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High-throughput chromatin screens to identify targets of senescence and aging

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This article describes the work presented in *Aging Science Talks Seminar Series* on April 14th, 2020. The findings summarized here were discovered by employing high-throughput epigenetics-focused screens and parallel aging assays. The screens pointed to two fundamental concepts in chromatin dysregulation that occurs during senescence and aging; the loss of transcriptional fidelity and the remodeling of the regulatory landscape that alters gene expression programs.

The yeast genome sequencing was completed in 1996 [1] and the human genome in 2003 [2]. The post-genome era ushered in a new paradigm of scientific study, forward genetic screens. In contrast to low-throughput gene-by-gene analysis, screens enabled the examination of all genes in the genome in a single assay. The yeast gene deletion library was completed in 2002 and afforded researchers the ability to perform standard assays in high-throughput, and identify genes that conferred sensitivity or resistance to interventions [3]. The technology quickly evolved to include mammalian systems through the design of large whole-genome panels of siRNAs, shRNAs, compound libraries and recently, CRISPR guide RNAs to induce loss-of-function [4]. These types of functional genomics screens were very popular in the cancer biology field and led to high impact discoveries of novel genes and cellular pathways for rational drug design [5–7].

However, the challenges of performing large-scale screens quickly became clear. siRNA screening would involve an arrayed format where individual hairpins would have to be spotted into separate wells. Multiple siRNAs against a single protein would have to be included in the assay for robust identification of targets. Consequently, to handle such a multi-well assay, expensive liquid handlers had to be employed which quickly exceeded the

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Declaration of competing interest

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budgetary reach of labs. This challenge was overcome by pooled format screens where shRNAs were introduced into cells as a mixed population and then deconvoluted post-assay through next-generation sequencing [4]. Deconvolution was possible because shRNAs unlike siRNAs, integrate into the genome, and hence serve as unique molecular barcodes for identification. Of course, this meant tweaking the experimental conditions to make sure only one shRNA infected one cell [8]. The pooling strategy was also adopted by yeast biologists to design entire mutational libraries [9]. These advances where the hairpin/mutation is integrated in the genome also meant that the perturbation was permanent and therefore could potentially be used in long-term assays such as aging. Yet, another challenge in screening was encountered when the scientific community realized whole-genome screens yielded a multitude of targets that presented diffuse information. Thus, there was much interest garnered to build more targeted screens. For example, shRNAs targeting genes in a specific pathway or subcellular location.

Technological advances in screening methodologies fueled my interest to build and use screens to understand chromatin pathways in senescence and aging. As a graduate student I studied chromatin remodeling in yeast while my postdoctoral work focused on replicative senescence and aging. I combined these two interests into one by executing two chromatin-based screens for factors that affected senescence and aging. In the Aging Science Talk Seminar Series, I described the key findings from two screens that revealed two thematic concepts in epigenetics in the context of aging.

The first screen was performed in budding yeast *Saccharomyces cerevisiae* using a library developed in the Boeke laboratory [9]. Created using a synthetic approach, this library of 486 systematic histone H3 and H4 mutants presented three features for high-throughput screening; (1) it contained a diverse set of substitution and deletion mutations, (2) each mutant was barcoded enabling identification by microarray hybridization or next-generation sequencing and (3) the mutants were integrated into the genome and thus provided stability in long-term assays. We used this library to perform an aging assay established in the Guarente lab in 1996 isolating old mother cells [10]. The assay takes advantage of the fact that yeast daughter cells perform *de novo* cell wall synthesis during budding and consequently if the cell surfaces of mother cells were biotin labeled, the label would be retained on those mothers through multiple rounds of cell division. Subsequent enrichment of labeled cells by streptavidin pull-down would allow isolation of pure populations of mother cells. By repeating the purification several rounds, we were able to isolate very old yeast cells [11]. We then isolated genomic DNA, performed barcode amplification followed by microarray and next-generation sequencing to identify specific barcodes with the notion that depletion in old yeast meant a short lifespan while enrichment meant longer lifespan. The library was designed to include two control strains: the short lived *sir2*Δ and the long-lived *SIR2-OE*. As expected, the barcode corresponding to the *sir2*Δ strain was depleted while that for *SIR2-OE* was enriched [12]. In this way, by carefully measuring barcode abundances, we identified multiple known and novel histone residues that regulate lifespan in yeast. We focused on the short-lived mutant of H3K36 (lysine 36 on histone H3) for further mechanistic studies. H3K36 is modified by the methyltransferase Set2p that can add up to three methyl groups co-transcriptionally [13]. Deletion of the methyltransferase Set2p resulted in a shorter lifespan while deletion of the only H3K36me3 demethylase, Rph1p,

extended lifespan. We thus concluded that the trimethylation of H3K36 confers protective lifespan benefits in yeast. H3K36me3 is normally found on gene bodies and prevents aberrant initiation of transcription (also known as cryptic transcription) [14]. We adopted a high-resolution RNA-sequencing method to directly measure cryptic transcription in yeast genes and found age-associated increases in cryptic transcription. Genes that had the highest incidence of cryptic transcription showed loss of H3K36me3 on their gene bodies by chromatin immunoprecipitation followed by sequencing (ChIP-seq). Importantly, when H3K36me3 was preserved by deletion of Rph1p, cryptic transcription was suppressed. To investigate if this was a conserved phenomenon, we went on to assess cryptic transcription in old worms [11], senescent cells (unpublished), and aged mouse livers (unpublished). To our surprise, we were able to detect cryptic transcripts across different species and consequently believe that loss of transcriptional fidelity is a conserved hallmark of aging.

The second screen used a related screening strategy, pooled shRNAs targeting epigenetic factors to investigate their effects on senescence [15]. In this case, we packaged the shRNAs into lenti-viruses and infected proliferating IMR90 cells at a low multiplicity of infection to ensure ~1 shRNA per cell. We then passaged the cells until senescence was established. We next isolated genomic DNA, amplified barcodes (in this case the shRNA sequence itself) and performed next-generation sequencing on proliferating and senescent cells. Hairpin abundance measurements revealed ~100 shRNAs enriched in the senescent cells that were proposed to delay the onset of senescence. We carefully selected targets from this list to validate in senescence assays and chose the p300 protein for follow-up studies. p300 is a well-known histone acetyltransferase that is recruited at enhancers to deposit H3K27ac and activate gene expression [16]. We performed ChIP-seq of a panel of histone acetylation marks (including H3K27ac) and found strong enrichments across the genome in senescent cells. In support, a mass-spectrometric estimation of histone modifications also revealed a general hyperacetylated state in senescent cells. Most the of the H3K27ac peaks were at enhancer locations and we identified *de novo* enhancer formation (both typical and super enhancer types [17]) in senescence. We further showed that these new enhancers were actively transcribing enhancer RNAs and reversibly regulated senescence gene expression. Our work establishes that formation of new regulatory elements in the genome might be one way of enforcing senescence. Preliminary analysis of published aged mouse ChIP-seq data [18] also alluded to new enhancer formation during liver aging (unpublished).

In conclusion, we believe that high-throughput focused screens can be adopted for long-term aging assays and may reveal novel targets of aging. Our chromatin-focused screens identified transcriptional paradigms and regulatory element formation during aging. Recent developments in CRISPR/Cas9 editing and epigenome modulation promise to expand the current capabilities of high-throughput screens and their use in aging biology.

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