NEURAL REGENERATION RESEARCH
www.nrronline.org

Regulation of aging by NELF-A and RNA polymerase II elongation

Chin-Tong Ong*, Zhen-Kai Ngian

Epigenetic regulation of aging: Aging is defined as the gradual decline of physiological function and cellular integrity, causing organismal vulnerability to age-onset diseases and morbidity. Studies in different animal models have led to the identification of twelve aging hallmarks that shared several features: its age-associated manifestation, how experimental manipulation of individual hallmark may alter the trajectory of aging, as well as their interdependence during the process of aging. These hallmarks include genomic instability, telomere attrition, loss of proteostasis, disabled macro-autophagy, deregulated nutrient-sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, chronic inflammation, dysbiosis, altered intercellular communication and epigenetic alterations (Lopez-Otin et al., 2023).

Epigenetic mechanisms control gene expression patterns without changes made to the underlying DNA sequences. The chromatin states of eukaryotic DNA sequences are highly dynamic during aging and may be regulated by myriads of epigenetic mechanisms such as DNA methylation, nucleosome occupancy, specific post-translational modifications at histone tails, non-coding RNAs, the association of DNA with nuclear lamina and specific nuclear proteins (Wang et al., 2022). Epigenetic mechanisms also play crucial roles in neurogenesis and brain homeostasis, with aberrant alterations often linked to different neurodegenerative disorders (Yao et al., 2016).

RNA polymerase II pausing at promoter is a key epigenetic mechanism: Promoter-proximal pausing of RNA polymerase II (RNAPII) occurs at widespread gene promoters in eukaryotes and is mediated by evolutionarily conserved negative elongation factor (NELF) complex (Core and Adelman, 2019). The release of stalled RNAPII for productive elongation and mRNA transcription is a tightly regulated process that involves the phosphorylation of the NELF complex by the post-transcription elongation factor-b (P-TEFb). Phosphorylation facilitates the eviction of the NELF complex and the subsequent release of RNAPII for productive transcription (Core and Adelman, 2019). *In vivo* studies indicated that NELF-mediated RNAPII pausing at the promoter is required for robust and coordinated gene activation in response to different stimuli. In accordance, perturbation of different NELF subunits or P-TEFb can impact transcriptional programs in a cell-type specific manner. For instance, *NELF* depletion in *Drosophila* S2 cells and larvae affected genes that are enriched for heat-shock and immune responses (Gilchrist et al., 2012; **Figure 1A**). Genetic disruption of *NELF* in murine macrophages enhanced transcription of AP-1 target genes and anti-inflammatory cytokine IL-10 (Yu et al., 2020). In human cells, P-TEFb regulates the transcription of key DNA damage response genes and multiple non-coding RNAs such that its interference provoked cellular hypersensitivity to genotoxic stress (Bugai et al., 2019; **Figure 1B**). As many of these target genes are linked to the process of aging, it is plausible that regulation of RNAPII pausing by NELF complex and P-TEFb may play a key role in animal healthspan.

Lower NELF-A promotes stress resistance and animal longevity: Genes encoding different NELF subunits are essential for animal viability. To test if NELF regulates animal healthspan, heterozygous *NELF-A* mutant *Drosophila* was first backcrossed ten generations to obtain offspring with highly similar genetic backgrounds. Heterozygous *NELF-A* mutant flies, which express lower levels of NELF-A protein, have extended lifespans with improved locomotor activity and resistance against oxidative stress when compared to their wild-type control siblings (Ngian et al., 2021). Conversely, ectopic *NELF-A* expression led to a drastic reduction in lifespan, indicating that *Drosophila* healthspan is dependent on NELF-A dosage. Interestingly, RNAi depletion of NELF-A in human neuroblastoma SH-SY5Y cells promotes survival against hydrogen peroxide-induced cytotoxicity, suggesting that NELF-A plays a conserved role in conferring resistance against oxidative stress (Ngian et al., 2021).

To define the organs by which NELF-A may regulate fly healthspan, tissue-specific RNAi depletion was performed using the GeneSwitch system. Pan-neuronal RNAi knockdown of NELF-A led to a modest yet statistically significant increase in fly lifespan and their locomotor activity, suggesting that NELF-A may impact longevity through its regulatory activity in the animal brain (Kounatidis et al., 2017). Transcriptomic analyses of head tissues harvested from Day 50 old flies revealed significant differential expression of several hundred genes between control siblings and heterozygous *NELF-A* mutants. Aged control siblings have elevated expression of genes that are enriched for different immune and defense responses, indicative of excessive age-onset inflammations that underlie neurodegeneration and morbidity in older flies (Kounatidis et al., 2017). On the other hand, *NELF-A* mutants exhibit pro-longevity transcriptional signatures with upregulation of genes that are involved in heat-shock response, protein folding, and different signaling pathways (Ngian et al., 2021).

