Principles of the Molecular and Cellular Mechanisms of Aging

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Aging can be defined as a state of progressive functional decline accompanied by an increase in mortality. Time-dependent accumulation of cellular damage, namely lesions and mutations in the DNA and misfolded proteins, impair organellar and cellular function. Ensuing cell fate alterations lead to the accumulation of dysfunctional cells and hamper homeostatic processes, thus limiting regenerative potential; trigger low-grade inflammation; and alter intercellular and intertissue communication. The accumulation of molecular damage together with modifications in the epigenetic landscape, dysregulation of gene expression, and altered endocrine communication, drive the aging process and establish age as the main risk factor for age-associated diseases and multimorbidity.

The molecular wear-and-tear of aging

Genetics and environmental and intrinsic factors play important roles in influencing aging. Damage accumulation causes common age-associated functional decline and increased multimorbidity with age (Figure 1) (Niedernhofer et al., 2018). Various aspects of multimorbidity can serve as independent predictors for mortality risk and functional outcome in the context of age-associated diseases (Chang et al., 2011; Wu et al., 2017).

The accumulation of stochastic errors, diverse lesions, and cellular damage derived from the normal cell metabolism is inevitable. The study of premature aging (progeroid) syndromes can greatly improve our understanding of the mechanisms underlying human aging and the etiology of age-associated diseases. Many of these disorders originate from mutations in DNA repair genes, and the pathology in these patients shares striking similarities with a physiological, age-associated, functional decline, highlighting the causal role of somatic genome damage accumulation as a driver of aging (Kubben and Misteli, 2017).

DNA damage accumulation. Driven by the onslaught of endogenous and exogenous genotoxins, accumulation of DNA damage is thought to be a natural driver of the aging process (da Silva and Schumacher, 2019; López-Otín et al., 2013; Moskalev et al., 2013;). Although a plethora of DNA damage types occur with an estimated frequency of tens of thousands of lesions—ranging from spontaneous deamination and oxidative base modifications to strand breaks and crosslinks—on a daily basis, the clearest evidence for increased DNA damage has been the age-dependent increase in mutations (Garcia et al., 2010; Vijg and Dollé, 2002; Zhang et al., 2019a). In contrast to the chemically distinct lesion types themselves, somatic mutations are readily quantifiable by sequencing of single somatic cell genomes (Gundry et al., 2012; Zhang et al., 2019a). Unrepaired lesions can have highly deleterious effects as they impair replication and transcription. Inaccurate repair or misreplication leads to accumulation and propagation of permanent mutations and chromosomal aberrations. Mutations can lead to cellular dysfunction but also cause cancer when tumor suppressor genes or oncogenes are affected (Blokzijl et al., 2016; Hoeijmakers, 2009; Jaiswal et al., 2014; Osorio et al., 2018; Xie et al., 2014). Consequently, cancer risk increases with age because of the increased accumulation of somatic mutations.

The DNA damage response (DDR) comprises highly specialized and conserved lesion-specific repair and signaling pathways that detect specific alterations in the DNA, arrest the cell cycle, and repair the lesion. These initial steps can decide a cell's fate: if lesions are successfully repaired, the DDR signaling is terminated and cells return to their original, prelesion state; if, however, lesions cannot be repaired, the DDR signaling persists, thus triggering cell senescence or cell death (d'Adda di Fagagna, 2008; Fitsiou et al., 2021). Both states prevent tumorigenesis but can eventually contribute to tissue aging (Hoeijmakers, 2009; Muñoz-Espín and Serrano, 2014).

