

Invited Mini Review

Multi-level remodeling of transcriptional landscapes in aging and longevity

Rochelle W. Lai^{1,#}, Ryan Lu^{1,#}, Prakroothi S. Danthi¹, Juan I. Bravo^{1,2}, Alexandre Goumba¹, Nirmal Kumar Sampathkumar¹ & Bérénice A. Benayoun^{1,3,4,*}

¹Leonard Davis School of Gerontology, University of Southern California, Los Angeles, CA 90089, ²Graduate program in the Biology of Aging, University of Southern California, Los Angeles, CA 90089, ³USC Norris Comprehensive Cancer Center, Epigenetics and Gene Regulation, Los Angeles, CA 90089, ⁴USC Stem Cell Initiative, Los Angeles, CA 90089, USA

In multi-cellular organisms, the control of gene expression is key not only for development, but also for adult cellular homeostasis, and gene expression has been observed to be deregulated with aging. In this review, we discuss the current knowledge on the transcriptional alterations that have been described to occur with age in metazoans. First, we discuss age-related transcriptional changes in protein-coding genes, the expected functional impact of such changes, and how known pro-longevity interventions impact these changes. Second, we discuss the changes and impact of emerging aspects of transcription in aging, including age-related changes in splicing, lncRNAs and circRNAs. Third, we discuss the changes and potential impact of transcription of transposable elements with aging. Fourth, we highlight small ncRNAs and their potential impact on the regulation of aging phenotypes. Understanding the aging transcriptome will be key to identify important regulatory targets, and ultimately slow-down or reverse aging and extend healthy lifespan in humans. [BMB Reports 2019; 52(1): 86-108]

INTRODUCTION

In multi-cellular organisms, the control of gene expression is key not only for development, but also for adult cellular homeostasis, and deregulation of gene expression correlates with aging (1-4). Aging is the main risk factor for many chronic diseases, such as neurodegeneration, cardiovascular disease, type II diabetes, osteoporosis, and cancer. Several conserved and interconnected pathways are deregulated during aging, delineating “hallmarks” or “pillars” of aging (5, 6). However,

*Corresponding author. Tel: +1-213-821-5997; E-mail: berenice.benayoun@usc.edu

[#]These authors contributed equally to this work.

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how these hallmarks are achieved throughout the life of an organism is still poorly understood.

In the laboratory, several models are used to study aging: (i) chronological aging (*i.e.* the time that passed in the organism's life), which most closely corresponds to the most intuitive definition of aging, (ii) replicative aging (*i.e.* the number of times a cell can divide; most used to study aging in yeast) (7), (iii) cellular senescence, which corresponds to the replicative limit of cells before they enter irreversible cell cycle arrest, also dubbed the ‘Hayflick limit’ (8), and (iv) progeria or premature aging syndromes (*i.e.* Hutchinson-Gilford or Werner Syndrome) (9). Although aging is a common feature of many taxa, the rate of aging differs between species, and accumulating evidence suggests that the process can be fine-tuned within a species based on environmental cues, inter-individual genetic variations, and even sex (10). In this review, we will focus primarily on transcriptional landscape alterations in chronological aging and pro-longevity models.

REMODELING OF THE PROTEIN CODING TRANSCRIPTIONAL LANDSCAPE WITH AGING AND IN LONGEVITY MODELS

In this review, we chose to compare age-related and longevity-related gene expression changes in different vertebrate species (*e.g.* mouse, human, rat, African turquoise killifish, *etc.*). Although this enabled us to draw more general comparisons, we understand that each species may have specific aging features, which limits our ability to draw a more general conclusion. Another general limitation in the field of aging genomics is the ill-defined age criteria for young and old animals that are profiled. For example, the age of “young” mice used in discussed studies ranges from age 1 to 4 months, while “old” mice range from age 18 to 30 months (Table 1). The lack of consensus age criteria makes reaching definitive conclusions on the effect of aging more difficult. Future studies should strive to use stricter age criteria for the study of aging, as well as include more time points along the life of animals to identify transcriptional trajectories with aging.

Table 1. Aging signatures in vertebrate tissues

Tissue	Upregulated pathways	Downregulated pathways	Species	Profiling method	Reference
Liver	Fibrosis Immune Response Inflammation Lipid Metabolism Protein Turnover Stress Response	Cell Cycle DNA Replication Drug Catabolic Processes Metabolic Pathways Oxidative Demethylation Protein Synthesis RNA Transport	Mouse	Microarray	(33, 241)
				RNA-seq	(29, 242, 243)
			Killifish	RNA-seq	(244)
			Rat	RNA-seq	(238)
Heart	Caspase Family Chemokines Immune Response Lysosome s100 Family	Electron Transport Chain Mitochondrion TCA Cycle Ubiquitin-dependent Catabolic Processes	Human	RNA-seq	(26)
			Mouse	Microarray	(245)
Pancreatic Islets	Extracellular Matrix Global Methylation Immune Response Inflammatory Response Oxidative Stress Wound Healing	Cell-cell Signaling Metabolic Pathways Neuron Recognition Nutrient Sensing Proliferation	Rat	Microarray	(25)
			Mouse	WGBS	(246)
				scRNA-seq	(247)
			Human	RNA-seq ChIP-seq	(49)
				scRNA-seq	(50)
Brain	<i>Cerebellum</i> Complement System Inflammatory Response Stress Response	Energy Metabolism Growth/Trophic Factors Inflammatory Response Protein Synthesis	Mouse	Microarray	(24)
Brain	<i>Whole Brain</i> Apoptosis Complement System Immune Response Inflammation Lysosome Ribosome Stress Response	Collagen Metabolic Pathways Mitochondrion Protein Synthesis Ribosomal Pathways RNA Transport Signal Transduction	Killifish	RNA-seq	(248, 244)
			Rat	RNA-seq	(238)
Kidney	Extracellular Matrix Immune Response Ribosome	Stress Response	Human	Microarray	(28)

Gene expression remodeling across cells and tissues in vertebrates

Transcriptional remodeling aging signatures across tissues: We summarized reported age-related transcriptional changes across key tissues that are known to undergo age-related functional decline (*i.e.* liver, heart, pancreatic islets, brain, kidney) (Table 1). For simplicity and exhaustiveness' sake, both changes in gene expression from microarrays and RNA-sequencing datasets are reported. As illustrated in Table 1, three common aging transcriptional signatures have been recurrently identified across different tissues and species. A prominent aging signature that affects all categories of tissues is increased expression of inflammatory pathways. More broadly, Gene Ontology (GO) categories of, 'immune response', 'stress response', 'chemokines', 'lysosome', 's100 family', 'caspase family', 'wound healing', 'fibrosis', and 'complement system' encompass genes that if left unattenuated, propagate an environment of oxidative stress

and vascular damage (Table 1) (11-16). Importantly, chronic activation of the immune system is the defining characteristic of 'inflamm-aging' (*i.e.* a low-level, sterile, chronic state of inflammation with age), and the global increase of pro-inflammatory gene expression may thus be a driving factor in the development of age-related diseases (17-19). For example, the inappropriate activation of immune cells can exacerbate cancer progression (20), pathogenesis of type II diabetes (21), and cardiovascular disease susceptibility (22, 23).

Another notable general aging signature is an overall decrease in expression of metabolic genes (Table 1). Disrupted metabolic pathways in the mitochondrion (*i.e.* TCA cycle, electron transport chain), protein synthesis (*i.e.* cysteinyl-tRNA-synthase, phenylalanine-tRNA-synthase), and nutrient sensing (*i.e.* *Cartpt*, *Npy*, *Ppy*) were observed in the liver, heart, pancreatic islets, cerebellum, and whole brain tissues (24-26). The reduced efficiency and ability to process nutrients may limit nutrient availability and decreased xenobiotic

metabolism may increase the event of drug toxicity in aging animals. Such a combination of metabolic decline may lay the foundation for the development of age-related diseases, such as cancer or Alzheimer's disease (27).

