Trends in Genetics

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Opinion

Time is ticking faster for long genes in aging

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Recent studies of aging organisms have identified a systematic phenomenon, characterized by a negative correlation between gene length and their expression in various cell types, species, and diseases. We term this phenomenon gene-length-dependent transcription decline (GLTD) and suggest that it may represent a bottleneck in the transcription machinery and thereby significantly contribute to aging as an etiological factor. We review potential links between GLTD and key aging processes such as DNA damage and explore their potential in identifying disease modification targets. Notably, in Alzheimer's disease, GLTD spotlights extremely long synaptic genes at chromosomal fragile sites (CFSs) and their vulnerability to postmitotic DNA damage. We suggest that GLTD is an integral element of biological aging.

Highlights

Transcript expression decreases with gene length in aging in humans and multiple animals.

We introduce the terminology gene length-dependent transcription decline (GLTD).

Polymerase stalling following DNA damage is a cause of GLTD in aging.

GLTD also appears in Alzheimer's disease, an age-associated disease.

Multiple known aspects of biological aging converge on GLTD.

GLTD in aging

Aging is characterized by gross phenotypic changes and micro- and mesoscale alterations in cell biology and tissue architecture and, ultimately, declining organ function. Various hypotheses attribute aging to programmed or entropic processes, such as DNA damage, progression of cellular clocks, free radical damage, and cellular stress response. A notion shared by multiple hypotheses is that factors such as damage to macromolecules, cell organelles, and the extracellular matrix cause a gradual loss of physiological balance in late life [1–3]. At the molecular level, genomic, epigenomic, and proteomic damage are postulated to contribute to various aging-related phenomena such as stem cell exhaustion, mitochondrial dysfunction, energy metabolism failure, altered signaling, and, ultimately, impairments of biological homeostasis [3]. Taking these together, aging appears to be a multifaceted phenomenon influenced by various biological processes. Thus, a traceable model that can identify a point of etiological convergence could enhance our understanding of aging [4].

While the epigenome and proteome have garnered most of the attention in aging research [5,6], recent technological advances and large-scale studies, especially in the transcriptomics domain, have enabled a data-driven search for gene expression signatures associated with aging. Initial transcriptomic measurements suggested that gene expression changes in aging are probably tissue and cell-type specific [7]. Several efforts have aimed to simplify, model, and explain such perturbations by identifying specific sets of genes whose expression trajectories are correlated with age [8–13]. Genes acting in multiple pathways exhibit altered expression in various aging tissues, such as genes involved in the inflammatory response [8,14,15], DNA repair and damage response [15], synaptic transmission [15], Wnt signaling [14,16], and Notch signaling [17]. However, few unique genes appear to be consistently up- or downregulated across organs and tissues [8,10,14,15], with a recent study reporting only 83 genes that change consistently across all inspected brain regions [18]. Further complicating this picture, the majority of genes (over ~90%) that are differentially expressed in young and aged tissues exhibit mild changes of less than onefold [14]. These observations, while perhaps partly reflecting cohort and platform variability, suggest that aging is not dictated by altered expression of a unique gene or a singular pathway. This fits

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the notion that aging has not been selected for in evolution and hence occurs by default [19,20], which implies that there may not be any uniform, specific gene expression program for aging.

In this opinion, we aim to summarize recent remarkable findings, including ours, on aging-associated genome-wide changes of the transcriptome with particular emphasis on the role of gene length on altered gene expression. The negative correlation between gene length and the expression of genes is a recently discovered phenomenon in aging research and has been robustly observed by multiple researchers in diverse organisms and in independent studies [21–26]. As most transcriptomic approaches do not provide absolute quantification, this phenomenon typically presents as a relative increase of the expression of short genes and a relative decrease of the expression of long genes. Since this phenomenon, however, originates in loss of transcription over the gene body [25], we refer to it as GLTD (Figure 1).

GLTD differs from gene- and pathway-centric descriptions of the aging transcriptome, as it reflects expression changes in almost the entire transcriptome. This novel phenomenon has been observed in most organs and tissues in organisms ranging from worms to mammals