UV-induced lesions are a particularly intriguing example of the mutagenic and cytotoxic outcomes of genotoxic stress. UV predominantly induces cyclobutane pyrimidine dimers (CPDs) that can be bypassed during replication by translesion synthesis polymerases (Powers and Washington, 2018) but also lead to replication fork breakdown resulting in double strand break (DSB) formation (Garinis et al.,
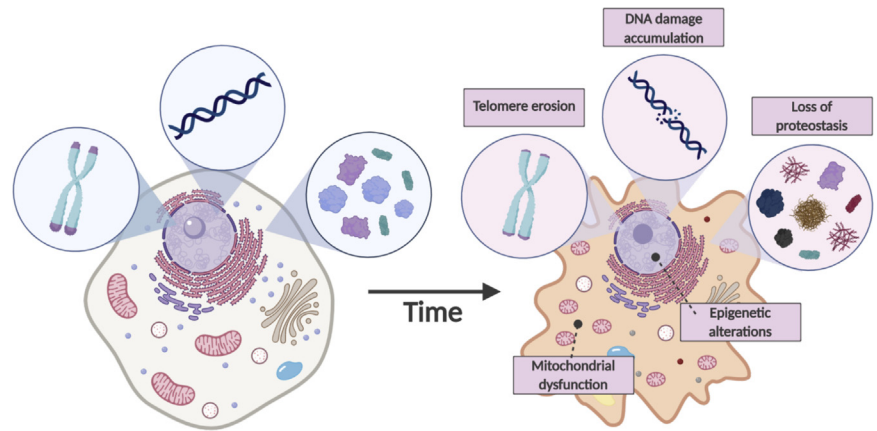
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Abbreviations: AD, Alzheimer disease; CPD, cyclobutane pyrimidine dimer; CS, Cockayne syndrome; DDR, DNA damage response; DR, dietary restriction; DSB, double strand break; HD, Huntington disease; H3K4me2, H3K4, demethylation; H3K4me3, H3K4 trimethylation; H3K9me3, H3K9 trimethylation; H3K27me3, H3K27 trimethylation; HGPS, Hutchinson-Gilford progeria syndrome; IIS, insulin/insulin-like signaling; NER, nucleotide excision repair; PD, Parkinson disease; SASP, senescence-associated secretory phenotype; TOR, target of rapamycin; XP, xeroderma pigmentosum

Figure 1. The molecular wear-and-tear of aging. Time-dependent accumulation of cellular damage, namely lesions and mutations in the DNA and misfolded proteins, can induce mitochondrial dysfunction and impair cellular function. Additionally, with each somatic cell division, telomeres shorten. Persistent DNA lesions and critically short telomeres are known to trigger a persistent DDR that can lead the cell into a senescent state. Gradual alterations in a cell's epigenetic landscape capable of altering a cell's fate or identity also accumulate over time. DDR, DNA damage response.

2005). During transcription, the RNA polymerases stall as they cannot bypass CPDs, ultimately leading to functional decline of the cells. The distinct consequences of mutagenic and obstructive DNA lesions become evident in rare congenital disorders caused by defects in nucleotide excision repair (NER), which is required for removing CPDs. Mutations primarily affecting global-genome NER lead to several thousand-fold elevated skin cancer susceptibility in patients with xeroderma pigmentosum (XP) (Rizza et al., 2021), displaying the oncogenic consequence of DNA damage–driven mutagenesis. In stark contrast, defective transcription-coupled NER defects underlie Cockayne syndrome (CS), which is characterized by growth failure and premature aging with neurodegeneration, atherosclerosis, and other typical age-related pathology during childhood, exemplifying the consequences of DNA lesions that obstruct transcription in promoting the aging process (Edifizi and Schumacher, 2015; Hoeijmakers, 2009). In addition to XP and CS, there is a plethora of genome instability syndromes that cause cancer susceptibility and premature aging: ataxia telangiectasia and Nijmegen breakage syndrome result from defects in DSB repair (Shiloh, 1997); Hutchinson-Gilford progeria syndrome (HGPS) and Néstor-Guillermo progeria syndrome are associated with defects in the nuclear envelope architecture (Kubben and Misteli, 2017); and defects in RecQ helicases, which are required during replication and recombination, can lead to Werner and Blooms syndromes (Croteau et al., 2014). These examples establish unrepaired DNA lesions as a driving factor for the aging process and the etiology of age-related multimorbidity.