A common signature of decreased membrane integrity also seems to be a common theme in the liver, pancreatic islets, and kidney through the increase of 'fibrosis', 'extracellular matrix', and 'wound healing' gene expression (Table 1) (25, 28, 29). The extracellular matrix is a lattice of polysaccharides and proteins that stem from the cell membrane, which provides structural support and scaffolding for physical communication between cells (30). In fibrosis and wound healing, the extracellular matrix expands and remodels to build upon or reestablish the cell barrier (30). However, an overgrowth of the extracellular matrix can hinder signaling between cells. This phenomenon is especially relevant for the liver, pancreatic islets, and kidney, as cells in these tissues heavily rely on membrane accessibility for metabolism, hormone release, and filtration. In the aging liver, the fibrotic extracellular matrix may contribute to the susceptibility to age-related diseases, since liver diseases involving inflammation, such as nonalcoholic fatty liver disease, are worsened with fibrosis (31). Concerning the pancreas, type II diabetes is a great model to understand the consequences of fibrotic islets, as fibrosis has been found to promote beta cell destruction, ultimately compromising the delivery of insulin (32). In the aging kidney, obstruction to the basement membrane may partially explain the age-related decline in glomerular filtration rate (28). Thus, the aging-associated loss in membrane integrity, predicted by the observed transcriptional remodeling, should be ultimately detrimental to the ability of cells to properly interact with the rest of the organism, and

lead to functional decline.

Tissue-specific transcriptional remodeling with aging: Although common dysregulated pathways can be identified across tissues, many aging transcriptional defects seem to be tissue-specific (Table 1). For example, the aging liver presents a characteristic signature of decreased oxidative demethylation gene expression (33) (Table 1), a process that reverses the effects of regulatory or mutagenic methylation by removing methyl adducts from DNA and RNA (34). Deterioration of this ability to regulate and repair the methylome can induce improper gene expression. The pancreatic islets also reveal a unique aging signature of decreased 'cell-cell signaling', 'nutrient sensing', and 'neuronal recognition' gene expression (25). The decreased capacity to perform these functions creates juxtaposing outcomes. On one hand, decreased expression of these genes can create issues because the pancreatic islets depend on nearby cell communication, glucose sensing, and vagal nerve stimulation to assess energy status (35). Inaccurate sensing and transduction of energy status can harm the aging metabolism by disrupting the timing of pancreatic islet hormone release (35). Contrary to this, Riera *et al.* demonstrates an advantage with the decline of cell communication. Through the ablation of TRPV1 receptors and inhibition of calcitonin gene-related peptide, an insulin secretion blocker, the aging pancreas can improve its insulin release, yielding a more youthful metabolic profile (36). In light of these conflicting responses, pancreatic islet cell communication genes are evidently pleiotropic and are perhaps yet to be fully understood.

Table 2. Aging signatures in adult mouse stem cells

Stem Cell	Upregulated pathways	Downregulated pathways	Profiling method	Reference
Neural (SVZ)	Cell Adhesion Cell-cell Signaling Cell Morphogenesis Nerve Impulse Transmission Neuron Differentiation	Angiogenesis Blood Vessel Development Blood Vessel Morphogenesis Development Lysosomes Regulation of Cell Motion Response to Growth Factors Vasculature	RNA-seq	(38, 39)
Muscle (Satellite Cells)	Apoptosis Regulation Immune Response Oxidation Reductin Vascular Development	Histone Genes	Microarray	(41, 249)
Hematopoietic	Cell Adhesion Cell Proliferation Inflammation Protein Aggregation Repetitive Elements Ribosome Stress Response	Cell Cycle Epigenetic Regulation Genomic Integrity	Microarray RNA-seq scRNA-seq	(42) (250) (44)

Age-related transcriptional remodeling: what about stem cells?: A hallmark of aging is the depletion and loss of function in adult stem cell pools (6). The ability of stem cells to preserve their quiescent state is central to maintaining a long-term functional stem cell pool for cell regeneration and tissue homeostasis. However, with aging, stem cell proliferation and the consequent rate of cell turnover have been observed to decline (37). The adult somatic stem cells that have been best studied at the genomic level during aging are: neural stem cells (NSCs), muscle stem cells or “satellite” cells (MuSCs), and hematopoietic stem cells (HSCs) (38-44) (Table 2). Interestingly, both HSCs and MuSCs display an upregulation of immune-related genes. This signature is akin to the one observed in bulk aging tissues (Table 1). Interestingly, a pro-inflammatory state resulting from an inappropriate immune response can both diminish and protect the viability and differentiation potential of the stem cell pool (45). This is also observed in NSCs and may be a product of an inflammatory microenvironment in the brain. In addition, aging HSCs and MuSCs share a transcriptional signature of decreased genome stability. For example, the decline in aging quiescent MuSCs may be explained by the silencing of genes through an increase in H3K27me3 (40). A decrease in epigenetic regulators and cell cycle genes was also observed with age in HSCs (43, 44), which is accompanied by a loss in self-renewal and differentiation capability (43, 44). Aging NSCs and HSCs share an increased expression of genes involved in cell adhesion (39, 43), which may have consequences on migration capacity and survival. Specific to NSCs, decreased lysosomal gene expression was observed, which may explain a reduced ability of aged NSCs to transition out of the quiescent state and into the active stem cell pool (38).

Lessons from bulk vs. single cell transcriptome profiling: We have discussed both bulk microarray and RNA-sequencing (RNA-seq) datasets, as well as published emerging single-cell RNA sequencing (scRNA-seq) (Table 1, 2). Thus far, a major confounding factor of aging ‘omics’ studies (*i.e.* genome-wide analyses such as transcriptomics and epigenomics) lies in the heterogeneous cellular make-up of mammalian tissues, which makes it difficult to disentangle the significance of transcriptomic changes (*i.e.* resulting from changes in the underlying cellular makeup, or from cell type-autonomous changes). Computational deconvolution algorithms can be used to evaluate the relative contribution of cell type composition changes in transcriptomes of heterogeneous cell population (*i.e.* as is generally the case for tissues) (46), although they require reference purified transcriptional profiles of the cell types present in the mixture. In contrast, cell sorting may be used to analyze the fate of specific cell types. However, sorting relies on the existence of well-established cellular markers (which may not always be readily available for all cell types of interest or in emerging model organisms), and expression of such

markers may itself be regulated with age, thus complicating data interpretation. Despite these limitations, bulk level RNA-seq is the more mature technology and enables the study of various aspects of transcription (*e.g.* strand-specificity, splicing isoforms, circRNAs, non-polyadenylated RNAs, etc.).

In contrast to bulk methods, advances in single-cell profiling techniques now allow direct efficient and high-resolution transcriptional mapping of the transcriptomes of thousands of cells from heterogeneous samples by harnessing the power of microfluidics (*e.g.* Drop-seq, 10xGenomics Chromium, etc.) (47). These advances have enabled profiling at an unprecedented resolution in term of cell-to-cell variations and cell type identification (48). However, few studies have harnessed these single cell technologies in comparative aging transcriptomics studies yet. In a pioneering study of the aging pancreas at the single cell level, a transcriptional signature of loss of cell identity captured in scRNA-seq could not be detected in previous bulk RNA-seq studies (49, 50). However, it is important to note that most single-cell profiling technologies do not provide information on transcription beyond gene expression level, and they cannot robustly detect lowly expressed genes. Despite these shortcomings, scRNA-seq provides important insights for understanding the heterogeneity of gene expression within a cell population, or heterogeneity in underlying cell types within a mixed population. Both bulk RNA-seq and scRNA-seq still share the limitations of amplification bias and poor cDNA synthesis efficiency (51).

Another important point to note is that current studies use either ribosomal RNA depletion (ribodepletion) or poly(A) selection to enrich for RNA species of interest prior to library construction and subsequent sequencing. Each method is likely to result in differences in both the amount, representation and quality of the profiled RNA. With ribodepletion, ribosomal-RNA (rRNA) is specifically depleted from an RNA sample so other RNA species (*e.g.* pre-mRNA and mature mRNA, circRNAs, lncRNAs, etc.) remain for downstream profiling. In contrast, poly(A) enrichment specifically isolates RNA species with (long) polyadenylated tails, a characteristic specific only to mature mRNAs. Thus, specific changes reported by various studies may be affected by the experimenter’s choices in target RNA selection. It will be important to determine the impact of RNA selection on observed signatures of the aging transcriptome.