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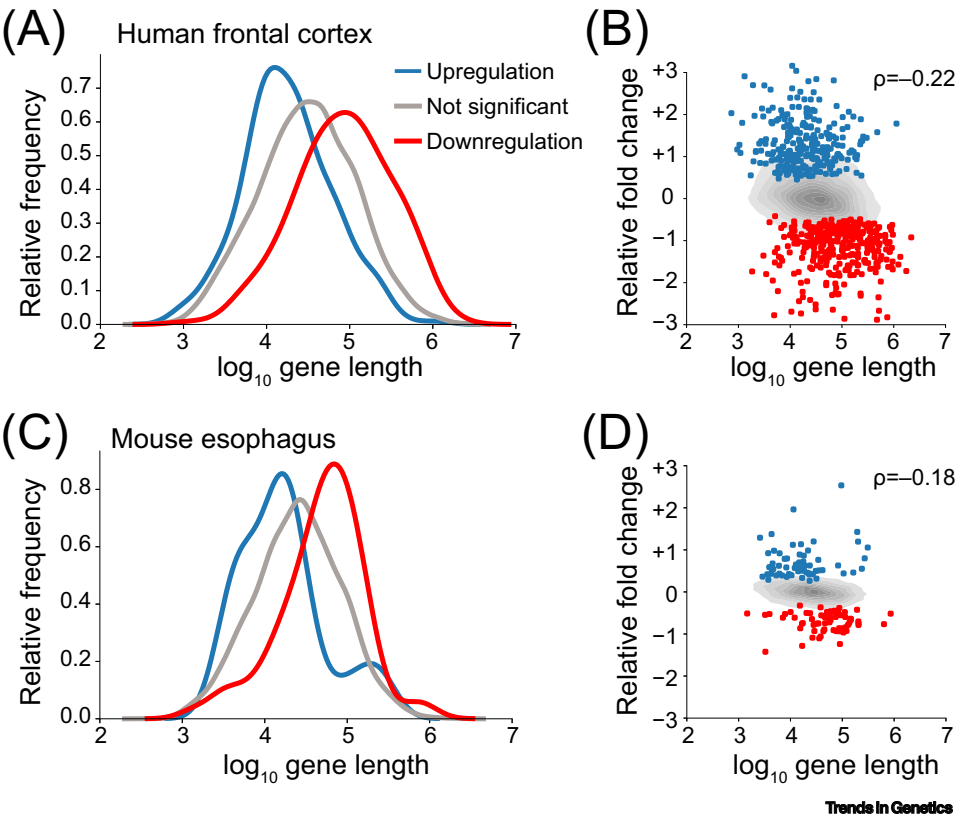

Figure 1. Gene-length-dependent transcriptional decline (GLTD) occurs in aging. (A,B) Gene expression differences in the frontal cortex of 60–79- (n = 53) and 20–39- (n = 7) year-old human donors of the GTEx cohort [105] measured by DESeq2. Smoothed histogram (A) and scatter plot (B) of significantly upregulated (blue) and downregulated (red) genes at P value of less than 0.05, superimposed on a kernel-density plot of the majority of genes that do not show significant changes in aging (gray, ~90%). (C,D) As panels (A,B), but for gene expression differences in the esophagus of 4- (n = 6) and 24- (n = 6) month-old male mice of Stoeger *et al.* [24] measured by DESeq2. Among all mouse tissues, esophagus has the most typical (median) GLTD [24]. ρ is the Spearman correlation. Note that length-correlated gene expression changes are not exclusive to genes exhibiting significant changes.

including humans and therefore appears universal and conserved in evolution [21–26]. Since GLTD affects the basic process of gene expression, essential for all cellular functions, we propose that GLTD contributes to aging and common diseases of (accelerated) aging, such as dementias, cardiovascular diseases, and common failures of metabolism such as diabetes, all involving organs and tissues in which GLTD is observed. A better understanding of GLTD may illuminate novel molecular aspects of aging and susceptibility to aging-related diseases and open perspectives for effective interventions.

GLTD is common in aging and responds to interventions

Protein-coding genes in the human genome vary greatly in length, from a few hundreds to several million base pairs (i.e., a difference of four orders of magnitude) (Figure 2). Notably, genes of different lengths are not uniformly expressed across cell types and organ systems [23,27], with neurons expressing some of the longest known genes [28,29]. We observed a length-dependent decline of gene expression in DNA-repair-deficient mouse models of premature aging, which exhibited strong age-dependent downregulation of long genes in their livers, revealing a causal link between unrepaired DNA damage and GLTD [21,30]. GLTD has subsequently been observed in the normal aging of multiple species, including humans [21,23–26], rats [21,24], mice [21,24–26], killifish [24], *Drosophila melanogaster* [22], and *Caenorhabditis elegans* [25]. Approaches to robustly measure GLTD are summarized in Box 1. In our pooled reanalysis of data from multiple transcriptomic experiments, approximately 70% of mouse tissues and approximately 60% of human tissues demonstrate GLTD, with the strongest effects in neural tissue (even after excluding genes with known neural function, consistent with the notion that the effects are genome wide) [24]. Although GLTD was first noticed in DNA-repair-deficient mice [21], we and others independently observed this phenomenon in other conditions associated with accelerated human aging, such as Alzheimer's disease [31,32], and exposure to tobacco smoke [26].

GLTD also responds to factors that delay aging. This was first observed on caloric restriction in mice, where caloric restriction partially mitigated the transcriptional downregulation of long genes in DNA-repair-deficient mice [21]. This extends to further antiaging interventions that increase the lifespan of mice in the Interventions Testing Program of the National Institutes of Health