In addition to the accumulation of DNA damage, telomeres shorten with each somatic cell division and, when they are critically shortened, trigger a DDR capable of inducing cellular senescence and mitochondrial dysfunction (Zhu et al., 2019) and promote stem cell aging (Behrens et al., 2014). Telomere shortening progressively occurs with aging (Canela et al., 2007), and telomere shortening rate has been used to predict species lifespan (Whittemore et al., 2019). Telomere shortening has also been observed in the context of numerous human diseases (Decker et al., 2009; Kong et al., 2013) and is thought to contribute to age-associated tissue dysfunction (Armanios et al., 2009; Rudolph et al., 1999).

Loss of proteostasis. Proteins must fold and assemble into precise three-dimensional structures. In a crowded cellular environment, it can be challenging to achieve and maintain a proper folded state (Bartlett and Radford, 2009). Protein homeostasis—proteostasis—requires coordinated action at multiple stages by a tightly regulated system composed of chaperones and protein-degradation machineries (Hipp et al., 2019; Klaips et al., 2018). The proteostasis mechanisms assist proteins to adopt and maintain their correct folding state during and after synthesis and, when this can no longer be achieved, ensure that misfolded proteins are degraded (Klaips et al., 2018). This can be particularly difficult when DNA mutations in components of proteostasis factors impair their function or alter residues that are important for a protein to fold correctly; when cells are exposed to acute stress; and, importantly, during aging (Santra et al., 2019). Proteostasis alters during aging, for example, in *Caenorhabditis elegans,* where collapse of proteostasis appears to be a relatively early event that promotes widespread proteome remodeling and aggregation (Ben-Zvi et al., 2009; David et al., 2010; Walther et al., 2015). Altered proteostasis function can then contribute to the genesis of age-associated diseases such as Alzheimer disease (AD), Parkinson disease (PD), and Huntington disease (HD) (Klaips et al., 2018).

To ensure that folding capacity is not overwhelmed, it can be beneficial to reduce translation. Reduction of protein synthesis extends lifespan and increases somatic stress resistance, as observed in *C. elegans ife-2* mutants (Syntichaki et al., 2007). Smaller proteins can often form fibrillary amyloid aggregates that develop into insoluble deposits, a hallmark of age-associated neurodegenerative diseases such as AD, PD, and HD (Hartl, 2017). To ensure proper folding and maintain that correct state, the cell utilizes different classes of molecular chaperones (Hipp et al., 2019). Overexpression of the heat shock proteins Hsp16 and Hsp22 extends the lifespan in *C. elegans* and *Drosophila melanogaster*, respectively (Morrow et al., 2004; Walker and Lithgow, 2003).

Finally, two major degradation pathways, the ubiquitin-proteasome system and the autophagy-lysosome system, have been reported to decline with aging (Chang et al., 2017; Vilchez et al., 2014). Stress response pathways trigger the upregulation of these proteolytic systems (Taylor et al., 2014).

In *C. elegans*, the FOXO transcription factor DAF-16 can upregulate the proteasome subunit RPN-6, contributing to stress resistance (Vilchez et al., 2012), and induction of autophagy can also extend lifespan and promote stress resistance, including to genotoxic stress (Edifizi et al., 2017; Kumsta et al., 2019,). Other important stress response pathways—unfolded protein responses—are triggered following accumulation of misfolded proteins in the endoplasmic reticulum and/or mitochondria (Taylor et al., 2014).