Impact of longevity interventions on the aging transcriptome

Analysis of known longevity-extending dietary, genetic, and drug interventions suggest that key pathways that decline transcriptionally with aging may be rescued at least partly by these interventions (52, 53) (Table 3). In this review, we focus on the transcriptional effects on common aging signatures of four well-characterized pro-longevity interventions: dietary restriction, *Ames* dwarfism, rapamycin supplementation, and metformin supplementation.

Dietary restriction (DR) refers to the limitation of either (i)

Table 3. Effects of longevity-promoting interventions on aging signatures

Intervention	Tissue	Effect on aging signatures	Species	Profiling method	Reference	
Dietary interventions						
Dietary Restriction	Liver	<i>Tissue</i>	Mouse	RNA-seq	(251)	
		Decreases cell differentiation and maturation		RNA-seq	(53)	
		Decreases protein metabolism		ChIP-seq		
		Decreases transcription		WGBS	(52)	
		Prevents age-associated methylation changes				
	Rat	<i>Tissue</i>	Western Blot	(252)		
		Site specific acetylation at H3K9, K27, and K56				
	Mouse	<i>Cell (Hepatoma model)</i>	RNA-seq	(253)		
		Alters SIRT gene expression				
		Modifies accessibility of SIRT regulator region				
	Brain	Hippocampus	<i>Tissue</i>	Mouse	RNA-seq	(254)
			Alters calcium signaling			
		Tissue	Alters axonal guidance signaling	Mouse	RNA-seq	(255)
			Alters corticotropin-releasing hormone signaling			
Alters synaptic long-term potentiation						
Alters neuronal CREB signaling						
Alters G-protein coupled receptor signaling						
Decreases senescence-associated secretory phenotype						
Decreases energy regulation						
Decreases inflammation						
Decreases phagocytosis						
Prevents age-related methylation changes						
Whole Brain			Western Blot			
Adipose	<i>Tissue</i>	Mouse	Microarray	(257)		
	Attenuates age-associated reduction in histone methylation					
	Decreases inflammation				Western Blot	(258)
	Decreases necroptosis					
	Increases stem cell maintenance					
Increases vascularization	RNA-seq					
Genetic						
Ames Dwarfism	Liver	<i>Tissue</i>	Mouse	RNA-seq	(53)	
		Alters methionine metabolism		ChIP-seq		
		Attenuates age-associated hypermethylation		WGBS		
		Decreases glycine-N-methyltransferase		Microarray		(68)
		Increases glutathione		Western Blot		(66)
		Increases mRNA expression of DNMT1 and DNMT3a				
		Increases urea cycle proteins				
		Prevents age-associated miRNA expression changes				
	Serum	<i>Cell</i>	Mouse	RNA-seq	(67)	
		Modulates insulin signaling				
		Modulates MAPK signaling				
		Modulates mTOR signaling				
		Modulates Wnt signaling				
Prevents age-associated miRNA expression changes						
Drug						
Rapamycin	Liver	<i>Tissue</i>	Mouse	WGBS	(52)	
		Prevents age-associated methylation changes		RNA-seq	(53)	
	Brain	<i>Tissue</i>	Mouse	Western Blot	(256)	
		Attenuates age-associated histone modifications				
Metformin	Liver	<i>Tissue</i>	Mouse	Microarray	(259)	
	Muscle	Attenuates expression of NF-kB				
		Increases anti-inflammatory genes				
		Improves stress response				
Increases antioxidant response						

the total caloric intake or (ii) specific nutrients, in each case without malnutrition (54). Typically, caloric restriction (CR) is achieved by a 25 to 60% reduction of calories from *ad libitum* controls in model organisms (54, 55). By altering nutrient sensing pathways, DR has been proposed to modulate downstream gene expression to extend longevity (56). CR-specific modulations may partly rescue transcriptional aging through upregulation of DNA methyltransferase activity, histone methylation, and histone deacetylation via HDAC1 and SIRT1 (57). These transcriptional changes have been observed to affect the development of cancer, diabetes, cardiovascular diseases, neurodegenerative diseases, and immune deficiencies in rodents, nonhuman primates, and humans (57). In the case of specific nutrient restriction, limitation of dietary protein or specific amino acids (*i.e.* asparagine, glutamate, methionine, tryptophan) yield the most robust longevity extension across metazoans (55, 58-62). Particularly with methionine restriction, the decrease in methionine-derivatives, such as methyl donor S-adenosylmethionine, may modulate DNA methylation in a protective manner against age-related neoplastic disease (63, 64). Notably, key downstream pathways reversed by these interventions at the gene expression level include a decrease in inflammatory processes, and the prevention of age-associated epigenetic changes (Table 3). These observations are compatible with the notion that dietary restriction can rescue aspects of aberrant age-related transcriptional remodeling.

The *Ames* dwarf mouse is a well-established longevity model (65). Because of a single nucleotide mutation in the *Prop1* gene, *Ames* dwarf mice lack the transcription factor responsible for pituitary gland cell differentiation (65). Thus, *Ames* dwarf mice exhibit reduced levels of circulating growth hormone, prolactin, and thyroid-stimulating hormone (66). These altered hormone levels can lead to non-autonomous changes in the transcriptional profile, potentially promoting longevity through increased insulin sensitivity and reduced oxidative stress (65). Most notably, these changes include DNA methylation and microRNA regulation (53, 66-68). Analogous to the effect of dietary restriction, the *Ames* dwarf mouse also displays a more stable epigenome throughout life (52).

Rapamycin and metformin supplementation are two of the most widely studied pharmaceutical pro-longevity interventions (69). These two drugs are thought to increase animal longevity by acting as CR mimetics (70). Rapamycin is an inhibitor of the mammalian target of rapamycin (mTOR), a kinase that regulates cell growth in response to nutrients, growth factors, cellular energy, and stress (71). In a fed state, mTOR is activated to initiate protein synthesis, whereas mTOR inhibition with rapamycin mimics a fasting state (70). Halting protein synthesis arrests cell growth, which may explain why rapamycin has been shown to slow aging and neoplastic proliferation (72). At the transcriptional level, rapamycin-induced mTOR inhibition slows the aging methylome (52, 53).

Metformin is a prevalent anti-hyperglycemic drug that primarily works by uncoupling the electron transport chain, thereby mimicking a fasted/low-energy state and stimulating adenosine monophosphate-activated protein kinase (AMPK) (73). When activated, AMPK phosphorylates key nuclear proteins, thereby regulating metabolic gene expression at the transcriptional level to make energy more available through catabolism in response to the fasted state (74). To note, AMPK activation is just one of the molecular effects of metformin, and it is thought that it may also act through other not fully understood pathways as well (70). In essence, rapamycin and metformin seem to mimic aspects of DR at both the translational and transcriptional level.

Limitations of creating a translational therapeutic derived from these animal interventions include difficulty in diet accountability, ethics of gene editing, pharmaceutical toxicity, and potential side effects. However, understanding the transduction pathways of longevity promoting interventions in animals will be key to ultimately apply and translate these interventions to humans.

Transcriptional variability in aging and longevity

Accumulating evidence supports a model where the transcriptome becomes less tightly reregulated throughout the aging process. Indeed, a progressive degradation of transcriptional networks robustness and integrity has been observed during aging in *C. elegans* (75) and in mouse tissues (76, 77). There is still a debate on the prevalence of increased cell-to-cell transcriptional noise in aging cells. Pioneering studies examined the impact of aging on the cell-to-cell levels of expression of a handful of genes (78, 79). Whereas increased transcriptional noise was observed in aging mouse cardiomyocytes (11 out of 15 tested genes) (78), no changes in transcriptional noise were detected in hematopoietic stem cells isolated from old mice (6 assayed genes) (79). Importantly, existing technical limitations limited the reach of these studies to few genes and cell types, thus making generalizations difficult.