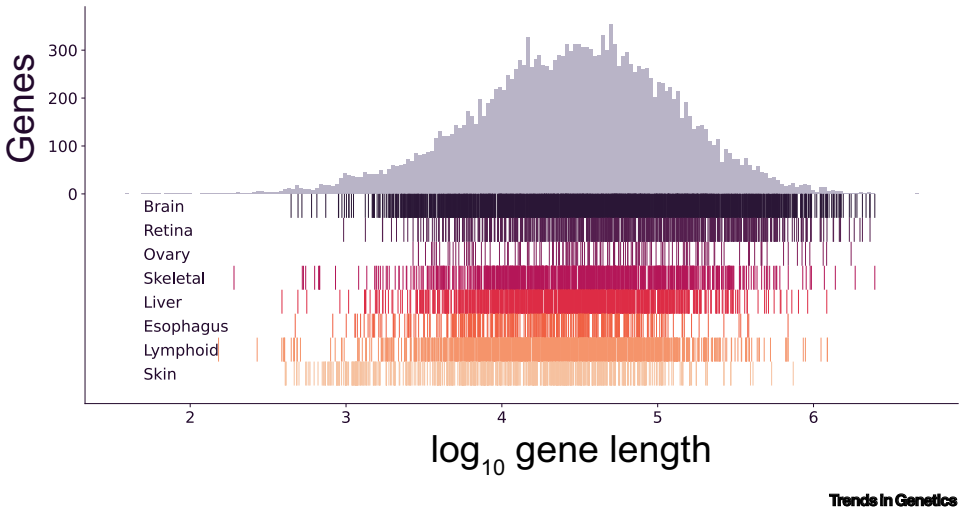

Figure 2. The lengths of protein-coding genes in the human genome. Gene lengths are calculated by calculating the difference between the transcription start and end coordinates of each gene (Ensembl GRCh38.p14). Data on tissue-specific expression of these genes are sourced from the Human Protein Atlas.

Box 1. How to measure GLTD

GLTD can be quantified by comparing the lengths of up- and downregulated genes, the decrease of transcripts of long genes, or the Spearman correlation between gene length and transcriptional changes [24]. The latter appears to be the most sensitive [24]. Although we here refer to the phenomenon as relating to 'gene length' to account for the hypothesis that led to its initial description [21,100], the length of mature (spliced) transcripts can be equally informative, from a statistical perspective [24].

Multiple biological factors could limit the ability to observe GLTD. For example, cell proliferation dynamics and tissue turnover dilute DNA damage and are highly variable across tissue types [47]. The shedding and apoptosis rate of older cells in tissues also probably varies, which could eliminate cells with accumulated damage [101] potentially resulting in survivorship bias in tissues with high turnover rates. GLTD readouts of bulk specimens may partly capture altered tissue cell composition, since aged tissues are often infiltrated by immune cells [13]. This can be addressed by studies on individual cell populations. For instance, sorted photoreceptors of *Drosophila melanogaster* show GLTD [22] as well as single human neurons afflicted by Alzheimer's disease [31] and multiple human tissue specimens studied by single-cell transcriptomics [26]. The use of spike-in sequences and unique molecular identifiers (UMIs) has recently shown promise in measuring absolute single-cell gene expression [102]. However, single-cell RNA-seq pipelines use normalization methods to harmonize gene expression across cells, usually based on total sequencing read counts per cell (read depth), which may introduce collider bias if global transcription in a cell is affected by the underlying biology. Another challenge is that most of the high-throughput single-cell RNA-seq methods are biased and primarily focus on sequencing reads from the 3′ or 5′ end of the genes. This issue is addressed by new long-read technologies such as MAS-seq, which enables direct full transcript measurements of even the longest human genes [103].

Technical biases related to gene length in RNA-seq [102,103] call for validation using alternative methods. While quantitative RT-PCR may be appealing, it may not be sensitive enough [104] to measure gene expression changes at the typical magnitudes observed in aging cells [14]. Here, meta-analyses or NanoString panels that contain long transcripts can increase reliability [24–26]. Interestingly, we have also recently observed a gene-length-dependent decline of the aging proteome [24], which may provide an orthogonal validation of this phenomenon.

(NIH) National Institute on Aging [33,34]. By reanalyzing published transcriptional data from mice, humans, and apes, we found increased transcription of long genes following fibroblast growth factor 21 excess, Myc heterozygosity, rapamycin, resveratrol, S6 kinase 1 deletion, senolytics, and mutation of pituitary-specific transcription factor 1 [24]. Further, we found that vitamin D reduces the magnitude of GLTD caused by UV irradiation [26]. Taking these findings together, GLTD appears to be widespread across species, tissues, and conditions and to correlate with the rate of aging. Critical inquiries emerge: could GLTD serve as a molecular marker of aging and represent an etiological factor?

Mechanistic interpretation of GLTD

DNA lies at the top of the cellular information hierarchy and is – by far – the largest and longest-living biomolecule in cells [35], and therefore prone to damage accumulation. Moreover, DNA solely depends on repair for maintenance throughout the lifespan, in contrast to other cellular components, which can be remade when damaged, based on genetic information in DNA. DNA damage has long been speculated to underlie aging [36,37]. In the 1960s, Wulff *et al.* reported a general decline of gene expression in aging tissues and postulated that it originates from damage at the sites of RNA synthesis [38]. This general decline was validated by others [9,39,40], and by us in diverse single-cell RNA-seq datasets of aging tissues [26] as well as following DNA damage accumulation in (prematurely) aging mice deficient in transcription-coupled repair (TCR), which removes DNA lesions that block elongating RNA polymerase II (RNA Pol II) in the transcribed strand of active genes to enable resumption of transcription [25,41,42]. Interestingly, we found that this damage does not result in a uniform and symmetrical decrease of all transcripts, but more strongly downregulates transcripts encoded by very long genes, consistent with the stochastic nature of DNA damage, affecting genes proportional to their length [21,25]. Surprisingly, in the livers of 2-year-old mice, ~40% of RNA Pol II appears to be stalled due to chemical lesions in the template strand blocking its movement [25]. Notably, this transcriptomic profile is similar to that of cells exposed to UV irradiation, which induces cyclobutane pyrimidine dimers that effectively block transcription. Such

lesions are poorly recognized by global genome repair but are removed by TCR, when they arrest RNA Pol II, allowing resumption of transcription [25,42].