Epigenetic alterations. An altered epigenetic landscape can modify a cell's response to damage or extracellular signals and alter a cell's fate or identity by alterations in transcription regulatory networks, thus affecting tissue function. Changes in transcription factor activity and binding can dysregulate gene expression, and several transcription factors have been identified as having key roles in aging, including the paradigmatic *C. elegans* DAF-16 (Kenyon et al., 1993; Ogg et al., 1997). A number of transcription factors affect lifespan including NRF2/SKN-1, regulating stress resistance (Blackwell et al., 2015), and HSF-1, regulating autophagy and proteostasis (Kumsta et al., 2017) and whose overexpression increases lifespan in *C. elegans* (Morley and Morimoto, 2004; Sural et al., 2019).

Chromatin state and structure change during aging (Booth and Brunet, 2016; Feser and Tyler, 2011). Particularly, histone marks such as decreased H3K27 trimethylation (H3K27me3) and H3K9 trimethylation (H3K9me3) and increased H3K4 trimethylation (H3K4me3), among a few others, can be regarded as age-associated epigenetic marks (Booth and Brunet, 2016). In *C. elegans*, genetic manipulations of different H3K27me3 demethylases can distinctly affect the insulin/insulin-like signaling (IIS) pathway and the heat shock response with opposite effects on lifespan (Jin et al., 2011; Labbadia and Morimoto, 2015; Maures et al., 2011). Knockdown of both H3K4me3 methyltransferases and demethylases affects the IIS pathway and fat metabolism and were reported to increase lifespan in worms (Greer et al., 2010; Han et al., 2017; Ni et al., 2012). H3K27me3 and H3K4me3 profiles have also been shown to alter when cells become senescent (Shah et al., 2013). Loss of heterochromatin is a hallmark of an aged epigenome: levels of the heterochromatin markers HP1 and H3K9me3 decline with age (Ni et al., 2012; Wood et al., 2010) and are reduced in HGPS cells (Shumaker et al., 2006). In *C. elegans,* H3K4 dimethylation (H3K4me2) is deposited after NER-mediated repair of transcription-blocking DNA lesions along open reading frames of genes regulating protein biosynthesis and homeostasis (Wang et al., 2020). Although failure of their deposition impaired developmental growth and shortened lifespan, elevated H3K4me2 deposition supported development and extended lifespan amid UV-induced DNA damage. These observations demonstrate how DNA repair can trigger epigenetic alterations that impact proteostasis and consequently affect longevity.

Members of the sirtuin protein family of NAD-dependent protein deacetylases have also been linked to aging. *Saccharomyces cerevisiae* Sir2 was shown to repress the formation of extrachromosomal ribosomal DNA circles that are driving yeast aging (Kaeberlein et al., 1999; Sinclair and Guarente, 1997). SIRT6, an H3K9 deacetylase, is involved in genome stability and NF-κB signaling (Kawahara et al., 2009; Mostoslavsky et al., 2006; Onn et al., 2020). SIRT6 deficiency increases levels of DNA damage, causes neurodegenerative changes, and reduces lifespan in mice (Kaluski et al., 2017; Mostoslavsky et al., 2006), whereas SIRT6-overexpressing mice display reduced IIS signaling and increased lifespan (Kanfi et al., 2012).

Differential methylation marks gradually accumulate (Bell et al., 2012; Bollati et al., 2009; Fraga et al., 2005; Hernando-Herraez et al., 2019), and the DNA methylation landscape can be used as an epigenetic clock for chronological age (Horvath and Raj, 2018). DNA methylation is a regulatory mark usually associated with transcriptional repression and, therefore, dysregulation of DNA methylation patterns during aging could have gradual effects on gene expression (Hernando-Herraez et al., 2019; Michalak et al., 2019).