As discussed above, recent advances in microfluidics have enabled genome-wide single-cell profiling across diverse cell types at high-resolution (47), and will be key to understand the biological impact of transcriptional noise regulation with age. Indeed, two recent studies have leveraged single-cell RNA-seq to query potential changes in cell-to-cell transcriptional noise with aging. Enge *et al.* profiled cells from the human pancreas, revealing that islet endocrine cells from older donors presented increased transcriptional noise, as well as signs of cell identity drift (50). Modulation of cell-to-cell noise with aging may also be context-specific. Indeed, a recent study in mice found that cell-to-cell transcriptional noise was increased upon immune stimulation in naive and effector memory CD4+ T cells from two subspecies of aging mice (80), whereas the opposite was observed in young cells. Together, these emerging discoveries are consistent with the notion that

increased cell-to-cell transcriptional noise may be an important feature of mammalian aging.

REMODELING OF THE TRANSCRIPTIONAL SPLICING LANDSCAPE WITH AGING AND IN LONGEVITY

Alternative splicing in the regulation of aging and longevity

A large majority of metazoan genes are transcribed into precursor mRNAs (pre-mRNAs), which are constituted of exons separated by introns, and are thus known as “multi-exonic genes”. Intronic regions are usually spliced to yield a mature mRNA transcript through the action of a nuclear enzymatic ribonucleoprotein complex, known as the “spliceosome”, which recognizes specific target sequences found at exon/intron boundaries (81). It is generally thought that most introns are spliced out constitutively. However, many splicing signals can be used in a context-dependent manner, which can lead to the emergence of alternatively spliced isoforms (81).

Importantly, nearly all multi-exonic genes are subject to some degree of alternative splicing in one or more cell types (> 95% of multi-exon transcripts in human cells), which increases the coding potential and regulatory complexity of metazoan genomes (77, 82). This mode of regulation depends on regulation by the core splicing machinery, which relies on sequence signals in *cis* and *trans* to either promote or repress splicing at such alternative sites (81). The coupling between multiple regulatory layers and context-driven selection of relevant splice sites results from exquisite regulation by input signals at multiple levels. Recent research advances have revealed that core components of the constitutive and alternative splicing machinery (*i.e.* the spliceosome) are actually involved in fine-tuning of the splicing landscape (reviewed in (81)).

Though splicing control may be thought of as a core essential process, emerging evidence suggests that its modulation may have key impacts on health and longevity. Indeed, splicing deregulation has been associated with a number of diseases, including age-related diseases (*e.g.* progeria, Alzheimer’s, etc.) (83, 84). In addition, functional enrichment analyses on age-related human transcriptome remodeling have revealed significant changes in the expression of genes associated to genesets ‘mRNA binding’, ‘mRNA processing’, and ‘RNA splicing’ (85). Profound changes in the alternative splicing landscape with aging have been observed in mouse and human brain regions (86–88), with significant splicing deregulation in ~40% of genes expressed in the human brain (88). Interestingly, Mazin *et al.* found that aging was associated to increased exon retention in human prefrontal and cerebellar cortex, which may result in increased targeting of the affected transcripts to Nonsense Mediated Decay (NMD) (88). To note, there seems to be little correlation between genes affected at the level of transcription and those affected at the level of splicing in aging (86).

Age-related splicing defects are also largely detected in brains from patients with neurodegenerative disorders compared to healthy controls (84, 87), which is consistent with the observation that these diseases have increased prevalence with age. Changes in the alternative splicing landscape with mammalian brain aging have been associated with carbohydrate metabolism and DNA repair (87), or neuronal function (86). Importantly, a recent study using hepatic tissue from rhesus monkeys subjected to 30% CR showed that alternative splicing was central to the response to nutrient restriction and integral to the associated metabolic reprogramming (89).

Supporting the hypothesis that splicing dysfunction may directly impact the aging process, a recent study found that differences in splicing factor spleen expression and changes to alternative splicing were associated with strain lifespan across 6 genetically distinct mouse strains (90). This finding is consistent with the fact that expression levels of splicing regulators hnRNPA1 and A2 were positively associated to increased parental longevity in the InCHIANTI human aging study (90). Importantly, the rewiring of the splicing machinery and potential imbalance of splicing factors may have a direct impact on organismal aging and longevity. Indeed, a recent study has found that, in the worm *C. elegans*, Splicing Factor 1 (SFA-1) plays a key role in modulating longevity upon DR (91). Importantly, stable overexpression of SFA-1 led to increased worm longevity, whereas SFA-1 depletion impacted the activity of components of the TORC1 pathway that are normally subjected to alternative splicing (91).

Thus, accumulating evidence suggests that splicing alterations may be a hallmark of aging transcriptome. This progressive loss of the ability to fine-tune gene expression in aging cells is likely to have a profound effect on the physiology of aging cells. Alternative splicing, as regulated by splicing factors, may represent an important regulator or early marker of longevity, which deserves further systematic study.

Long non-coding RNAs in the regulation of aging and in longevity

Thousands of long noncoding RNA (lncRNA) transcripts have now been identified, most of which with yet unknown biological functions (92). Although these transcripts were initially thought to be non-coding (hence their name), accumulating evidence has shown that they in fact harbor a wealth of small open reading frames that can give rise to small functional peptides (93, 94). In addition, emerging evidence suggests that long non-coding RNAs (lncRNAs) may act as aging biomarkers, and perhaps even influence the aging process (95). Emerging studies have focused on exploring the roles of specific lncRNAs in aging of different cell types. Specifically, the *H19* lncRNA was recently demonstrated to be involved in vascular endothelial cell aging through inhibition of the Stat3 signaling pathway (96). In addition, control of *H19* levels is key to maintain adult HSC quiescence (97), thus

preventing a premature depletion of the stem cell pool. Moreover, the lncRNA *FLJ46906* may regulate the expression of various aging-associated genes (e.g. *TGFB1*, *CXCL8*, *IL1B*, *IL6*, and *ELN*) through modulation of the NF- κ B and AP-1 transcription factors in human fibroblasts (98). Thus, because of the abundance of lncRNAs in vertebrate genomes, it is likely that many of them may play important roles in adult tissue homeostasis and regulation of the aging process. Future studies are needed to shed light on lncRNAs as candidate regulators of the aging transcriptional landscape.

Circular RNAs: exciting new players of the aging transcriptome?

Circular RNAs (circRNAs), which are covalently closed circular RNAs without a free 5'- or 3'- end, were first identified in viruses by electron microscopy (99). A few years later, they were found in eukaryotic cells, although they were then dismissed as likely insignificant byproducts (100). With the recent emergence of ultra-high-throughput Next Generation Sequencing (NGS), further investigation into transcriptomic landscapes has become possible on an unprecedented scale. Indeed, deep RNA-seq in human cells revealed that circRNAs may even sometimes represent the major transcript isoforms of many genes (101). Indeed, circRNAs are abundant, and largely expressed in a tissue-specific manner (102, 103). Moreover, recent studies have identified circRNAs across tissues and species (e.g. human, mouse, *Drosophila*, *C. elegans*, Tree Shrew, etc.) (101, 104-106). Circular RNAs are thought to be generated through an original alternative splicing mechanism, known as "back-splicing", where the spliceosome covalently bonds the downstream 5' donor site of an exon to the upstream 3' acceptor site of another (upstream) exon (107), which may be a promoter by the proximity of transposable element sequences (102). Since the rediscovery of circRNAs in mammalian cells, the cellular roles of these RNAs have been under scrutiny. Studies have found evidence that circRNAs can act as 'microRNA sponges' (108), promoting microRNA-directed RNA degradation (109), trapping RNA-binding proteins (110), or even act as templates for protein translation through the presence of Internal Ribosome Entry Sites (IRES) (111-113). For example, one of the best-studied circRNA species to date is derived from antisense transcription from the *CDR1* locus, and known as *CDR1as*, which harbors ~70 conserved miR-7 target sites, and can function as a miRNA sponge (108). Because of the ability of some circRNAs to act as miRNA sponges, they are now widely thought to constitute a class of competing endogenous RNA (108).