Mechanisms of NELF-A regulation and its impact on aging hallmarks: The transcriptome of old heterozygous *NELF-A* mutant head tissues shows remarkable similarity to *Drosophila* S2 cells where RNAi depletion of NELF-A facilitates the release of RNAPII pausing and productive transcription of many heat-shock protein (Hsp) genes (Gilchrist et al., 2012). Chromatin immunoprecipitation showed higher RNAPII occupancy in the gene bodies of *Hsp68* and *Hsp83* genes in heterozygous *NELF-A* mutants which correlated with their elevated mRNA expression when compared to control siblings. In accordance, pan-neuronal RNAi depletion of NELF-A or NELF-E subunits led to elevated expression of *Hsp* genes such as *Hsp27* in fly brains. These results suggest that lower NELF-A in heterozygous mutants facilitates the release of RNAPII pausing and elevates the expression of many *Hsp* genes, which are well-characterized molecular chaperones that modulate protein homeostasis and reactive oxygen species production (**Figure 1A**). Consistent with this notion, brains harvested from aged heterozygous *NELF-A* mutants have significantly lower reactive oxygen species levels when compared to control siblings. In addition, tissue lysates prepared from heterozygous *NELF-A* mutant heads revealed significantly lower levels of double-stranded DNA breaks marker γH2AX and an insoluble fraction of Refractory to Sigma P, Ref(2)P protein. Ref(2)P, the fly ortholog of mammalian p62 protein, is a major component of aged-onset protein aggregates which label ubiquitinated proteins for autophagic or proteosomal degradation. These results indicate that the reduction of reactive oxygen species, DNA damage, and protein aggregation in the brain by elevated HSPs might contribute to the improved locomotor activity and the extended healthspan observed in heterozygous *NELF-A* mutant flies (Ngian et al., 2021).

Unexpectedly, there is also a reconfiguration of repressive histone modification marks between aged *NELF-A* mutant flies and their control siblings. Immunofluorescence and immunoblot experiments revealed significant elevation of repressive H3K9me2 and H3K9me3 heterochromatin marks in the brain sections of old *NELF-A* mutant flies as compared to their control siblings (Ngian et al., 2021). Accumulated evidence demonstrated the loss of H3K9me2/3 heterochromatin marks during aging can lead to de-repression of retrotransposons and genome instability (Lopez-Otin et al., 2023). Consistent with these observations, genome-wide profiling of head tissues uncovered the presence of several thousands of H3K9me2 peaks, differentially elevated in heterozygous *NELF-A* mutants, which were distributed across known retrotransposons integration sites. Furthermore, enrichment of H3K9me2 at these loci in heterozygous *NELF-A* mutants correlates with the lower expression of retrotransposons such as *Gypsy* and *DIVER* when compared to their control siblings. Taken together, these results suggest that lower NELF-A levels may promote H3K9me2-mediated silencing of transposable elements during aging (**Figure 1A**).

Glycine N-methyltransferase (*Gnmt*) converts S-adenosylmethionine into S-adenosylhomocysteine while S-adenosylmethionine is utilized as the methyl donor to methylate histone H3K9 residues by histone methyltransferase. The reduced *Gnmt* expression and the concomitant increased S-adenosylmethionine concentration in aged heterozygous *NELF-A* mutants likely contribute to the maintenance of H3K9me2-enriched heterochromatin in their heads/brains. However, it remains unclear if *Gnmt* is a direct transcriptional target of the NELF complex.

Changes in transcriptional elongation impact aging: While elevated *HSPs* expression may account for the reversal of several aging hallmarks and the extended healthspan in heterozygous *NELF-A* mutants, the maintenance of H3K9me2/3-marked heterochromatin during aging suggests that NELF-A may also impact the epigenetic landscapes directly through transcriptional control (Ngian et al., 2021).

Indeed, the productivity and rate of transcription have been implicated in the regulation of the aging process by several studies. For instance, the H3K36me3 histone modification mark is deposited by elongating RNAPII and enriched at the gene bodies of actively transcribed genes. Besides regulating RNAPII elongation, H3K36me3 is crucial to prevent cryptic transcription and aberrant splicing. In worms and *Drosophila*, the H3K36me3 level at gene bodies is necessary to suppress age-dependent alteration in mRNA expression (Pu et al., 2015). Consequentially, depletion of the H3K36me3 mark by mutating methyltransferase *met-1* can result in the loss of gene expression

stability during aging and a shortened animal lifespan (Pu et al., 2015).