The aforementioned changes in transcription factor binding and activity, histone marks, chromatin state and structure, and DNA methylation patterns likely contribute to the transcriptional drift observed during aging (Bryois et al., 2017; Hernando-Herraez et al., 2019; Lai et al., 2019; Maures et al., 2011; Rangaraju et al., 2015; Stegeman and Weake, 2017). Suppression of this age-associated transcriptional drift extended the lifespan in *C. elegans* (Rangaraju et al., 2015). Gene expression hallmarks of cellular aging have also been proposed (Frenk and Houseley, 2018), opening the door for transcriptomic clocks of aging to classify chronological and biological age (Meyer and Schumacher, 2020[1]).
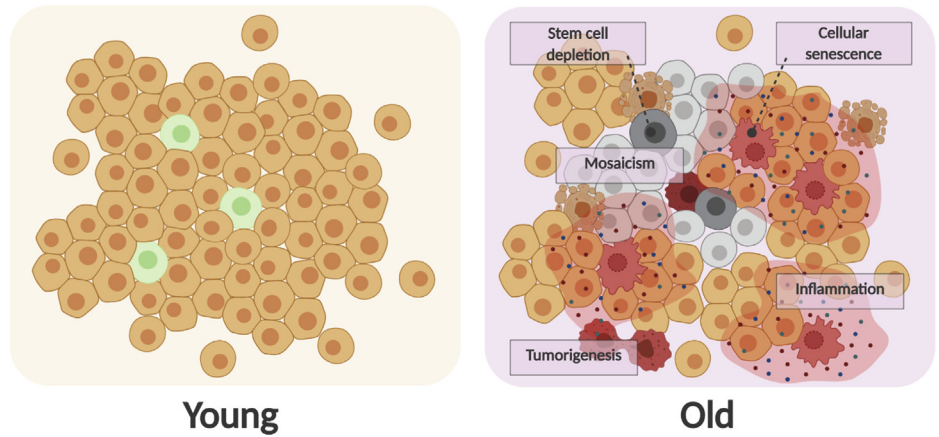
Mitochondrial dysfunction. The accumulation of molecular damage in the cell can lead to organelle dysfunction including mitochondria, which have been associated with multiple age-associated pathologies (Frazier et al., 2019; Koopman et al., 2012). Age-dependent mitochondrial dysfunction stems from many sources, including accumulation of mtDNA mutations, dysfunction of mitochondrial proteins, structural alterations in mitochondrial membranes, imbalance between fission and fusion events, and defective clearance of damaged mitochondria by mitophagy (López-Otín et al., 2013). Mitochondrial dysfunction was suggested to be a consequence of nuclear DNA repair deficiencies (Chang et al., 2015; Hussain et al., 2021) and might account for some of the degenerative pathologies such as neurodegeneration, for example, in CS (Fang et al., 2016; Lopes et al., 2020).

The efficacy of the respiratory chain decreases, resulting in increased electron leakage, reduced ATP generation, and increased ROS production (López-Otín et al., 2013), and thus, it is not surprising to observe that impaired mitochondrial function results in disruption of cellular homeostasis and has consequences in terms of cell function. Mitochondrial dysfunction is a known driver of cell senescence and stem cell exhaustion (Correia-Melo et al., 2016; Fujimaki and Kuwabara, 2017; Korolchuk et al., 2017). Conversely, improving mitochondrial function via NAD$^+$ repletion

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[1] Meyer DH, Schumacher B. A transcriptome based aging clock near the theoretical limit of accuracy. bioRxiv 2020.

Figure 2. Alterations in cell function and fate drive tissue dysfunction.
Accumulation of molecular damage drives an age-dependent increase in cell-to-cell variability and introduces cellular heterogeneity within tissues. Over time, the pools of stem cells within tissues (left panel, green) become depleted and numbers of senescent cells increase (right panel, red). Senescent cells, via the SASP (right panel, small dots), establish a proinflammatory microenvironment (right panel, red areas) affecting neighboring cells. Accumulation of genomic mutations can cause cells to become tumorigenic (right panel, dark red cells) and explains the age-dependent increase in clonal mosaicism of somatic cells within tissues (right panel, gray). SASP, senescence-associated secretory phenotype.

Young | Old

promotes stem cell function and increases lifespan (Zhang et al., 2016).

