi trafficking (Cooper et al., 2006) and Golgi fragmentation (Gosavi et al., 2002). Aggregates that accumulate in axons can also simply present a physical blockage to axonal transport (Sinadinos et al., 2009; Volpicelli-Daley et al., 2014).

Considering the potential toxic effects of the aggregates, their targeted clearance may hold potential for pharmacotherapeutic intervention. Although a recent clinical trial using an antibody to target amyloids in AD failed, this does not disprove that amyloids have detrimental effects because the antibody might not have reached its target in the brain (Abbott and Dolgin, 2016). Nevertheless, this and other failed recent trials also reflect the complexity of treating neurodegenerative diseases. For example, how does the drug pass the blood–brain barrier and reach its targets? When is the critical time point to start drug treatment? Can the cellular functions that are disrupted by aggregates be restored after removing the aggregates? Understanding the roles of protein aggregates in aging and associated diseases has been the focus of studies for the past century; however, it remains unclear whether aggregate formation is a cause or effect of aging, or indeed both. It would be important to understand what aspects of aggregate toxicity

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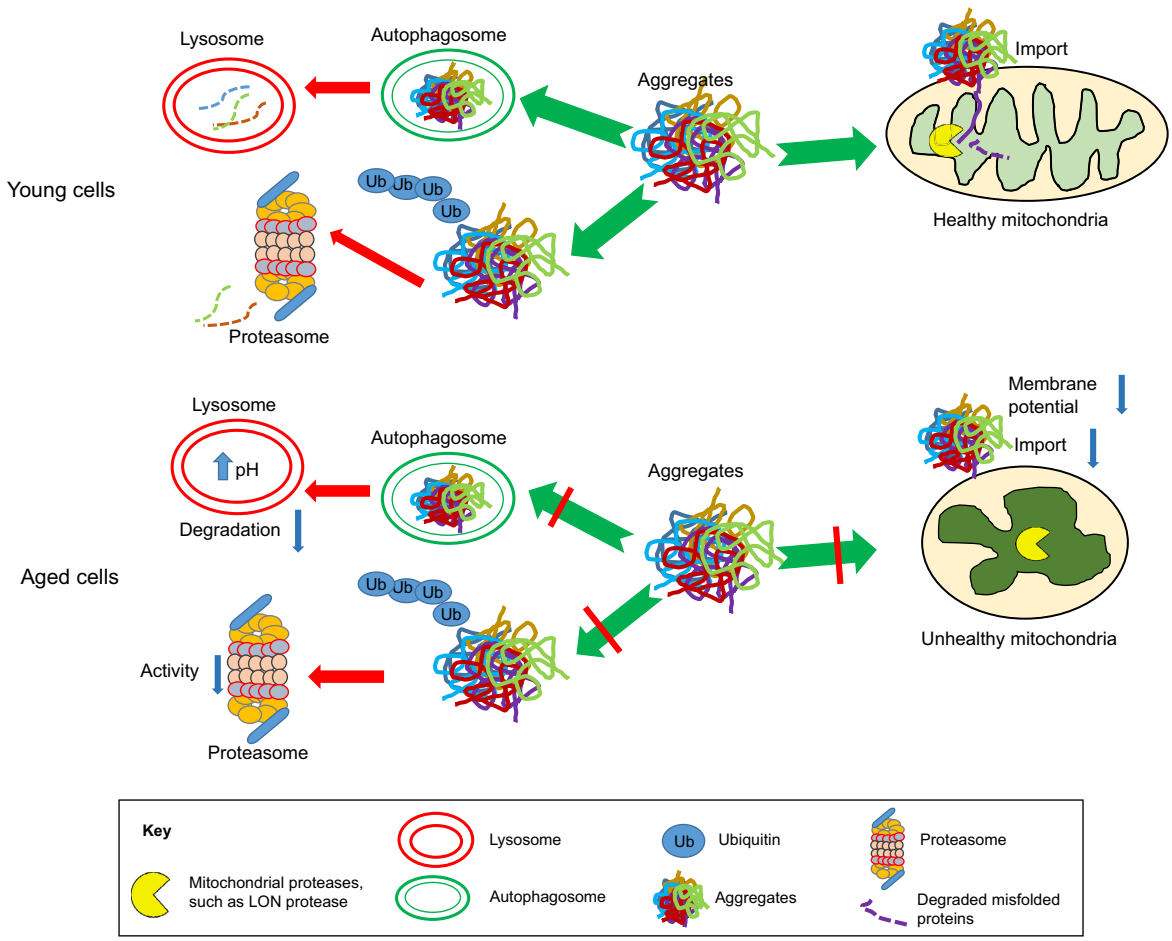

