RESEARCH HIGHLIGHT

Accelerated transcriptional elongation during aging impairs longevity

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Increased transcriptional errors in aged organisms have been demonstrated for multiple species; however, mechanistic details remained elusive. In a recent issue of *Nature*, Debès et al. reveal a novel form of aging-associated molecular damage, demonstrating that Pol II transcriptional elongation rate increases with age, presumably due to reduced nucleosomal density, whereas targeted reduction of Pol II speed can partially ameliorate this deleterious phenotype and extend lifespan in animals, such as roundworms and fruit flies.

Aging is associated with systemic accumulation of molecular damage leading to physiological decline, development of chronic diseases and increased mortality.[1] Among others, deleterious age-related changes include DNA lesions, mutations, epigenetic aberrations and chromatin reorganization, causing deterioration of crucial biological processes, such as gene regulation and protein synthesis.

During aging, animal cells undergo substantial remodeling of gene expression profiles.[2] Error rate during transcription also increases with age, affecting cellular proteostasis, shortening lifespan,[3] and impacting co-transcriptional gene regulation processes, such as alternative splicing. This results in the accumulation of non-functional mRNAs and changes in gene isoform abundance, which may lead to cellular senescence, insulin resistance and development of age-related diseases.[4] Although transcription plays a crucial role in the maintenance of cellular homeostasis, little has been known about mechanisms of aging-associated damage occurring during this process.

Recently, aging was shown to be accompanied by stalling of RNA polymerase II (Pol II) during transcriptional elongation of mRNAs, presumably caused by the accumulation of DNA lesions in gene bodies.[5] This defect may partially contribute to the global reduction of RNA synthesis, especially for long genes, leading to a generally increased relative expression of short genes with age.

Now, Debès et al.[6] examined in detail age-related changes in transcriptional elongation rate in various invertebrates and mammalian species. Since intronic RNA is generally spliced and degraded co-transcriptionally, it is possible to estimate the elongation rate based on the gradient of intron reads from RNA-seq data.[7] Slow Pol II takes more time to transcribe an intron, resulting in the accumulation of reads prior to splicing and more steep RNA density slope throughout the intron, while fast Pol II results in a lower read gradient due to rapid splicing and degradation of intronic RNA (Fig. 1). Employing this method, the authors observed an average increase in the transcriptional elongation rate with age in tissues of multiple species, including fruit flies (*Drosophila melanogaster*), roundworms (*Caenorhabditis elegans*), rats (*Rattus norvegicus*), mice (*Mus musculus*) and humans (*Homo sapiens*). Remarkably, this feature was shared by senescent human cells, and also confirmed with the traditional method of Pol II elongation rate estimation, utilizing reversible chemical inhibition of transcription combined with 4sU-based RNA-seq.[8] In contrast, lifespan-extending interventions, such as dietary restriction and mutations that inhibit insulin-IGF signaling, resulted in a lower elongation rate, suggesting that this feature may be associated with longevity.

To test this hypothesis, Debès et al. assessed the lifespan of fruit flies and roundworms carrying point mutations in the main Pol II subunit, which reduced its elongation speed. Indeed, mutants showed an extension of median lifespan by approximately 10% and 20% in *D. melanogaster* and *C. elegans*, respectively. Interestingly, mutant roundworms also exhibited higher pharyngeal pumping rates at older age, suggesting that their healthspan was also improved by slow Pol II. Since most pre-mRNAs are spliced co-transcriptionally, an optimal transcriptional elongation rate is required for the proper splicing.[7, 9] Consistently, the authors found more rare splice events and circular RNAs in aged *D. melanogaster* and *C. elegans*, indicating that the faster elongation rate during aging may diminish quality of RNA splicing (Fig. 1). In contrast, these types of damage were reduced by slow Pol II. Interestingly, rare splice events and circular RNAs were not enriched in tissues of old mammals, presumably due to a more efficient splicing regulation in long-lived species. Finally, similar to the other examined longevity interventions, mutations that decreased transcriptional elongation rate in invertebrates also resulted in fewer mismatches in transcribed mRNAs, pointing to a potential deterioration in proofreading capacity of fast Pol II.