Alterations in cell function and fate
Because cells are individually affected by damaging insults and epigenetic modifications, cell-to-cell variability on an epigenomic and transcriptomic level increases with age (Bahar et al., 2006; Cheung et al., 2018; Enge et al., 2017; Nikopoulou et al., 2019). These stochastic changes in gene expression might be responsible for the progressive age-dependent cellular heterogeneity and the clonal mosaicism of somatic cells and decline in organ function (Figure 2) (Machiela and Chanock, 2017; Milholland et al., 2017). Age-dependent accumulation of somatic mutations and clonal expansion have been detected in blood cells and in other tissues such as the skin and the esophageal epithelium (Colom et al., 2020; Genovese et al., 2014; Jaiswal et al., 2014; Martincorena et al., 2018, 2015; Xie et al., 2014; Yokoyama et al., 2019). The pattern of mutations in skin from aged donors closely resembles what is expected for UV exposure and is observed in skin cancers (Martincorena et al., 2015). In the esophagus, mutation rates are lower than in skin; nevertheless, clonal expansion, particularly those carrying mutations in cancer-associated genes, is accelerated by alcohol consumption and/or smoking (Martincorena et al., 2018; Yokoyama et al., 2019). This time-dependent accumulation of somatic mutations might be directly linked to cell fate alterations such as the age-associated increase in senescent cells and loss of stem cells.

Senescence and inflammation. Cellular senescence describes a state of irreversible cell-cycle arrest, with cells displaying resistance to apoptosis, morphological abnormalities, changes in gene expression, and a complex senescence-associated secretory phenotype (SASP) (Campisi and D'Adda Di Fagagna, 2007; Coppé et al., 2008; Fitsiou et al., 2021). Cellular senescence can be triggered by a persistent DDR, for example, induced by critically short telomeres (d'Adda di Fagagna, 2008), oncogene activation, or stressing agents

(Campisi and D'Adda Di Fagagna, 2007; Serrano et al., 1997; Toussaint et al., 2000); epigenomic changes; and mitochondrial dysfunction (Correia-Melo et al., 2016; Shah et al., 2013; Wiley et al., 2016).

Senescent cells have been identified mainly based on their irreversible cell-cycle arrest and consequent overexpression of the p21 (CDKN1a/CIP1) and p16 (CDKN2a/INK4) cyclin-dependent kinase inhibitors (Campisi and D'Adda Di Fagagna, 2007; Liu et al., 2019, 2009); the presence of senescence-associated β-galactosidase activity (Dimri et al., 1995); and, more recently, loss and/or redistribution of Lamin B1 (Freund et al., 2012), accumulation of lipofuscin (Evangelou et al., 2017; Georgakopoulou et al., 2013), telomere-associated DNA-damage foci (Hewitt et al., 2012), senescence-associated heterochromatin foci (Narita et al., 2003), distinct genome-wide methylation profiles (Lowe et al., 2015), and senescence-associated mitochondrial dysfunction (Korolchuk et al., 2017). Several of these features have been reported in multiple types of postmitotic cells, including neurons (Jurk et al., 2012), glial cells (Bussian et al., 2018), osteocytes (Farr et al., 2016), myofibers (da Silva et al., 2019), and cardiomyocytes (Anderson et al., 2019).

Senescent cells accumulate in multiple aged and diseased tissues (Bussian et al., 2018; Jeyapalan et al., 2007; Muñoz-Espín and Serrano, 2014; Ogrodnik et al., 2017; Schafer et al., 2017; Wang et al., 2009; Xu et al., 2017); however, the accumulation rates appear to be tissue-dependent, with tissues such as skin, spleen, liver, and testis showing accelerated age-associated accumulation of senescence-associated DNA-damage foci (Jeyapalan et al., 2007; Wang et al., 2009). The causal role of senescent cells in age-associated phenotypes and pathology in vivo has been demonstrated by studies using transgenic mouse models in which senescent cells were selectively eliminated, for example, by genetic clearance of p16-positive cells in progeroid BubR1 mutant mice (Baker et al., 2011). In addition, clearance of naturally occurring p16-positive cells in a non-progeroid genetic background increased both the healthspan

and lifespan of the animals (Baker et al., 2016). Removal of senescent cells with senolytic drug treatments has been shown to improve tissue function in animal models of multiple age-associated pathologies (Jeon et al., 2017; Ogrodnik et al., 2019, 2017; Paez-Ribes et al., 2019; Palmer et al., 2019; Roos et al., 2016; Schafer et al., 2017; Xu et al., 2018).