DNA damage accumulation is extensively linked with aging. In replicating cells, DNA damage such as crosslinks, double-strand breaks, and telomere shortening result in permanent cell cycle arrest, premature differentiation, cellular senescence, and associated proinflammatory status and cell death [43]. Particularly, genetic defects in double-strand break and crosslink repair pathways (e.g., Fanconi anemia) and in telomere maintenance (e.g., dyskeratosis congenita) are associated with features of accelerated aging, mostly in proliferative tissues such as the hematopoietic system. Additionally, DNA lesions causing replication stress enhance mutagenesis and thereby increase cancer risk. However, the accumulation of DNA damage may be even more severe in postmitotic tissues, as cell turnover is (virtually) absent and the damage is not diluted by *de novo* DNA synthesis. Several multisystem premature aging syndromes in humans and mouse models that affect non-dividing tissues arise from deficient DNA repair systems [44]. In particular, compromised TCR, further exacerbated by additional defects in global genome repair systems such as in Cockayne syndrome and trichothiodystrophy and corresponding mouse mutants, exhibits numerous features of accelerated *bona fide* aging in postmitotic tissues (e.g., liver, kidney, skeleton and, most prominently, the neuronal system) [45,46]. These findings associate defects in specific repair pathways with aging in specific subsets of organs and tissues [47] and reveal a dose–response relationship with the rate of acceleration of segmental multimorbidity pointing to causality. In support of this notion, mouse mutants in multifunctional repair genes such as *Ercc1* affecting all repair pathways mentioned earlier, critical for proliferative and postmitotic organs, exhibit virtually all-round systemic aging phenotypes [45]. Interestingly, they also display accelerated aging as measured by epigenetic aging clocks in multiple tissues and *N*-glycan clocks in serum [48,49], further extending the parallels with natural aging. Furthermore, they exhibit progressive, dose-dependent, genome-wide GLTD [25]. Indeed, GLTD was first disclosed in TCR-deficient mouse mutants before being discovered in physiological aging [21,41]. Finally, in humans with intact repair systems, exposure to genotoxic agents in chemo- and radiotherapy accelerate several aging-related features [50] and has been associated with GLTD, as shown by studies on heavy smokers [26] and human cell lines exposed to genotoxic agents that induce bulky DNA lesions [51].

In particular, (i) the association in mouse mutants and human syndromes between the type of repair defect (repair pathways related to replication and cell cycle versus transcription) and the type of segmental premature aging (affecting proliferative versus postmitotic organs, respectively) and (ii) the strict dose–response relationship between the degree of the DNA repair defect (TCR alone or aggravated by additional deficiencies in global genome repair) and the acceleration of multiorgan features of aging [30,45,46] convincingly link DNA damage accumulation with the process of aging. Moreover, our finding that compromised TCR accelerates both GLTD and aging, in a dose-dependent manner [25], and our observations that robust antiaging interventions, such as dietary restriction (DR), do the converse [21,25] identify DNA damage as the origin of both GLTD and aging and makes GLTD the logical causal intermediate by which DNA damage exerts its numerous accelerated but genuine aging features. Since, transcription is the start of every cellular process, the secondary effects of GLTD are, by definition, widespread.

Taking these findings together, DNA damage appears causally linked with GLTD and impacts systemic aging in multiple ways.

Extremely long synaptic genes at fragile sites: a double-edged sword?

Currently, it is unclear whether GLTD directly scales with gene length (see Outstanding questions). Some DNA motifs and chromosomal structures are more prevalent in longer genes.

Hints toward such motifs or structures can be found in cancer and Alzheimer's disease, where long genes show increased vulnerability to distinct types of chromosomal damage.

Cancer, a prominent contributor to aging-related mortality, is commonly caused by DNA damage and consequent somatic mutations in tumor suppressors or protooncogenes. Many tumor suppressor genes reside at CFSs, regions known for their high frequency of DNA strand breaks and focal deletions [52,53]. These fragile sites often intersect with extremely long genes, which not only function as tumor suppressors [54] but also are associated with common aging-related disorders beyond cancer. This may imply a possible connection between the vulnerability of long genes to DNA damage and/or GLTD. Two examples include the *PARK2* gene of Parkinson's disease and a receptor of the Alzheimer's disease ApoE locus *LRP1B*, both acting as extremely long tumor suppressors at fragile sites FRA6E and FRA2F. Further review of the 20 longest human genes indicates that most map to CFSs and have dual roles in oncogenesis and disorders of the nervous system (Table 1). A significant knowledge gap exists regarding CFS vulnerability in postmitotic cells and in neurodegenerative disorders [55–64].

An extremely long gene may render local genomic organization prone to fragility. This effect has been recently ascribed to topologically associating domains (TADs), which are 3D units

