TRANSCRIPTION

Spurious transcription: A hallmark of aging?

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ABSTRACT

Epigenetic changes are a driver of senescence and occur during aging. Herein, a new study in Nature Aging shows how chromatin-mediated loss of transcription fidelity, previously shown to happen in yeast and worms, also occurs in mammalian cells and could constitute a new hallmark of senescence and aging.

MAIN TEXT

Senescence is a stress response characterized by a stable cell cycle arrest, changed morphology, and secretion of a pro-inflammatory secretome called the SASP (Senescence Associated Secretory Phenotype) [1]. As we age, senescent cells accumulate and contribute to tissue dysfunction and progression of age-associated diseases. [2].

Chromatin remodelling and epigenetic modifications are key drivers of the transcriptional changes needed to execute the various senescence phenotypes. For example, chromatin remodelling helps establishing domains (termed senescence-associated heterochromatin foci, SAHFs) that repress E2F target genes and keep senescent cells arrested [3], while remodelling of superenhancers regulates the expression of the SASP [4, 5]. In addition to epigenetic changes that actively drive senescence, there is an increasing realisation that the loosening of tight epigenetic control observed during senescence and aging is also important. For example, retrotransponsable elements and endogenous retroviruses get derepressed and contribute to activate the inflammatory phenotypes associated with senescence and aging [6, 7]. However, the complex interplay between chromatin dysregulation and the altered transcriptome of senescent and aging cells remains unclear. Here, Sen and colleagues [8] propose that spurious transcription is also a consequence of the epigenetic drifting observed in senescence and aging and suggest that cryptic transcription might constitute a novel hallmark of senescence and aging.

These findings build upon previous work showing that trimethylation of lysine 36 of histone H3 (H3K36me3) protects against intragenic cryptic transcription occurring during aging in *C. elegans* and *S. cerevisiae* [9]. To investigate cryptic transcription in mammalian cells and tissues, Sen and colleagues [8] take advantage of advanced methods for precision run-on RNA sequencing (PRO-seq and PRO-Cap) that allow the detection of newly formed transcription starting sites (TSS) on IMR-90 human lung fibroblast undergoing replicative senescence. This initial survey allowed them to identify cryptic TSSs in both proliferating and senescent cells.

By studying chromatin accessibility and analysing histone modifications, the authors identified that the cryptic TSS arise predominantly at intragenic enhancer sites in both proliferating and senescent cells. Upon senescence induction, these enhancers are converted into promoter-like elements, as suggested by a changed chromatin profile that includes increased H3K4me3 and H3K27ac. In addition, cryptic TSSs found in senescent cells show increased histone acetylation (H3K5ac, H3K18ac, H3K23ac, H3K122ac), decreased DNA methylation, improved chromatin accessibility, and enhanced deposition of the histone variant H2A.Z (Figure 1). In contrast, cryptic TSSs found specifically in proliferating cells arise from intragenic enhancers that become delicensed in senescence.

As a possible diver for this increase in cryptic transcription, the authors identify members of the Activator Protein-1 (AP-1) transcription factor (TF) family. AP-1 factors are known to play pioneering roles and contribute to enhancer remodelling during senescence [4]. To validate their function as a driver of cryptic transcription, the researchers overexpressed BATF, an AP-1 family member, in IMR-90 cells. Overexpression in proliferating cells leads to increased cryptic transcripts of Senescent-not-Proliferating TSS as well as reduced cell proliferation. Further experiments will be needed to show whether AP-1 are the key pioneering factors driving cryptic transcription on senescence or whether they are just able to cause a senescence-like phenotype by alternative mechanisms and the increase on cryptic transcription is a consequence of that.

An important question is whether cryptic transcription is just a side effect of the epigenetic changes occurring during senescence and aging or if it also has functional

consequences as suggested by the extension of lifespan observed in *C. elegans* when cryptic transcription is suppressed [9]. Sen and colleagues [8] demonstrate that cryptic transcripts do not suppress or interfere with the transcription of the corresponding canonical TSS upon senescence induction. Gene ontology (GO) analysis on the cryptic transcripts highlights pathways associated with cell adhesion, cell signalling, and cell differentiation. But enrichment of GO-terms linked to senescence from cryptic transcripts might be a consequence of the increased expression of these genes and cannot be taken as an indication of function. The authors speculate that age-related intragenic transcripts add to the transcriptional noise of aged and senescent cells and might use up energy and resources in already weakened and fragile cells, further weakening transcriptional integrity. But in contrast to the de-repression of retrotransponsable elements and endogenous retroviruses that activate cGAS/STING and inflammation [6, 7], there is no evidence of cryptic transcription being sensed as damage and contributing to senescence.