This emerging class of RNAs has recently been gaining traction in the aging field, notably because many circRNAs have been observed to be differentially expressed in aging neural tissue across species (104, 106, 114, 115). In *Drosophila*, 262 circRNAs expressed in the Central Nervous Systems (CNS) were found to be upregulated between 1 and 20 days of life (104). Accumulation of circRNA in neural tissue

was also observed in mice (115), with ~5% of brain circRNAs significantly up-regulated with age whereas only ~1% were significantly downregulated with age (1 vs. 22 months) (115), although the young control of that study may have still been showing transcriptional signatures of a developing brain. Those results are consistent previous observations did in *Drosophila*, but, the young mice used can be considered as pre-developmental mice. All the changes in circRNA levels may be due to development and not aging at this age. Future studies would require young post-developmental mice for any comparisons. A recent study found that a global, age-associated, accumulation of circRNAs can also be observed in *C. elegans* (105). About 90% (1052/1166) of measured circRNAs were observed to be up-regulated at day 10 of life compared the L4 larval stage, whereas 37 circRNAs were observed to be significantly down-regulated (105). Cortes-Lopez et al. proposed that this accumulation of circRNAs may be the result of their increased stability and the fact that most of *C. elegans*' cells are post-mitotic cells (105). It is important to note that, so far, circRNAs have only been observed to accumulate in adult *C. elegans* and mammalian brains, both of which are enriched in post-mitotic cells. However, those studies do not include spike-in to control the analysis and to conclude accumulation of circRNAs with aging (116).

While functional studies of the roles of circRNAs are still lacking in the context of aging research, the downregulation of circRNA *circPVT1* was found to be critical for the establishment of cell senescence in human fibroblasts, through its interaction with microRNA let-7 (117). Although this is not strictly representative of organismal aging, senescent cells do accumulate over the course of lifespan (118), and clearance of these cells leads to improved health and lifespan (119). More generally, the functional impact of circRNA regulation on cells and organisms is still very much an open field of study. Importantly, emerging work suggest that they may be key for normal brain function, since the specific depletion of *Cdr1as* in mice was associated with neuropsychiatric dysfunction (120). The study of circRNA regulation and its impact on organismal physiology is still very much in its infancy, and additional gain and loss-of-function studies for circRNA genes will be needed in adult organisms to shed light on their potential role in health and lifespan.

TRANSPOSABLE ELEMENTS AND ENDOGENOUS RETROVIRUSES: THE SILENT KILLERS WITHIN THE EUKARYOTIC GENOME?

Genomic instability is characterized by an accumulation of somatic mutations, including *de novo* insertions of transposable elements (TEs) (121). TEs, which have also been dubbed "jumping genes," are a type of repetitive DNA that has the capacity to transpose from one genomic location to another (122). TEs constitute anywhere from 3% in *Saccharomyces cerevisiae* to 77%, in *Rana esculenta*, of eukaryotic genomes

Table 4. Impact of transposable elements in health and disease

Organism	Cell lines / Cell Types / Tissues	Transposon name	How were they measured?	Changes observed in Transposons	Phenotypes associated with the listed change	Reference
<i>Drosophila melanogaster</i>	Fat body	Multiple Retro transposons	qPCR, RNA- seq, Immunofluorescence microscopy	Increase in expression with age	Aging	(260)
<i>Drosophila melanogaster</i>	Heads	LINE-1, Gypsy	qPCR, Immunochemical staining	Increase in expression of TEs	Aging	(169)
<i>Mus musculus</i>	Tissue (C57BL/6) from Aged Rodent Tissue Bank & NIA Aged Rodent Colonies	LINE-1 & MusD	Nuclease Sensitivity, oligo-dT immuno-FISH staining, RNA-seq, qPCR	Increase in copy numbers of LINE-1 & MusD retrotransposons in liver and muscle	Increase in somatic retro-transposition with age	(261)
<i>Mus musculus</i>	ES cell culture, embryos, 2C-GFP ES cell line	LINE-1	RNA-FISH, RNA-seq, RT-qPCR, ChIP Assay, Immunofluorescence analysis, co-immunoprecipitation	LINE-1 inhibition	Inhibited embryonic stem cell self-renewal and impaired embryo development	(175)
<i>Homo sapiens</i>	Oocyte and embryo	HERVL retrotransposons	RNA-seq, Immunofluorescence Imaging	HERVL and MLT2A1 repeat elements are transiently expressed during the cleavage stage	DUX4 potentially activating the cleavage stage genes and repetitive elements	(262)
<i>Homo sapiens</i>	iPSC culture	HERVL retrotransposons	RNA-seq, Luciferase Assay, ChIP-Seq, RT-qPCR	HERVL and MLT2A1 repeat elements are transiently expressed during the conversion of cleavage stage	DUX4 potentially activating the cleavage stage genes and repetitive elements	(262)
<i>Mus musculus</i>	ES cell culture	MERV1 retrotransposons	RNA-seq, ATAC-seq, ChIP-Seq and Immunofluorescence and Imaging	Conversion mESCs to a 2C-like state when MERV1 peaks	Murine DUX activating the cleavage stage genes	(262)
<i>Homo sapiens</i>	HeLa M2cell line	LINE-1	Mass Spectrometry, Immunofluorescence staining, RNA-FISH, Western	Peaks during S phase of the cell division	N/A	(263)
<i>Homo sapiens</i>	Adipose derived stem cells (ADCS)	Alu/SINE	Immunofluorescence, qPCR, ChIP-Seq, Northern Hybridization	Knockdown of Alu/SINE in senescent cells	Initiates self-renewal of the cells	(264, 265)
<i>Drosophila melanogaster</i>	dAgo2 mutated heads	LINE-1, Gypsy	Western Blots, qPCR Immunochemical staining, GFP Imaging	Increase in expression of TEs	Neuronal decline	(169)
<i>Homo sapiens</i>	H9 ESCs cell line	LINE-1	RT-qPCR, Retro transposition Assay, TUNEL analysis, Western Blot	LINE-1 increases when TREX1 decrease	Neuroinflammation	(266)
<i>Homo sapiens</i> (ROSMAP project)	Brain	Multiple TEs	RNA-seq, PCR, H3KAc ChIP-seq	Tau levels correlate with transposon levels	Alzheimer's	(180)
<i>Drosophila melanogaster</i> (Transgenic flies)	Brain	Multiple TEs	qPCR, RNA-seq	As Tau is expressed, transposon expression increases	Alzheimer's	(180)

Table 4. Continued

Organism	Cell lines / Cell Types / Tissues	Transposon name	How were they measured?	Changes observed in Transposons	Phenotypes associated with the listed change	Reference
<i>Drosophila melanogaster</i> (Transgenic flies)	Head Tissues	gypsy ERV	qPCR, RNA-seq	Neuronal and glial hTDP-43 expression exhibits elevated expression of gypsy ERV	Neurodegeneration in diseases such as Amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTDL)	(183)
<i>Homo sapiens</i>	Brain	Multiple TEs	RNA-seq, RT-qPCR	Increased repetitive element accumulation in C9orf72 positive cases	Neurodegeneration in diseases such as Amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTDL)	(267)
<i>Rattus norvegicus</i>	Cortical neurons	Multiple TEs	RIP-seq	TDP-43 binds to transposons	N/A	(182, 268)
<i>Mus musculus</i>	Brain	Multiple TEs	mRNA-seq, CLIP-seq	Depletion of TDP-43 in mice increases transposon expression, TDP-43 binds to transposons	N/A	(182, 269)
<i>Mus musculus</i> (transgenic)	Spinal cord	Multiple TEs	mRNA-seq	Increase in expression of transposons when TDP-43 was overexpressed	N/A	(182, 270)
<i>Homo sapiens</i>	Brain	Multiple TEs	CLIP-seq	Decreased binding of transposons to TDP-43	Frontotemporal lobar degeneration (FTDL)	(182)
<i>Mus musculus</i>	Lymphoma and hepatocellular carcinoma tumors	LINE-1 and MusD	qPCR	Copy number increases compared to control tissue	Cancer	(261)
<i>Homo sapiens</i>	Many tissue types	LINE-1	Immunohistochemistry	Present in the cancerous tissue types; mostly absent in healthy tissue	Cancer	(271)
<i>Homo sapiens</i>	Blood	AAlu/SINE and LINE-1	MethylLight PCR	Transposon methylation status is inversely correlated with menopausal age	Earlier menopause is associated with earlier onset of age-related disorders	(140)