To uncover possible mechanisms of the age-related increase in elongation rate, Debès et al. assessed nucleosome density in proliferating and senescent human cells, since chromatin structure can affect transcriptional elongation and splicing. Utilizing mononucleosomal DNA sequencing, they observed a significant decrease of nucleosome occupancy in gene introns of senescent cells along with lower precision of nucleosome positioning across gene body compared to proliferating cells. Interestingly, higher chromatin accessibility in senescent cells was accompanied by lower protein levels of histone H3. Histone expression may affect

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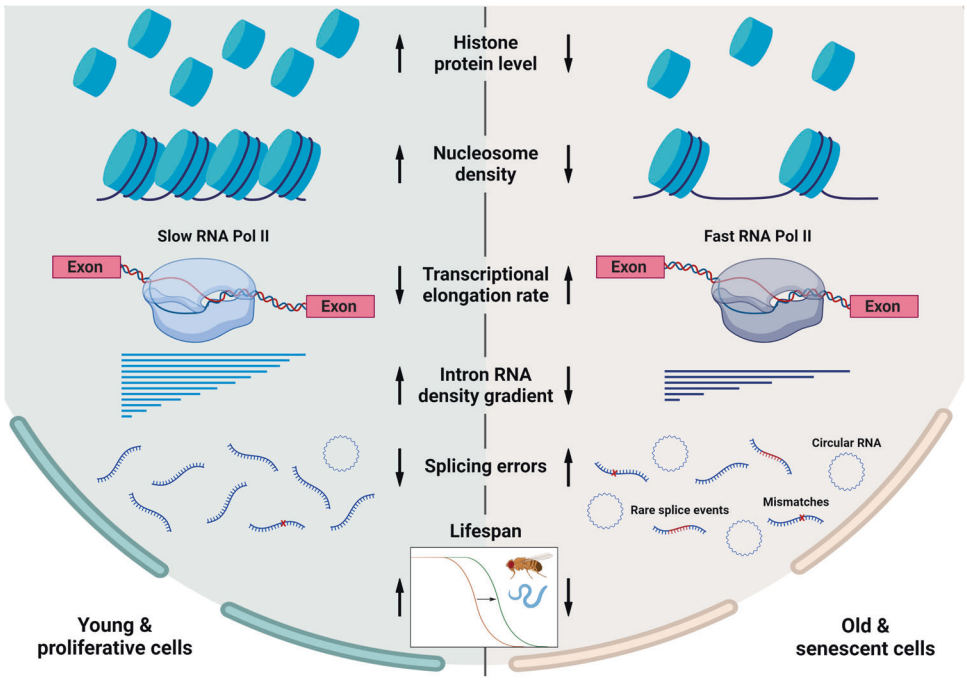

Fig. 1 Association of the transcriptional elongation rate with aging and longevity. RNA Pol II elongation speed is elevated in old and senescent cells, presumably due to reduced nucleosome occupancy partially driven by the decreased level of histones. Higher rate of elongation is associated with flatter intron RNA read gradient and accumulation of splicing errors. Mutated roundworms and fruit flies with slower elongation rate exhibit fewer splicing errors and increased lifespan.

chromatin structure and lifespan of various species. Thus, overexpression of histone H4 in *C. elegans* increased global compaction of both nuclear and mitochondrial chromatin, and extended worm lifespan by up to 25%.[10] Histone H4 also mediated the longevity effect of heat shock factor-1 overexpression in roundworms. In agreement with previous studies, Debès et al. showed that overexpression of histone H3 in *Drosophila* glial cells increased nucleosome density and lifespan of fruit flies. Consistent with the authors' hypothesis, overexpression of either histone H3 or H4 in human fetal lung fibroblasts (IMR90) led to a significant decrease of Pol II elongation speed together with reduced levels of senescence-associated markers, including β-galactosidase and p21. Therefore, lower nucleosome density in aged cells may be driven by lower expression of histones, resulting in higher chromatin accessibility and faster transcription elongation (Fig. 1).

Taken together, Debès et al. revealed a novel aging-associated mechanism of damage accumulation at the level of cellular transcription. Their results suggest that the higher average rate of Pol II elongation is a deleterious hallmark of aging shared across multiple invertebrates and mammalian species. This feature may be partially mediated by remodeling of nucleosome organization driven by lower histone levels, while interventions targeting elongation rate can extend animal's lifespan and ameliorate the detrimental aging-associated phenotype, pointing to new approaches for discovering geroprotectors. In the light of recent data,[5] it is intriguing to find out whether the higher elongation speed of Pol II with age discovered by Debès et al. may drive its stalling during transcription, or whether these hallmarks of aging share a common cause. Future studies may shed light on the relationship between various transcriptional changes co-occurring with age and reveal the impact of different forms of molecular damage on the loss of cellular homeostasis and the development of aging-associated pathologies.

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ADDITIONAL INFORMATION

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