Finally, given that an increase of cryptic transcription has been observed during aging in *C. elegans* and *S. cerevisiae* [9], the authors interrogate whether the spurious intragenic transcription observed on senescent cells extends to aged mouse livers. Intriguingly, cryptic transcripts are detected in the livers from old female mice but not in livers of male mice. IMR90 human fibroblasts, the cells used for the senescence experiments are also derived from a female donor. Whether cryptic transcription is a sexual dimorphic phenotype, or the discrepancies are linked to difference in the age of the old mice (the females analysed were 3 month older than the males), remains to be established. Since the proportion of senescent cells present in most old tissues is usually small, it will also be interesting to investigate whether the signal for cryptic transcription observed in the liver comes exclusively from those senescent cells.

Overall, the authors conclude that aberrant intragenic transcription is a feature of senescence and aging. It is tempting to speculate that emerging rejuvenation strategies such as partial reprogramming [10] could supress these cryptic transcripts. Whether aberrant transcription has functional implications for senescence and aging, and whether its suppression might be relevant in a therapeutic context remains unclear but worthy of future investigations.

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COMPETING INTERESTS

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REFERENCES

1. Gorgoulis, V. *et al.* Cellular Senescence: Defining a Path Forward. *Cell* **179**, 813-827 (2019).
2. Baker, D.J. *et al.* Naturally occurring p16(Ink4a)-positive cells shorten healthy lifespan. *Nature* **530**, 184-189 (2016).
3. Criscione, S.W., Teo, Y.V. & Neretti, N. The Chromatin Landscape of Cellular Senescence. *Trends Genet* **32**, 751-761 (2016).
4. Martinez-Zamudio, R.I. *et al.* AP-1 imprints a reversible transcriptional programme of senescent cells. *Nat Cell Biol* **22**, 842-855 (2020).
5. Tasdemir, N. *et al.* BRD4 Connects Enhancer Remodeling to Senescence Immune Surveillance. *Cancer Discov* (2016).
6. De Cecco, M. *et al.* L1 drives IFN in senescent cells and promotes age-associated inflammation. *Nature* **566**, 73-78 (2019).
7. Liu, X. *et al.* Resurrection of endogenous retroviruses during aging reinforces senescence. *Cell* **186**, 287-304 e226 (2023).
8. Sen, P. *et al.* Spurious intragenic transcription is a hallmark of mammalian cellular senescence and tissue aging. *Nat Aging* (2023).
9. Sen, P. *et al.* H3K36 methylation promotes longevity by enhancing transcriptional fidelity. *Genes Dev* **29**, 1362-1376 (2015).
10. Mahmoudi, S., Xu, L. & Brunet, A. Turning back time with emerging rejuvenation strategies. *Nat Cell Biol* **21**, 32-43 (2019).

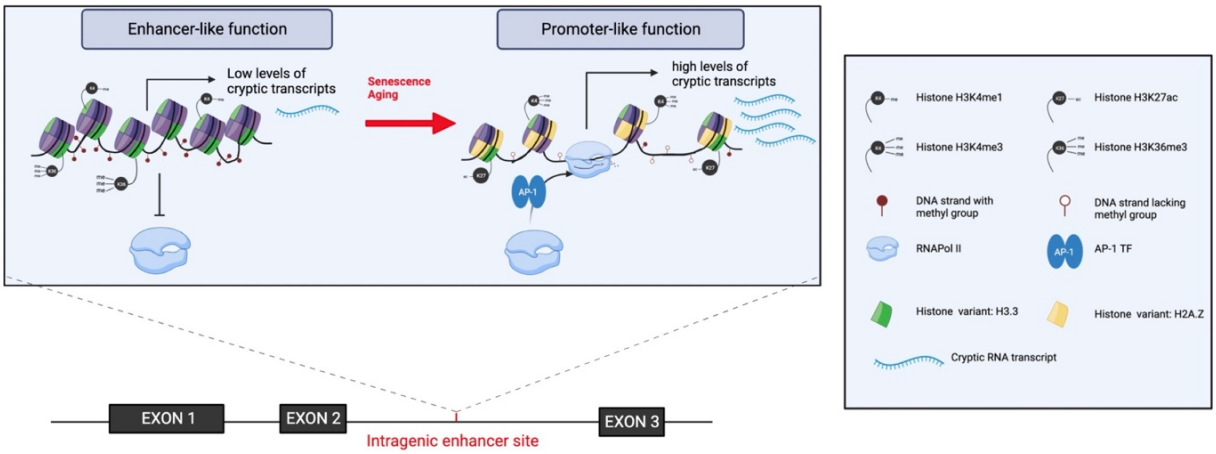


Figure 1. Conversion of intragenic enhancer to promoters drives cryptic transcription in senescent cells. Intragenic enhancers are converted into promoter-like elements, characterised by increased histone acetylation (in H3K27ac, H3K18ac, H3K23ac, H3K122ac), H3K4me3 decreased DNA methylation, improved chromatin accessibility, and enhanced deposition of the histone variant H2A.Z. Transcription factors of the AP-1 family are thought to drive this process. The enhancer to promoter-like element conversion generates cryptic TSS responsible for spurious intragenic transcription in senescent cells.