(123). In vertebrates specifically, the proportion ranges from 6% in the green spotted pufferfish to 55% in the zebrafish (124). These elements are highly diverse in terms of DNA sequences, mobilization mechanisms, and regulatory mechanisms (reviewed in (125, 126). Two main classes of transposons have been described: (i) Class I transposons, or RNA-mediated transposons, and (ii) Class II transposons, or DNA-mediated transposons (127). Class I TEs transpose through a “copy and paste” mechanism, generating RNA intermediates that can be reverse transcribed and integrated into the genome at a new site (127). In contrast, Class II transposons rely on enzymes that “cut” the transposon DNA sequences out of their current loci, and “paste” them elsewhere in the genome (127). Because of their mobility, TEs

can act as mutagens and have the potential to disrupt genes by inserting themselves into coding sequences and to change gene expression through insertion into regulatory sequences (128). From a bioinformatics perspective, the repetitive nature of TEs have complicated genomic analyses, as short-read NGS technologies have limited ability to distinguish different insertions of the same element family (129). Though > 44,000 sequences have been catalogued on Repbase, a database for repetitive elements (130), it is likely that many more remain to be characterized. Fortunately, the emerging availability and reduced cost of long-read sequencing technologies (e.g. Pacific Biosciences SMRT sequencing (131), Oxford Nanopore (132)) should help overcome these technical hurdles in the coming years (133, 134). Ultimately, the high sequence and functional

variability of transposons means that much remains to be discovered in terms of their mechanisms, roles, and biological consequences, and accumulating evidence suggests that they play a key role during aging and in the pathogenesis of age-related diseases (Table 4).

Transposon landscapes in traditional and emerging model systems

A number of invertebrate animal models, including *C. elegans* and *D. melanogaster*, and some vertebrate models, such as the mouse and the zebrafish, have been used in aging and transposable element research (135). Noteworthy, TEs vary in type and in frequency across different organisms. For example, 12% of the *C. elegans* genome is composed of TEs (136), most of which (~95%) are DNA transposons (124). In contrast, TEs encompass ~20% of the *D. melanogaster* genome (137), and LTRs from the gypsy, copia, and Pao families are the most abundant (138). In humans, ~50% of the genome is repetitive, with the majority of human TEs being LINE-1 elements (~17% of the genome) (139). Although invertebrate models have been widely used in studies on transposon and aging processes due to, among other factors, their short lifespans, this has come at the expense of knowledge concerning the relationship between these processes and vertebrate-specific genes, organs and tissues, and physiological processes. For example, methylation status of transposon loci in human blood has been identified as a predictor of menopausal age (140), and transposons have also been shown to induce responses mediated by interferons (141), which are modulators of innate and adaptive immunity.

Though aging studies can be, and have been, carried out in vertebrate models such as the mouse and the zebrafish, their relatively long lifespans in the laboratory (3-4 and 5 years, respectively) make aging studies costly and time-consuming. Consequently, there has been a need for a tractable short-lived vertebrate model to address these limits. The African turquoise killifish (*Nothobranchius furzeri*) is a naturally short-lived vertebrate (~0.5 year), which was first found and collected from ephemeral ponds in Mozambique and Zimbabwe (142-145). Other advantages of this model are the availability of several wild-derived strains (146-148), an annotated draft genome sequence (142, 149) and a rapid genome-editing toolkit (143). In addition, despite a compressed lifespan, the turquoise killifish shows classical phenotypes of vertebrate aging (e.g. cognitive impairment, histological changes, telomere shortening, and mitochondrial dysfunction) (150-153). Relevant to the study of TEs in vertebrate aging, 45-65% of the turquoise killifish genome is composed of TEs, a proportion which is similar to that of the human genome (154). Moreover, the elements seem to be transcriptionally active, compatible with the notion of active transposition across tissues in this species (142). Given all of these characteristics, we anticipate that the African turquoise killifish will be an important model to understand the mechanisms and impact of TE regulation in

aging and age-related diseases.

Mechanisms and breakdown of TE regulation throughout lifespan

Given the mutagenic potential of unfettered transposition, cells have evolved layers of pre- and post-transcriptional mechanisms to restrain transposon activity in the germline and somatic tissues (125, 126). Known pre-transcriptional mechanisms mainly include DNA methylation and TE heterochromatinization (125, 155). For instance, in female *Drosophila* head tissues and fat bodies, Wood *et al.* found that overexpression of genes encoding chromatin modifying enzymes *Sir2* and *Su(var)3-9* led to repression of the age-related increase in TE expression (156). *Sir2* is an NAD-dependent deacetylase involved in gene silencing at heterochromatic regions in yeast and flies (157-159), while *Su(var)3-9* catalyzes the methylation of Lysine-9 of histone H3 (H3K9) to repress transcription (160). The study also found that overexpression of *Su(var)3-9* was associated with increased lifespan (156). Another study noted a global increase in heterochromatin in the livers of old mice (based on DNase I sensitivity assay and comet tail assay), but associated increased expression of TEs with relaxed heterochromatic regions (161). Although the global increase in heterochromatin in this specific context runs counter to the "loss of heterochromatin" model of aging (162), TE activation has, in contrast, been shown to be recurrently associated with heterochromatin loss in *C. elegans*, *D. melanogaster*, mice, and mammalian cells (163, 164). Additionally, in senescent human fibroblasts, chromatin accessibility was shown to increase at TE insertion loci, thus promoting TE transcription and active transposition (161).

Current research is partially focused on identifying novel genetic regulators of transposon activity. A genome-wide CRISPR-Cas9 screen in the K562 and HeLa cancer cell lines was recently performed in order to identify novel regulators of LINE-1 retrotransposition unbiasedly (165). However, how these new TE regulators respond to aging and longevity intervention remains unknown. Accumulating evidence also highlights the interaction between the interferon response and TE activity. Type I interferons (IFNs) are a family of cytokines that modulate immunity against viruses and intracellular pathogens, notably by inducing genes that promote an "antiviral state", sensitizing these cells to apoptosis, and by activating immune cells that clear infected cells (166). Importantly, Yu *et al.* showed that LINE-1 stimulates IFN- β expression in *in vitro* human and mouse cell models (141). Conversely, treatment with IFN- β could repress endogenous LINE-1 retrotransposition, with endogenous IFN- β signaling counteracting replication of LINE-1 elements (141). An additional study looking for regulators of cell survival in human acute myeloid leukemia cell lines found that loss of heterochromatin regulator SETDB1 associated with lower cell viability, induction of TEs, and induction of viral response genes, including interferon stimulated genes (167).

Post-transcriptional mechanisms of regulation rely on the degradation of TE RNAs or inhibition of their translation. These rely on siRNA, miRNA, and piRNA pathways (125, 155). Work by Wood *et al.* also assessed the relationship between siRNA activity and TE levels in *Drosophila*. They observed a repression of the age-associated TE upregulation in mutant flies overexpressing *Dicer-2*, an enzyme necessary for siRNA processing, or in flies hypomorphic for the *Adar* gene, which encodes the main RNA-editing enzyme and functions as a negative regulator of the siRNA pathway (156). Importantly, the presence of Alu elements is a major factor for selecting RNA-editing targets by ADAR in human cells (168). In another *Drosophila* study focusing on the central nervous system, increased TE expression and transposition events were observed with aging using a *gypsy* GFP reporter (*gypsy*-TRAP reporter) (169). Interestingly, mutation of *Ago2*, another gene involved in RNAi pathways, led to increased TE activity and shortened lifespan (169). To note, although associations between RNAi pathways and TE activity have been noted, the results should be interpreted with caution, as dysregulation of RNAi pathways are likely to lead to a general misregulation of many downstream processes. Canonically, siRNA pathways and piRNA pathways have been thought to be primarily restricted to regulatory roles in somatic and germ line cells, respectively (138). Emerging evidence, however, suggests that piRNA mechanisms may also be active in somatic cells (170). In one type of mushroom body neuron in *Drosophila*, suppression of the piRNA proteins Aubergine and Argonaute3 was associated with elevated TE expression (171), and another study proposed that piRNA components may play a role in alternative splicing of transposable elements in both soma and germ line cells of the fly (172). A final form of regulation which remains loosely characterized involves tRNA-derived fragments which have been shown to target retrotransposon primer binding sites in mouse embryonic and trophoblast stem cells and may, more generally, inhibit transcription or induce RNAi pathways (173). Though multiple layers of native regulatory mechanisms for transposon control exist, research has also been focused on identifying dietary and pharmaceutical interventions that can supplement cellular mechanisms.