Genome-wide profiling of young and aged 2 years mouse liver with different types of RNAPII antibodies showed that about 40% of elongating RNAPII are stalled by the accumulation of stochastic endogenous DNA damage along the active genes (Gyenis et al., 2023; **Figure 1C**). The stalling of RNAPII lowers productive transcription, skews transcriptional output in a gene-length-dependent manner, and specifically impacts genes that are associated with different aging-related pathways (Gyenis et al., 2023; **Figure 1D**). On the other hand, RNA-sequencing of total RNA depleted of ribosomal RNA from five metazoan species revealed an age-dependent increase in the transcriptional elongation speed and errors by RNAPII (Debes et al., 2023; **Figure 1D**). Dietary restriction and attenuated insulin/insulin-like growth factor signaling, two pro-longevity interventions, reversed the rate of RNAPII elongation and aberrant splicing events during aging. Similarly, expression of RNAPII variants with reduced elongation speed or additional histone components, which impede the rate of RNAPII elongation, increased lifespan in *Drosophila*, and reduced senescent in human cells (Debes et al., 2023). Taken together, these studies indicate that the regulation of RNAPII elongation is one of the fundamental molecular mechanisms underlying animal aging.

Concluding remarks: Fine-tuning the rate and processivity of RNAPII elongation is crucial to ensure the integrity of transcription such that its dysregulation is tightly linked to the process of aging (Debes et al., 2023; Gyenis et al., 2023). Moreover, controlled release of paused RNAPII by both NELF-A and P-TEFb at selected genes elicits pro-survival transcriptional responses that promote proteostasis and resistance against oxidative stress, as well as attenuate DNA damages and inflammations (Bugai et al., 2019; Yu et al., 2020; Ngian et al., 2021). Collectively, these results indicate that regulation of RNAPII dynamics can impact different hallmarks of aging. High-resolution mapping and characterization of major factors that govern RNAPII elongation in healthy and neurodegenerative brain tissues will provide mechanistic insight into its roles in neurological aging and disorders (Yao et al., 2016).

This work was supported by core funding provided by Temasek Life Sciences Laboratory (to CTO).

Chin-Tong Ong*, Zhen-Kai Ngian
Temasek Life Sciences Laboratory and Department of Biological Sciences, National University of Singapore, Singapore, Singapore (Ong CT)
Department of Genetics, Harvard Medical School, Boston, MA, USA (Ngian ZK)
Institute of Molecular and Cell Biology, Agency for Science, Technology and Research, Singapore, Singapore (Ngian ZK)
***Correspondence to:** Chin-Tong Ong, PhD, chintong@tll.org.sg.
https://orcid.org/0000-0002-5450-4760 (Chin-Tong Ong)

Date of submission: July 5, 2023
Date of decision: September 15, 2023
Date of acceptance: September 29, 2023
Date of web publication: November 8, 2023

https://doi.org/10.4103/1673-5374.387989
How to cite this article: *Ong CT, Ngian ZK (2024) Regulation of aging by NELF-A and RNA polymerase II elongation. Neural Regen Res 19(7):1415-1416.*