Table 1. Top 20 longest genes and their mapping to CFSs

Rank	Gene	Gene length (bp)	Exon count	Exon length	Fragile site	Disorder/pathway
1	RBFOX1	2 473 538	20	3651	16p13.3[a]	Synaptic RNA splicing
2	CNTNAP2	2 304 996	24	9454	FRA7I	Autism spectrum
3	PTPRD	2 298 756	17	1697	–[b]	–
4	DMD	2 241 932	79	13 992	FRAXC	Muscular dystrophy
5	DLG2	2 173 323	28	7959	FRA11F	Schizophrenia, Parkinson's disease
6	CSMD1	2 059 619	70	14 417	FRA8B	Schizophrenia, cognitive function
7	MACROD2	2 057 828	17	4994	20p12.1[a]	Autism spectrum, attention deficit hyperactivity disorder (ADHD), schizophrenia
8	EYS	1 987 246	43	10 590	–	–
9	LRP1B	1 899 593	91	15 850	FRA2F	ApoE receptor
10	CTNNA3	1 851 180	18	10 696	FRA10D	–
11	ROBO2	1 743 269	27	5919	FRA3R	Axon guidance
12	NRXN3	1 697 918	21	12 048	FRA14C	Autism spectrum
13	PDE4D	1 553 082	17	2478	–	–
14	DAB1	1 551 956	15	5301	FRA1B	ApoE receptor signaling
15	GRID2	1 506 191	16	5783	FRA4G	Synaptic transmission
16	FHIT	1 504 182	10	3116	FRA3B	Tumor suppressor
17	AGBL4	1 491 100	14	2989	–	–
18	CCSER1	1 477 901	11	5847	–	–
19	GPC5	1 475 061	8	2943	FRA13D	–
20	CTNNA2	1 463 630	22	4349	FRA2E	Synaptic adhesion

[a]Recurrent deletion site (not a CFS) [107].
[b]The '–' indicates absence of known fragile site.

of the genome packing adjacent genes. At average lengths of ~300–900 kb in different cell types [55], a single TAD can accommodate multiple medium-sized genes but usually cannot completely contain even a single long gene in the megabase range. As a result, long genes often cross TAD boundaries, a feature that reportedly creates fragile sites [56]. Intronic expansion has promoted functional complexity and tissue specialization of genes that act in neurobiology. This may explain the extreme lengths of some neuronal and synaptic genes [57,58] and their possible contribution to the emergence of fragile sites, which can be seen as a double-edged sword.

DNA damage and somatic mutations undergo different dynamics in mitotic cells (e.g., cancer) and postmitotic cells (e.g. neurons). Cell division cycles effectively remove most of the DNA lesions following *de novo* DNA synthesis, unless they are tolerated and trigger somatic mutations. By contrast, postmitotic cells solely rely on DNA repair for life-long maintenance of gene expression, which makes such cells more vulnerable to DNA damage and GLTD. DNA lesions of various types occur in mammalian cells, estimated at up to 10^5 per cell each day. Spontaneous hydrolysis alone can generate approximately 10^4 abasic sites per day [65]. Besides the vulnerability of DNA to exogenous or endogenous genotoxic agents (e.g., high-energy photons, reactive metabolic byproducts), even mechanical stress has been reported to induce DNA damage [37].

Some DNA lesions are not recognized while others are irreparable and accumulate, or become subject to erroneous repair. The burden of somatic mutations is reported to increase, with ~10–20 single-nucleotide variants and approximately three small insertion–deletions per year in aging postmitotic neurons [66]. Interestingly, larger deletions seem to be depleted in late-life neurons of human brains, suggesting their stronger influence on cell survival [67]. Since neurons, synapses, and their relevant biological adhesion ontologies represent the most prominent function of long genes, we speculate that synaptic function may be highly sensitive to the effect of GLTD in aging [29] (Figure 3).

Since very long genes have exceedingly long introns, most likely DNA damage will occur in an intron. When a DNA lesion stalls an elongating RNA-polymerase for too long, the nascent RNA will engage with the complementary (and underwound) DNA, leading to very long R-loops, which in turn may stall the next RNA polymerase as well, aggravating the problem. The very long stretch of DNA–RNA hybrids, which are more stable than DNA–DNA hybrids, and the displaced single-stranded DNA, likely covered with replication protein A (RPA), may form a bulky and abnormal chromatin structure. Such R-loops are one of the explanatory mechanisms for DNA damage and chromosomal fragility at some sites [68]. In this context, a fragile site may interfere with the expression of long genes through RNA polymerase stalling and GLTD, or rather directly contribute to DNA strand breaks and gene copy number loss [69].

Long-read sequencing offers promising prospects for future research into uncharted territories at the single-cell resolution in neurodegenerative disorders. This technology enables simultaneous measurements of DNA damage and the calling of large insertions and deletions, copy number variations, and chromosomal rearrangements. A recent study described gene-length-dependent fusion events in Alzheimer's disease neurons, which may reflect frequent double-strand breaks in long genes and perhaps fragile sites [70]. Future investigation of DNA strand breaks, deletions, and rearrangements at the sites of long genes in conditions such as Alzheimer's and Parkinson's disease may offer new insights into their etiological drives. Most importantly, it remains to be determined how these vulnerabilities relate to GLTD (see Outstanding questions).