Transposon activity modulation by dietary and chemical interventions

One of the focuses of ongoing research is to identify dietary interventions or novel drugs that inhibit transposon activity and determining what impact these have on lifespan and health. In female *Drosophila* heads and fat bodies, DR suppressed the age-related increase in TE expression that was observed in the high calorie (HC) diet control group (156). Additionally, DR seemed to counteract the increase in expression of genes located in constitutive heterochromatin regions that occurred with age in HC-fed flies (156). A high-fat diet similarly promoted TE expression in the livers of middle-aged male mice, and the authors suggest that CR may

inhibit these effects (174). Interestingly, knockdown of the chromatin-remodeler *Chd1* led to similar transposon activation as the high-fat diet and aging conditions, supporting a key role for chromatin-level regulation of TE activity (174). Pharmacologically, Wood *et al.* demonstrated that treatment of flies with the reverse transcriptase inhibitor lamivudine repressed retrotransposition in *Drosophila* fat bodies and increased the lifespan of *Dicer-2* mutants (156). Looking forward, the effects of this drug, and other anti-retroviral drugs, should be assessed when administered at different times in an organism's life to determine which dosing periods contribute most towards improving organism function and lifespan. In particular, embryogenesis may be an informative period to test, given that transposition events occurring during this time are likely to lead to large bulks of mutated cells in the body, thus leading to somatic chimerism (138). Importantly, since both avenues for TE regulation (*i.e.* inhibiting expression and inhibiting transposition) may not necessarily lead to the same outcome, both avenues should be explored in the treatment of aging and age-related diseases. For example, inhibition of LINE-1 with a reverse transcriptase inhibitor does not have the same developmental consequences for mouse embryonic stem cells as does a genetic knockdown (175). Since accumulating evidence suggests that transposon reactivation occurs with and in various age-related diseases (Table 4), it will ultimately be important to identify robust methods to modulate TE activation *in vivo* throughout life.

Transposons: a blessing and a curse?

The idea that TEs play both positive and negative roles, based on factors such as the time of activation and the integration site within the host genome, has been gaining traction (Table 4). Historically, TEs have been seen as 'genetic parasites' and 'selfish DNA' elements (176). Though some TEs can be described as such, others are non-functional and thus neutral, or have helped shape new functions at the cellular and organismal level (129). Since TEs contain regulatory elements that differentially respond to environmental and stress cues, TE insertions may help dynamic rewiring of gene regulatory networks, and generate novel spatiotemporal gene expression programs (126). Examples to illustrate the potential multi-pronged effects of transposons concern the human Endogenous Retrovirus (HERV) family of transposons. On the one hand, the proteins syncytin-1 and syncytin-2 – encoded by the HERV-W and HERV-FRD *env* genes – are required for fusion of trophoblast giant cells to form the syncytiotrophoblast, a key step for placental formation, and contribute to fetal immune tolerance (177). On the other hand, the viral properties of ERVs render them capable of activating the immune system, and this may contribute to the development of autoimmunity and other pathologies (178, 179).

Thus, the ERV families of transposons, and perhaps others, may have antagonistic pleiotropic effects, playing important roles in early development, but contributing to pathogenesis

and functional decline post-development. A number of associations between transposons and different age-related disease states have been identified (see Table 4). In an intriguing example, Guo *et al.* identified an association between global TE levels and Tau pathological burden (180). Moreover, they found that Tau expression induces TE expression in a *Drosophila* model (180), raising the possibility that TEs may be causative agents or drivers of Alzheimer's disease and neurodegeneration. However, the mechanisms underlying this link are still unclear, and will deserve further consideration. Similarly, functionally abnormal TAR DNA-binding protein 43 (TDP-43) is observed in many cases of amyotrophic lateral sclerosis (ALS) and some cases of frontotemporal lobar degeneration (FTLD) (181). Experimentally, TDP-43 has been shown to bind transposable elements in mouse and rat models, differentially bind TEs in healthy and FTLD patients (182), and induce expression of a panel of transposons when human TDP-43 is expressed in *Drosophila* and mouse models (182, 183). The data in *Drosophila* suggests hTDP-43 toxicity may stem from gypsy ERV activating DNA damage-mediated programmed cell death (183), but whether this mechanism is conserved and whether it is the sole driving mechanism remains unclear.

Future work on the role of TE activity in aging and age-related diseases should seek to clarify this relationship by generating precise genomic tools. Ultimately, the field will need to address whether transposon activation is a byproduct or a driver of aging and disease and, if the latter is true, uncover the mechanisms by which this occurs.

THE EMERGING IMPACT OF SMALL ncRNAs IN AGING AND LONGEVITY

miRNAs in the regulation of aging and longevity

MicroRNA (miRNA) are a class of endogenous eukaryotic small non-coding RNAs, ~22 nucleotides long, that play a key role in RNA silencing and post-transcriptional regulation of gene expression (184). miRNAs are abundant and have been proposed to target, and potentially regulate, ~60% of mammalian genes (185). Accumulating evidence suggests that remodeling of the miRNA expression landscape may be a biomarker, or even a driver, of the aging process (95).

Several pioneering studies have identified age-related changes in the miRNA transcriptional landscape across cells and tissues from various evolutionary taxa (*i.e.* worms, mouse, rats, human, macaque, African turquoise killifish, etc.) (186-198). Interestingly, miRNA with age-specific changes in *C. elegans* were shown to either promote or repress normal lifespan and stress resistance in the adult organism (186, 199). Further, expression levels of specific miRNA early in life can predict *C. elegans* longevity (187), supporting the notion that these changes may be responsible for aging phenotypes at least partly. Maturation of miRNA sequences from precursors requires *Dicer* expression (200). Intriguingly, a fat-specific *Dicer* ablation was found to lead to an accelerated aging

phenotype in mice, and counteracted positive effect of DR (201). Moreover, transfection of age downregulated miRNAs into 18-month-old mice led to a partial rejuvenation of liver expression profiles, at least in terms of aging markers (e.g. *p16INK4a*) (190).

An interesting case is that of the miR-29 microRNA family. Indeed, members of this family were found to be upregulated with age in various tissues, including brain, muscle, skin, vascular tissue, heart, liver, lung and kidney (191, 193-198, 202). Age-related accumulation of DNA-damage has been proposed to activate miR-29 and p53 (195). It has also been proposed that miR-29 may be involved in a compensatory response limiting an age-related neuronal iron accumulation (196). Although miR-29 upregulation with aging seems ubiquitous, conditional knockdown for miR-29 in mouse brains led to an increase in male lifespan, while female lifespan was decreased (202), suggesting a complex interaction between miR-29 functions in health and lifespan and other factors (e.g. sex). Interestingly, a recent study found that TGF- β could signal via miR-29 to promote repress the translation of the main H4K20me3 methyltransferases (203). Deficit in cellular H4K20me3, a chromatin mark which plays an important for DNA-damage repair, led to cellular senescence, and might contribute to mouse cardiac aging *in vivo* (203). These observations support the notion that a complex interplay of signaling between non-coding RNAs and the epigenome may be driving aspects of the aging process.