One mechanism through which senescent cells contribute to tissue dysfunction is the SASP, composed of an array of proinflammatory cytokines, chemokines, growth factors, and matrix-remodeling enzymes capable of altering their microenvironment (Coppé et al., 2010). The SASP, as well as ROS signaling, additionally induces senescence in adjacent bystander cells (Nelson et al., 2018, 2012). This, coupled with slower removal rates (Karin et al., 2019), helps explain the reported age-associated build-up of senescent cells in vivo and increase in low-grade systemic inflammation (inflammaging) (Franceschi et al., 2000).

Stem cell depletion. Age-related tissue dysfunction has been associated with a decline in stem cell number and function and consequent loss of regenerative potential (Ermolaeva et al., 2018; Ren et al., 2017). In particular, accumulation of DNA damage, telomere shortening, loss of proteostasis, epigenetic modifications, and mitochondrial dysfunction are known factors driving stem cell decline (Behrens et al., 2014; Flores et al., 2005; García-Prat et al., 2016; Oh et al., 2014; Rossi et al., 2007), whereas autophagy and dietary interventions have been shown to help maintain stemness (Cerletti et al., 2012; Ertl et al., 2008; García-Prat et al., 2016). Thus, stem cell depletion arises as consequence of multiple sources of damage and highlights how insults at the molecular and cellular levels translate into the tissue and organismal level (Ermolaeva et al., 2018).

The plasticity of aging

The hallmarks of aging are interconnected: different types of molecular damage affect organelles, modifying cell function and even cell fate, which, in turn, will affect tissue homeostasis and have consequences for the whole organism. Tissues are affected differently in the same individual and individuals are affected differently, suggesting personalized patterns that could only result from intricate combinations of effects from multiple sources generating specific systemic responses.

Nutrient-sensing pathways and intertissue communication. Under the molecular wear-and-tear paradigm, aging is an unavoidable trait: a fixed expiration date for each organism, set by the specific set of repair mechanisms with a limited capacity to counteract the natural abrasion of cells and tissues. Even though aging appears to be a nearly universal feature of life, the existence of certain species without observable time-dependent functional decline and correspondent decrease in fertility and increase in mortality (Buffenstein, 2008; Finch, 2009; Jones et al., 2014; Ruby et al., 2018) hints that aging is more than just the result of molecular wear-and-tear.

Roughly three decades have now passed since the isolation of *age-1* and *daf-2* mutants, the first long-lived *C. elegans* strains (Johnson, 1990; Kenyon et al., 1993), which proved that longevity and tissue function have some degree of plasticity and can be genetically manipulated. Similar genetic manipulations in flies (Tatar et al., 2001) and mammals have the same effects (Bartke, 2008), and mutations in *FOXO3* (the human *daf-16* ortholog) are associated with centenarians (Flachsbart et al., 2009; Willcox et al., 2008), supporting the idea that these mechanisms are conserved in humans. A complex network of multiple and interconnected longevity pathways, such as the IIS and target of rapamycin (TOR) pathways, regulates nutrient sensing and energy metabolism and mediates, at least in part, the health benefits of dietary restriction (DR) (Kapahi et al., 2010; Mair and Dillin, 2008).