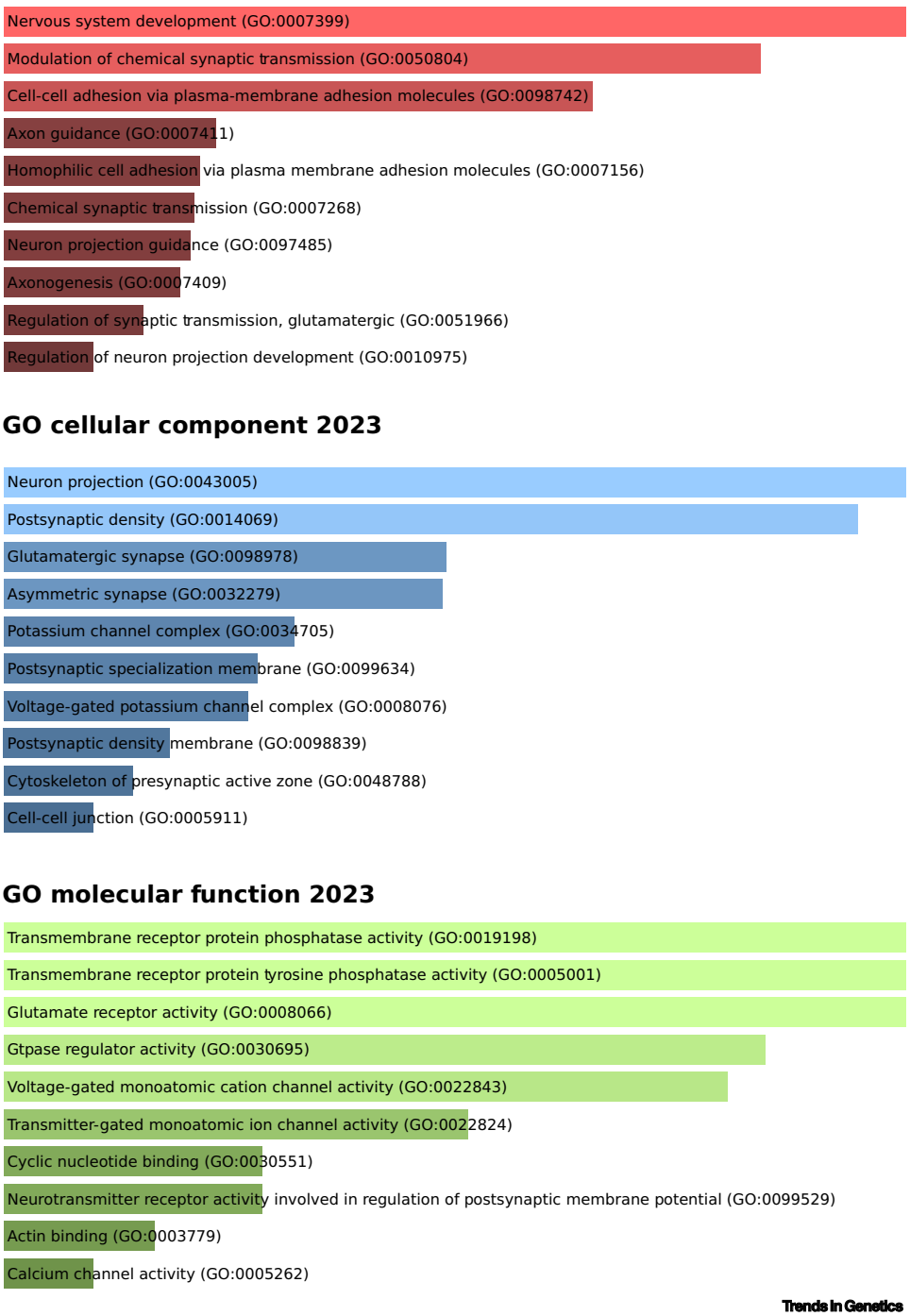

Figure 3. Gene Ontology (GO) terms overrepresented in the 500 longest genes in the human genome. The top ten GO terms associated with the longest genes (adjusted *P* value <0.001) align with key neuronal and synaptic functions and biological adhesion at transmembrane receptor complexes.

Length-dependent transcription beyond aging

Hitherto, DNA damage is the only demonstrated cause of GLTD in aging [25]. However, length-dependent regulation of transcription has also been observed elsewhere. It is presently unclear how these observations relate to GLTD in aging.

(i) Interference with the function of U1 small nuclear ribonucleoproteins (snRNPs) shows that longer genes are more susceptible to premature cleavage and polyadenylation [71] that is prevented by U1 snRNP telescripting [72–74]. Exposure of human cell lines to a heat shock produces an increase in the RNA Pol II elongation rate and premature termination resembling insufficient telescripting [75]. As recently shown, this also results in GLTD (supplemental figures in [75]). An additional role of U1 snRNP, as part of the RNA Pol II elongation complex, is increasing the speed of transcription through AT-rich introns, here facilitating the transcription of long genes [76]. Although not demonstrated to be mechanistically linked, RNA Pol II speed increases in animal aging [77], raising the question of whether premature polyadenylation sites are preferentially used in aging.

(ii) Decreased RNA Pol II speeds can lead to GLTD, demonstrating that not only increased speeds can lead to GLTD [75]. Specifically, in mice *in vivo* the introduction of a point mutation in RNA Pol II to decrease the elongation speed leads to embryonic lethality, reduced renewal of neuronal stem cells, alternative splicing, and GLTD [78]. This superficially opposes observations in *D. melanogaster* and *C. elegans*, where slowed transcriptional elongation increases lifespan by 10% and 20%, respectively [77]. While species-specific differences may exist, a complementary hypothesis is that GLTD restricts the effect of existing antiaging interventions, if maintenance of intermediate 'just-right' RNA Pol II speeds is required to permanently prevent GLTD (see Outstanding questions).