A potential role for tRNAs in aging and longevity regulation?

The genetic code is degenerate, with the set of 20 amino acids encoded by 61 base triplets or codons (204). Hence, multiple synonymous codons can be used in coding sequences, giving rise to the inclusion of the same amino acid in the final protein product. Traditional studies of disease genetics and evolutionary selection have focused on non-synonymous variants, which lead to a change in protein sequence and, presumably, protein function. However, accruing evidence now supports the idea that codon choices, while not affecting the protein sequence, can impact translation by interacting with the tRNA pool (205), thus functionally modulating final protein products (206, 207). Recent examples of the consequences of codon usage biases include modulation of the transcription rate (208), of translation rate (209) or selection against protein misfolding (210). In turn, these processes have been involved in the pathogenesis of neurodegenerative diseases (211-213), and their modulation may be involved in the age-related proteostasis collapse (6). Almost 50 years ago, researchers theorized that changes in the tRNA pool with life or development may result in an otherwise unnoticed role for codon usage bias in aging (214). However, the importance of changes in tRNA pools, and their interactions with codon usage, on aging and lifespan is still largely unknown.

Accumulating evidence showing time and tissue variability of the tRNA pool have led to rekindled interest in this

intriguing hypothesis (215, 216). Because of the generally accepted importance of translation regulation and proteostasis with aging (6), the impact of alterations of the tRNA pool on ribosomal protein synthesis and co-translational folding, interacting with differences in codon usage, may have profound impact on the proteome throughout life. Indeed, proliferation-enriched and differentiation-enriched tRNAs preferentially have anticodons that are enriched to be represented respectively among cell-autonomous vs. intercellular communication genes, suggesting exquisite coordination between transcriptional and translational programs (217). Interestingly, tRNA-Leu overexpression can enhance phosphorylation of S6-kinase under amino acids starvation condition independent of mTOR signaling (218). This observation is consistent with the notion that tRNAs may also play more general regulatory functions.

Recent evidence has revealed the existence of a new class of small non-coding regulatory RNAs derived from tRNAs, known as tRFs (*i.e.* tRNA-derived fragments) (219). Although the biological roles of tRFs are still poorly understood, they have been suggested to play roles in regulating cell proliferation, gene expression and RNA processing, priming viral reverse transcriptases, and modulating the DNA damage response, tumor suppression, and neurodegeneration (219). For instance, inhibition of a tRF species derived from the 3' half of tRNA-Leu (CAG) can induce apoptosis and enhance the translation of two ribosomal protein (RPS28 and RPS15) (220).

The expression of tRNA genes is under the control of a specialized polymerase, RNA polymerase III (RNA Pol III) (221). Transcription of specific tRNA genes is thus likely to be influenced by remodeling of the chromatin landscape, notably during aging (1), which may disrupt the homeostasis of tRNA gene pools. Although RNA Pol III genes are generally thought of as 'housekeeping genes', recent evidence suggests that RNA Pol III target selection must be regulated at least in part (reviewed in (222)). Indeed, while a large majority of RNA Pol III target genes are shared across cell types and tissues, private targets exist and could be involved in tissue- or context-specific responses (223). Interestingly, a recent study has shown that a reduction in RNA Pol III levels is associated to increased longevity downstream of TOR activity in yeast, worms and flies (224), supporting the idea that RNA Pol III (and its targets, including tRNA genes) may be a crucial mediator of the nutrient-signaling longevity pathway.

A systematic assessment of tRNA pools, derived tRFs, as well as RNA Pol III targets with mammalian aging will be crucial to understand the role of these phenomena on health and lifespan.

GENERAL DISCUSSION AND IMPLICATIONS

This review has painted a broad picture of the current knowledge in various aspects of transcriptional regulation in aging and longevity models. Understanding gene regulation

and misregulation with aging will be of great use in order to help mitigate or reverse aging-related functional decline.

In addition to humans, sex differences in lifespan have been observed across taxa (10), and mammalian females across species are also generally longer-lived than males, including pilot whales, lions, red deer, prairie dogs, monkeys and apes (10). In most reported cases, female laboratory rats live longer than males (10). Although the existence of a similar female advantage for lifespan in laboratory mice is still debated (10), in standardized husbandry conditions developed for the NIA Interventions Testing Program (ITP), female individuals consistently outlive males at 3 independent sites (10), suggesting that the human female advantage may be recapitulated in laboratory mice in controlled conditions. Many health parameters differ between male and female mice with aging (225). Several reports have indicated that adult stem cell populations (*i.e.* HSCs, NSCs, MuSCs) show increased self-renewal and regenerative capacity in females compared to males (226). Moreover, females exhibit increased abilities for wound healing and liver regeneration (226). Surprisingly, interventions that successfully extend the health- and lifespan of laboratory mice often do so in a sex-dimorphic manner (10, 55, 227). For instance, treatment with rapamycin preferentially extends female lifespan, whereas treatment with 17- α -estradiol only extends male lifespan (55). More surprisingly, genetic manipulations can also extend lifespan in a sex-dimorphic manner, leading to either greater lifespan extension in females vs. males (*e.g.* *Igf1r* haploinsufficiency), or to lifespan extension exclusively in males (*e.g.* *Sirt6* overexpression) (10, 55). Though it has been proposed that differences in the bioavailability or metabolism of longevity-promoting compounds may be responsible for sex-dimorphic longevity effects, the existence of sex-dimorphic longevity effects upon genetic manipulations suggests that other underlying mechanisms are at work. Remarkably, thousands of genes can be regulated in a sex-dimorphic manner across a range of youthful healthy mouse and human somatic tissues (*e.g.* brain, liver, heart, muscle) (228-231). Genes expressed in a sex-dimorphic manner are located on autosomes as well as on sex chromosomes (228, 230-232), and many of these genes are not directly targeted by sex hormones (233). Functional enrichment analyses of sex-dimorphic gene regulation have identified differentially enriched pathways between male and female tissue transcriptomes, including immune response, oxidoreductase activity, and lipid metabolism (232). In addition, genes expressed in a sex-dimorphic manner in the human brain were proposed to act as mediators of stress susceptibility and depressive symptoms (234), consistent with the idea that sex-dimorphic expression can broadly impact human health and physiology. Studies in *Drosophila* have revealed that the sex-dimorphism in the expression of the mitochondrial Lon protease mediates sex- and age-specific adaptation to oxidative stress, and the sex-dimorphic expression of this protease may be conserved in mice (235).

Interestingly, DR leads to a feminization of the gene expression profile of male mouse livers (*i.e.* rendering the gene expression profile of the organ more similar to that of females) (236). These observations raise the intriguing possibility that sex-dimorphic gene expression may play a key role in aging and response to longevity interventions. However, despite accumulating evidence of widespread sex-dimorphism in aging and lifespan across species as well as in mechanisms of gene regulation, few studies have investigated age-related sex-dimorphisms at the molecular level. Thus, systematically understanding the transcriptional underpinnings of sex differences in aging and longevity will be crucial to develop therapeutic strategies to slow down age-related functional decline and diseases which work in both males and females.

An important caveat to note for all studies of aging and longevity-associated transcriptional remodeling is that the penetrance of such transcriptional changes to cellular and organismal phenotypes may be limited (237). However, proteomics still has a lower sensitivity threshold and higher technical noise than RNA profiling. Thus, it will be important to systematically combine transcriptional mapping approaches to “proteome” and “translatome” mapping, notably through ribosome-profiling and mass spectrometry, to understand the relationship between mRNA levels and translated proteins (238).

Finally, transcriptional changes are the end-result of changes in upstream regulators, including chromatin remodeling (1, 2) and modulation of transcription factor activity (e.g. FOXOs, NRF2, *etc.*) (239, 240). The accumulating evidence showing that longevity-interventions can slow-down or reverse aspects of transcriptional aging suggests that aging can be modulated at the molecular level by environmental cues and genetic factors. Understanding the regulatory mechanisms leading to transcriptional defects with aging will be key in the design of therapeutic strategies to slow-down age-related decline, and delay the onset of age-related diseases.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

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