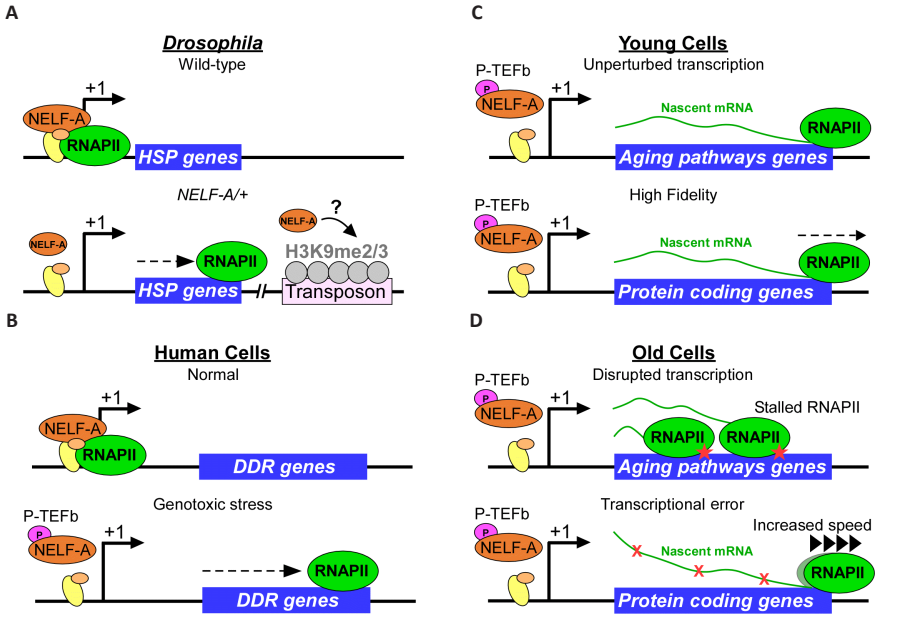

Figure 1 | **Regulation of releasing paused RNAPII and its elongation impacts aging.**
(A) Lower NELF-A level in heterozygous mutants promotes *Drosophila* healthspan by facilitating productive transcription of *HSPs* genes and ensures H3K9me2/3-mediated silencing of transposable elements through uncharacterized mechanisms. The grey circle denotes nucleosomes carrying H3K9me2/3 modifications. Yellow and birch ovals denote other transcription factors. NELF complex contains multiple subunits and is represented by NELF-A orange oval. Adapted from Ngian et al. (2021). (B) Phosphorylation of NELF-A complex by P-TEFb, depicted by a violet circle, leads to its eviction and facilitates RNAPII transcription of DDR genes upon genotoxic stress in human cells. "P" indicates phosphorylation of NELF-A subunit. Adapted with permission from Bugai et al. (2019). (C) In young cells, RNAPII elongates unperturbed across the genes to ensure complete mRNA transcripts (top) with high fidelity (bottom). (D) In old cells, stochastic DNA damage across gene bodies, denoted by a red star, leads to the stalling of RNAPII and the transcription of incomplete mRNA transcripts (top). The increased speed of RNAPII elongation also introduces transcriptional errors, denoted by red "X", and aberrant splicing events in the mature mRNA species (bottom). Adapted with permission from Debès et al. (2023) and Gyenis et al. (2023). Created using CANVAS (version 12). DDR: DNA damage response; H3K9me2/3: Di/Trimethylation of lysine 9 on histone H3; HSP: heat shock proteins; NELF-A: negative elongation factor A; P: phosphorylation; P-TEF-b: positive transcription elongation factor; RNAPII: RNA polymerase II; +1: transcription start site.

References

Bugai A, Quaresma AJC, Friedel CC, Lenasi T, Duster R, Sibley CR, Fujinaga K, Kukanja P, Hennig T, Blasius M, Geyer M, Ule J, Dolken L, Barboric M (2019) P-TEFb activation by RBM7 shapes a pro-survival transcriptional response to genotoxic stress. Mol Cell 74:254-267.

Core L, Adelman K (2019) Promoter-proximal pausing of RNA polymerase II: a nexus of gene regulation. Genes Dev 33:960-982.

Debès C, Papadakis A, Grönke S, Karalay Ö, Tain LS, Mizi A, Nakamura S, Hahn O, Weigelt C, Josipovic N, Zirkel A, Brusius I, Sofiadis K, Lamprousi M, Lu YX, Huang W, Esmaillie R, Kubacki T, Späth MR, Schermer B, et al. (2023) Ageing-associated changes in transcriptional elongation influence longevity. Nature 616:814-821.

Gilchrist DA, Fromm G, dos Santos G, Pham LN, McDaniel IE, Burkholder A, Fargo DC, Adelman K (2012) Regulating the regulators: the pervasive effects of Pol II pausing on stimulus-responsive gene networks. Genes Dev 26:933-944.

Gyenis A, Chang J, Demmers J, Bruens ST, Barnhoorn S, Brandt RMC, Baar MP, Raseta M, Derks KWJ, Hoeijmakers JHJ, Pothof J (2023) Genome-wide RNA polymerase stalling shapes the transcriptome during aging. Nat Genet 55:268-279.

Kounatidis I, Chtarbanova S, Cao Y, Hayne M, Jayanth D, Ganetzky B, Ligoxygakis P (2017) NF-kappaB immunity in the brain determines fly lifespan in healthy aging and age-related neurodegeneration. Cell Rep 19:836-848.

Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G (2023) Hallmarks of aging: an expanding universe. Cell 186:243-278.

Ngian ZK, Lin WQ, Ong CT (2021) NELF-A controls Drosophila healthspan by regulating heat-shock protein-mediated cellular protection and heterochromatin maintenance. Aging Cell 20:e13348.

Pu M, Ni Z, Wang M, Wang X, Wood JG, Helfand SL, Yu H, Lee SS (2015) Trimethylation of Lys36 on H3 restricts gene expression change during aging and impacts life span. Genes Dev 29:718-731.

Wang K, Liu H, Hu Q, Wang L, Liu J, Zheng Z, Zhang W, Ren J, Zhu F, Liu GH (2022) Epigenetic regulation of aging: implications for interventions of aging and diseases. Signal Transduct Target Ther 7:374.

Yao B, Christian KM, He C, Jin P, Ming GL, Song H (2016) Epigenetic mechanisms in neurogenesis. Nat Rev Neurosci 17:537-549.

Yu L, Zhang B, Deochand D, Sacta MA, Coppo M, Shang Y, Guo Z, Zeng X, Rollins DA, Tharmalingam B, Li R, Chinenov Y, Rogatsky I, Hu X (2020) Negative elongation factor complex enables macrophage inflammatory responses by controlling anti-inflammatory gene expression. Nat Commun 11:2286.

C-Editors: Zhao M, Sun Y, Qiu Y; T-Editor: Jia Y