(iii) Ablation or downregulation of the multifunctional RNA-binding protein SFPQ in neurons and muscles has resulted in decreased transcription of very long (>100 kbp) genes [79,80]. SFPQ mediates CDK9 recruitment to the transcription elongation complex, which in turn activates RNA Pol II [79,80]. However, SFPQ is also involved in DNA double-strand break repair [81]. SFPQ is one of the most differentially spliced genes during human aging [82] and one of the most robustly downregulated genes during murine aging [83]. A custom bibliometric tool to identify under-studied areas of aging biology and strategies to approach them [83] prioritized SFPQ [83] and motivated one of us (T.S.) toward subsequent research into GLTD [24].

(iv) Dysregulation of RNA Pol II turnover, such as by inhibition of its polyubiquitination, that targets the protein for degradation also results in GLTD [84,85]. This appears to be an integral of part of TCR of transcription-blocking lesions [84,85].

(v) While there is presently no direct evidence to connect DNA methylation with GLTD, Rett syndrome [86], caused by mutations in methyl-CpG binding protein 2 (MeCP2), yields an increase of long transcripts. MeCP2 decreases the rate of transcriptional initiation of highly methylated long genes [87]. However, as mentioned earlier, the aging clock based on altered DNA methylation in specific genomic sites [88] is also accelerated in prematurely aging mouse mutants and patients carrying TCR defects [48], linking this phenomenon to DNA damage. As Rett syndrome patients have shortened life expectancy, typically restricted to their 40s to 60s [89], increased expression of long transcripts beyond the decrease encountered in aging may shorten health- and lifespan.

(vi) Inhibition of topoisomerase I has been demonstrated to lead to GLTD [90]. As inhibition of topoisomerase I maintains DNA strand breaks [91], GLTD following topoisomerase I inhibition may be functionally related to GLTD in aging [25].

(vii) TATA boxes are an optional element of promoters, whose strength is inversely correlated with gene length [92]. In a first approximation, this runs against current insights into GLTD in aging, where there is a decline of transcription over gene bodies [21].

Interventions against GLTD

Currently, it is yet to be understood whether GLTD is only a marker of aging or whether it also actively plays a role in the aging process itself [23–25]. We consider this to be the greatest outstanding question on GLTD (see Outstanding questions). To measure the potential impact of GLTD on aging, it would be necessary to identify interventions or experimental schemes that only affect GLTD. However, no such intervention or experimental scheme is currently known. To quantify the magnitude of their impact, we may further need to first apply them to animal models. Even in such studies, it would remain challenging to attribute effects toward aging to GLTD rather than any single subsets of genes that change their transcription as part of GLTD. Nevertheless, we cautiously suspect a causal contribution, as we observed that orthologs of the longest human genes increase lifespan in model organisms (i.e., increase lifespan following gain of function or decrease lifespan following loss of function), whereas orthologs of the shortest human genes shorten it [24].

While it remains to be thoroughly demonstrated whether GLTD is a cause rather than a consequence of aging (see Outstanding questions), further circumstantial support stems from the realization that, downstream of GLTD, genes involved in most of the hallmarks of aging become dysfunctional [24,25]. Similarly, age-associated signaling pathways such as IGF1, mTor, PGC1α, and TGFβ have demonstrated effects on RNA metabolism, and six of the seven biological pathways most robustly associated with age in transcriptomic data of human peripheral blood leukocytes relate to RNA metabolism [93]. Although initial evidence indicates that GLTD may be reversible and thus amenable for therapeutic amelioration, this needs to be more conclusively shown in duly designed prospective studies optimized for biosafety and effective delivery.

Interventions against GLTD are tempting as it is extremely difficult to improve DNA repair, in view of the complexity of at least six major and several minor repair pathways, which operate in balance and in coordination, involving hundreds of genes [37]. However, as mentioned earlier, 30% DR dramatically extended the lifespan of progeroid TCR mutants by 200% and strongly delayed all features of accelerated aging, most notably the neurodegeneration. This tremendous benefit correlated with a significant rescue of the GLTD [21]. GLTD in TCR mutants is due to accumulating DNA damage blocking transcription. The genome-wide reduced GLTD implies that DR must lower the DNA damage load, because DNA repair in the mutant is deficient. Diminished DNA damage explains why TCR mutants overrespond to DR as they are hypersensitive to DNA damage. DR may reduce endogenous DNA damage levels by metabolic redesign as part of the 'survival response', induced by attenuation of the IGF1/GH somatotrophic axis, which suppresses growth but boosts resilience. A similar response is observed in long-lived 'dwarf' mouse mutants [30,94,95]. It may be postulated that the survival response through IGF1 decrease plays a role in rescuing GLTD and thereby decelerates aging.

In senile neurodegenerative disorders, GLTD warrants rescuing of the expression and function of extremely long neuronal and synaptic genes that may be lost in aging (Table 1). For example, an underlying proteinopathy of Alzheimer's disease [5] may be the result of an asymmetrical loss of large subunits of protein complex in aging [96] and exposure of the remaining units such as APP to catabolism. Examples include APP-LRP1b [63] and CSMD1 (M.L. Baum, PhD thesis, Harvard University, 2018) and their engagement in the β1-integrin cell adhesion pathway of Alzheimer's disease.

Overall, we envision that further research into GLTD will facilitate the development of biomarkers and interventions for aging and help in the formulation of new models for aging-associated diseases.