Food availability is known, for many decades now, to influence the aging process (McCay et al., 1939). DR has proved to increase both lifespan and healthspan in multiple animal models (Fontana and Partridge, 2015; Kapahi et al., 2017; Mair and Dillin, 2008; Mattison et al., 2017) and might promote health in humans as well (Belsky et al., 2017). DR might promote somatic maintenance by reducing DNA damage (Vermeij et al., 2016; Wang et al., 2018), promoting autophagy (Aris et al., 2013) and mitochondrial biogenesis (Civitarese et al., 2007), and reducing the SASP of senescent cells (Wang et al., 2018). TOR is a conserved nutrient sensor whose function is to integrate environmental signals and coordinate multiple output responses, such as transcription and translation, autophagy, and mitochondrial function (Kapahi et al., 2010). Mutations in the TOR pathway have been shown to mimic the effects of DR (Kapahi et al., 2004), and therefore, TOR signaling appears to play important roles in life- and healthspan regulation.

Acting through DAF-16, the *C. elegans* insulin-like growth factor receptor DAF-2 coordinates stress response networks by regulating the expression of genes involved in proteostasis, immunity, metabolism, and, importantly, neuronal function (Edifizi et al., 2017; Lee et al., 2003; Lin et al., 2018; Webb et al., 2016). DAF-16 responds to DNA damage to coordinate responses at the organismal level (Bianco and Schumacher, 2018; Edifizi et al., 2017; Mueller et al., 2014) and the capacity of FOXO3 to bind to DNA depends on the pre-existing chromatin context (Eijkelenboom et al., 2013). The consequences of such DDRs can be systemic, as demonstrated by the consequences of cell type–specific DNA damage in worms and flies (Ermolaeva et al., 2013; Karpac et al., 2011). In *C. elegans*, DNA damage in the germline triggers a systemic innate immune response to induce somatic stress resistance (Ermolaeva et al., 2013). In *D. melanogaster*, epidermal UV-induced DNA damage triggers an immune response that leads to IIS repression to limit said immune response and promote survival (Karpac et al., 2011). NER mouse mutants also display attenuation of IIS activity (Niedernhofer et al., 2006; van der Pluijm et al., 2007; Schumacher et al., 2008), thus linking DNA damage accumulation with longevity pathways that elevate stress resistance amid increasing molecular damage (Garinis et al., 2009).

Neuronal signaling plays a crucial role in regulating stress responses. In mice, the hypothalamus is important for whole-body aging: age-associated inflammation in the hypothalamus, with consequent reduction in gonadotropin-releasing hormone and decrease in hypothalamic stem cells, was shown to accelerate aging and distal tissue dysfunction (Zhang et al., 2017, 2013). In *C. elegans*, several studies have

reported an important role of the neuronal system and glial cells in the regulation of proteostasis and mitochondrial function in distal tissues and organismal lifespan (Alcedo and Kenyon, 2004; Berendzen et al., 2016; Burkewitz et al., 2015; Durieux et al., 2011; Frakes et al., 2020; O'Brien et al., 2018; Taylor and Dillin, 2013; Zhang et al., 2019b, 2018).

Concluding remarks

The past decades since the unraveling of the first genetic mechanisms of aging have provided ample insight into the causal role of molecular damage in the aging process and into response mechanisms that not only repair the damage but impinge on multiple regulatory mechanisms that maintain cellular and tissue homeostasis. Only recently, systemic response mechanisms to the accumulation of molecular damage have started to appear and allow the assessment of biological age and multimorbidity while providing new therapeutic targets for slowing aging and thus reducing the risk for age-related diseases. The aging process and the mechanisms that govern longevity are complex, and each one of the distinct aspects of aging is being pursued in the development of therapeutic strategies that aim at health maintenance. It will be pivotal to explore how such interventions affect the multimodal interactions between the aging mechanisms. The influx of scientists from distinct fields into the field of aging is a good prerequisite for tackling the many outstanding questions on mechanisms, their interrelations, and the most effective intervention strategies.

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CONFLICT OF INTEREST
The authors state no conflict of interest.

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