Fig. 2. The ability of cells to degrade aggregates declines during aging. Structurally unstable proteins misfold under stress and form heterogeneous aggregates. Chaperone machineries assist in the refolding of misfolded proteins and participate in sending them to different degradation systems (not shown in the figure). In young cells with highly functional cellular machineries, aggregated proteins are efficiently degraded by autophagy through the lysosome system, by the ubiquitin-proteasome system (UPS), and by mitochondrial import and degradation (MAGIC). However, in aged cells, these machineries decline in function. For example, the reduced membrane potential of mitochondria in the aged cell may also lead to reduced import. The activities of UPS and lysosomal proteolysis may also decline during aging due to a decrease in cellular ATP levels and increased lysosomal pH, respectively. The accumulation of aggregates in aged cells then can impair other cellular functions and lead to fitness decline and cell death.

are the early events that lead to cellular and physiological dysfunction. Animal models unfortunately do not always mimic human aging and age-related pathologies, and thus might not yield the answers.

It is also puzzling that aggregate accumulation and disease symptoms only occur after adulthood, but individuals may bear the causal mutations since birth. One explanation could be that mechanisms that normally prevent the accumulation of aggregates deteriorate and are less active during aging. Indeed, overexpression of molecular chaperones, such as Hsp16 in *C. elegans* and Hsp22 in *Drosophila* (Morrow et al., 2004; Walker and Lithgow, 2003), to increase their activities, can restore proteostasis and extend lifespan, whereas reducing the chaperone activities can reduce lifespan (Kaushik and Cuervo, 2015; Morley and Morimoto, 2004; Swindell, 2009). Another possible explanation could be that in aged tissues, an altered cellular environment promotes protein aggregation. Indeed, *in vitro* experiments have shown that acidic conditions help the formation of toxic Aβ aggregates (Pfefferkorn et al., 2010; Su and Chang, 2001) and the acidic molecular wastes might gradually change the cellular pH during aging. The acidic cellular environment may facilitate the aggregation of specific proteins, such as Aβ. It is clear that more research is needed to elucidate the early events that disrupt proteostasis and the crosstalk among the different mechanisms that maintain proteostasis.

Senescent cells

Cellular senescence refers to the irreversible exit from the cell cycle and changes in cellular products and/or components, including an altered secretome and chromatin (van Deursen, 2014). Senescence can be caused by various stressors encountered during aging, such as telomere erosion, DNA damage, oxidative stress, oncogene activation and/or tumor suppressor loss, and others. It is typically viewed as beneficial to the organism because of its roles in tumor suppression, embryogenesis, wound healing and host immunity (He and Sharpless, 2017). However, depending on the context, the accumulation of senescent cells during aging can be detrimental to the organism, as it may cause anatomic lesions, disrupted tissue homeostasis and loss of regeneration capacity (He and Sharpless, 2017; Munoz-Espin and Serrano, 2014). In light of recent findings on the importance of circulatory proteins during aging (Castellano et al., 2017), the altered secretome of senescent cells could play a role in tissue or cell homeostasis during aging. Indeed, several classes of proteins are exclusively secreted from senescent cells and not from non-senescent cells, such as the redox factors

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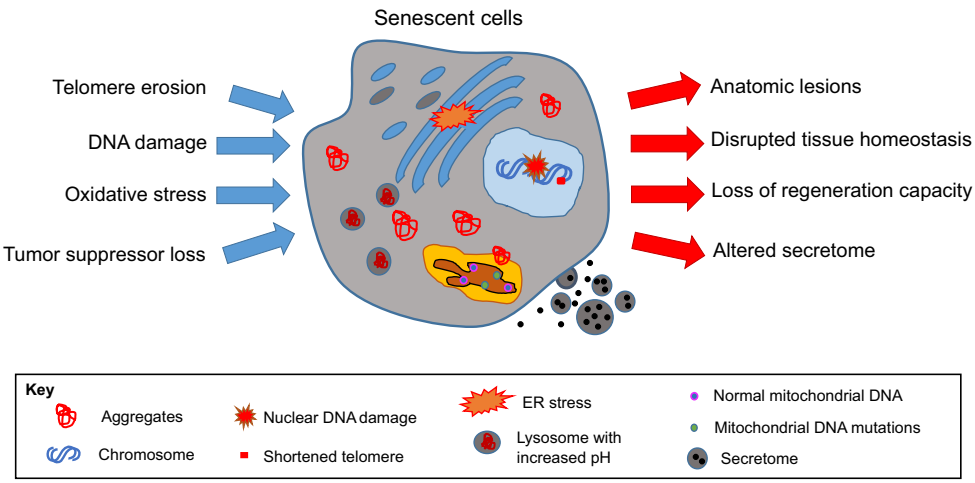