Concluding remarks

A pervasive GLTD has been observed in aging by several independent research groups in diverse experimental settings. In the light of these new data, we suggest a reevaluation of previous studies and the formulation of a model of aging focused on long genes and their vulnerability to various insults. A strong etiological candidate for GLTD is the accumulation of transcription-blocking lesions in the aging DNA, but other causes must be explored to understand whether GLTD always is a subset DNA damage as an aging mechanism (see Outstanding questions). GLTD integrates well into existing insights about aging as it can be triggered by multiple factors that affect aging and by itself inherently preferentially affects genes implicated in aging [23–25] (Figure 4, modified after an excellent infographic by Cohen and colleagues [97,98], licensed under CC BY 4.0). These initial observations around GLTD invite the hypothesis that, over the passage of time, the different factors that accelerate aging may converge toward a common gene expression profile [99] characterized by GLTD. Several opportunities for future research remain to be explored (see Outstanding questions).

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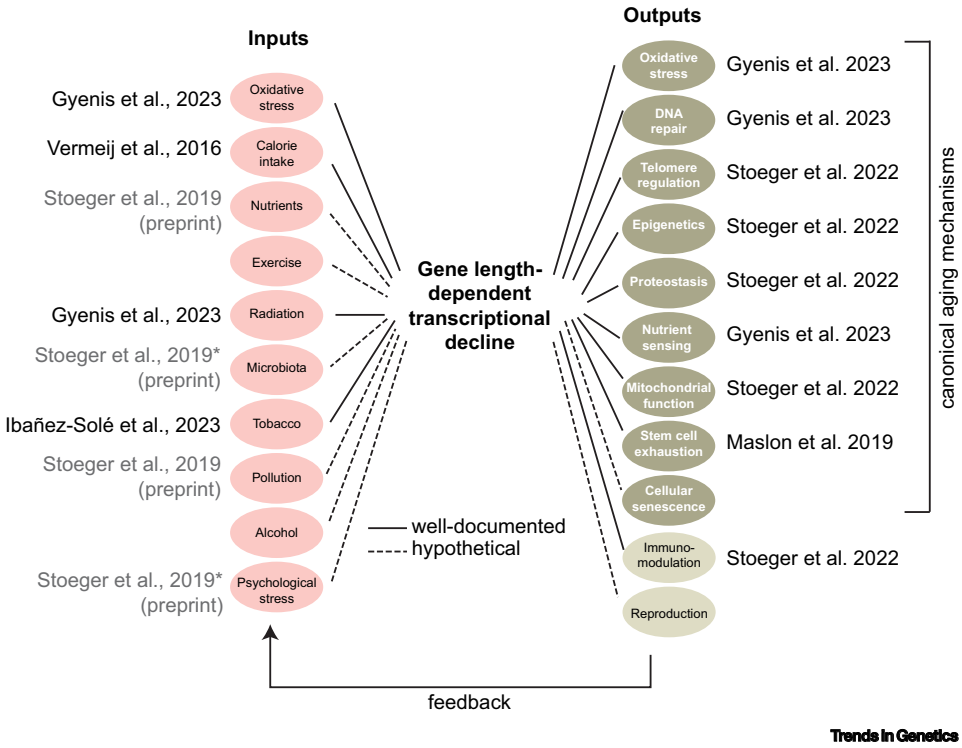

Figure 4. Gene-length-dependent transcriptional decline (GLTD) connects several known aspects of aging. GLTD connects inputs and outputs of various aging processes. This figure, adapted from [97,98] (licensed under CC BY 4.0), illustrates inputs and outputs of various aging-related phenomena connected via GLTD, which substitutes multiple, complex signaling pathways. References for the specific inputs and outputs are provided [21,24–26,78,106]. This figure includes references to an extended analysis contained in the preprint version [106] of a meta-analysis of transcriptional datasets. Asterisk marks indirect support (sleep deprivation and specific microbes).

Outstanding questions

What are the cellular and physiological consequences of GLTD? While GLTD is responsive to antiaging interventions and correlates with accelerated aging in transcription-coupled DNA repair mutants, demonstration of the net consequences of GLTD is experimentally challenging as it involves thousands of genes. Does GLTD contribute to aging? Could some of the consequences of GLTD even be beneficial to health or adaptive?

Is GLTD preventable? Hitherto, the presence of GLTD has been reported only in animals that demonstrate aging.

What is the relationship to RNA Pol II speeds? The latter have recently been shown to increase during aging. Surprisingly, in contexts other than aging, both slowed and increased RNA Pol II speeds have been reported to lead to GLTD.

Does GLTD scale with gene length or with motifs and chromosomal structures that scale with gene length?

Is GLTD a marker of cellular stress or insult-mediated active aging, a marker of accumulated age, or both? Most analyses of GLTD compare 'average' transcriptomes of young and old individuals. However, there also appears to exist interindividual variability as well as intertissue variability in GLTD. Does it relate to the high metabolic rates of tissues such as brain, heart, and skeletal muscle?

How is GLTD related to terminal differentiation? In mice, slowing of transcriptional elongation leads to GLTD and embryonic lethality by impairing the differentiation of embryonic stem cells toward the neuronal lineage by limiting their self-renewal.

Is there a correlation of cellular chronological age with GLTD phenotype? GLTD appears to be most pronounced in postmitotic neuronal tissues.

How does GLTD reconcile with other prevailing theories of aging? It remains, for instance, unclear how GLTD relates to evolutionary theories of aging or whether it always is a subset of DNA damage as an aging mechanism.

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Declaration of interests

The authors declare no conflicts of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT in order to ensure correct spelling and grammar. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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