Fig. 3. Detrimental effects of senescent cells in aged organisms. Different stressors encountered during aging, such as telomere erosion, DNA damage, oxidative stress, oncogene activation and/or tumor suppressor loss, and others, can all cause cellular senescence. The failed clearance of the senescent cells during aging can be detrimental, as they may cause anatomic lesions, such as build up of an atherosclerotic plaque, disrupt tissue homeostasis by occupying physiological niches or result in a loss of the regeneration capacity of the tissue, such as in T cells and pancreatic β cells (for details please see the review, He and Sharpless, 2017). Furthermore, the secretome of senescent cells can be toxic to other cells.

peroxiredoxin 6 (PRDX6) and Parkinson disease protein 7 (PARK7) and 14-3-3ε, among others (Ozcan et al., 2016) (Fig. 3). Below, we briefly discuss the detrimental effects of senescent cells in aged organisms, but we will not focus here on the mechanism of cellular senescence per se (Childs et al., 2017).

Senescent cells are often removed by immune-mediated clearance (Katlinskaya et al., 2015; Munoz-Espin and Serrano, 2014). During aging, the percentage of senescent cells increases in different organs, such as the lung, spleen, dermis, liver and gut (Wang et al., 2009), possibly as a result of their increased rate of production or decreased rate of removal, or both. Recent studies have suggested that the targeted clearance of senescent cells in aged mice can delay aging (Baar et al., 2017), whereas the induction of cellular senescence during adulthood leads to accelerated aging and reduced healthy lifespan (Baker et al., 2016; Keyes et al., 2005; Zhang et al., 2017).

These findings suggest a causative role of senescent cells in aging. Therefore, there has been much interest in understanding the detrimental effects of senescent cells and their targeted elimination in aged organisms. Targeted clearance of p16 (Ink4a or CDKN2A)-positive cells, which is a biomarker for senescence, not only delayed age-related pathologies, such as a decrease in muscle fiber diameter and fat depots, but also attenuated the progression of already acquired disorders in aged mice (Baker et al., 2011). Furthermore, a recent screen identified the drug ABT263, which inhibits the anti-apoptotic proteins BCL-2 and BCL-xL (encoded by *BCL2L1*), selectively eliminates senescent cells in normally aged mice by apoptosis; this improved the regeneration abilities of the aged bone marrow HSCs and muscle stem cells (MuSCs) (Chang et al., 2016).

In addition to removing senescent cells themselves, the specific elimination of their secretion products could also be beneficial in aged organisms. For instance, reducing the level of circulating activin A, which is produced by senescent cells, in 22-month-old mice alleviated age-related dysfunctions, such as reduced fat mass and insulin sensitivity, and increased lipotoxicity (Xu et al., 2015). In summary, these studies demonstrate the therapeutic potential of targeting senescent cells with the aim to extend healthy lifespan, although the potential side effects in humans, such as increased incidence of cancer or mis-targeting of normal non-senescent cells, need to be carefully evaluated.

Perspectives

Taken together, it has become clear that investigating the loss of beneficial factors and accumulation of detrimental factors provides a framework for future studies on the molecular and cellular basis of aging. Some of the examples provided above also highlight the notion that the loss and gain of aging determinants are intrinsically connected. For example, the accumulation of aggregates reflects the gradual loss of the beneficial mechanisms that maintain proteostasis during aging. In the past few years, there have been many exciting advances in aging research, including methods that successfully increased the lifespan of model organisms, such as reintroducing or increasing the level of the beneficial factor TIMP2, or the targeted clearance of senescent cells in aged animals (Baar et al., 2017; Baker et al., 2016; Castellano et al., 2017), although none of these approaches have so far been tested in humans. Many outstanding mechanistic questions are still waiting to be explored. For example, among the age-related changes taking place, what are the primary causal factors, and what are secondary factors or only the consequence of aging? Apart from revealing the identities and specific mechanisms of factors that either drive or slow down aging, future research should also be aimed at disentangling their interconnections and deciphering the complex combinatorial effects on aging. Ultimately, prolonging healthy aging and delaying age-related diseases may be achieved by replenishing certain beneficial factors, while blocking any counter-acting detrimental factors.

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Competing interests

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