T(ell)TALE signs of aging

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Transcriptional activator-like effectors (TALEs) have emerged as powerful tools for genome editing. A recent study published by *Cell Research* reports that fusion of thioredoxin to TALEs unlocks their full potential in live-cell imaging to accurately analyze genome instability, telomere attrition and epigenetic alterations that are hallmarks of aging.

Now commonly accepted as the major risk factor for a wide range of chronic diseases, aging is accompanied by correlative and/or causative changes in the genome and epigenome [1]. While genome sequencing and whole genome monitoring of epigenetic changes are possible, these approaches are not viable *in vivo* or in live cells in culture. Accurate tools in live single cells to track chromosome dynamics in three dimensions (3D) could dramatically enhance fundamental knowledge of the aging process. Here, Ren *et al.* [2] develop a promising new technique to monitor the genome in live cells and exploit it to understand aging-related changes at locations previously implicated in modulation of lifespan.

Fluorescent *in situ* hybridization (FISH), fluorescent Lac or Tet operator and repressor systems, engineered zinc finger proteins and the CRISPR/Cas9 system have each been used to visualize genome organization [3–6]. However, each of these are hampered by restrictive labeling to certain sequences, low signal-to-noise ratio, poor transfection efficiency, and/or formation of non-specific protein aggregates. These factors also limit the utility of current editing techniques to visualize changes in genome topology.

Transcriptional activator-like effectors (TALEs) are proteins secreted by *Xanthomonas* that have 33–35 amino acid repeats, each of which specifically recognizes and binds to a DNA base [7]. They function as transcriptional activators in plant species that are targets of infection by the bacteria. Importantly for this study, TALEs can be engineered to bind to specific DNA sequences and are functionally validated in mammalian cells [7, 8]. Compared to CRISPR/Cas9, TALEs overcome technical difficulties, such as low transfection efficiency due to the high molecular weight of Cas9 and low signal-to-noise ratio. Fluorescent TALEs have recently been used for live imaging of centromeres and telomeres to visualize chromatin dynamics *in vitro* and *in vivo* [9, 10]. However, TALEs tend to aggregate, resulting in their inaccurate localization. They are thus not optimal as a live-cell imaging tool to visualize human telomeric and centromeric DNAs, or other sequences in the genome.

Ren and colleagues [2] have now taken a creative approach to improve upon conventional fluorescent TALEs by fusing the proteins to thioredoxin. Thioredoxin catalyzes steps in oxidative protein folding, facilitating the reduction of disulfide linkages in other proteins [11]. Thioredoxin is often fused to other proteins during recombinant expression as a means to assist their protein folding and prevent aggregation, which it can achieve independently of its latter activity in some cases. It is this redox activity that may be most beneficial in the case of TALEs. By fusing fluorescent TALEs to thioredoxin (TTALEs), Ren *et al.* [2] found a dra-

matic improvement of signal-to-noise ratio in imaging studies.

TTALEs were assessed in a variety of contexts, including not only human cancer cell lines, but also human embryonic stem cells, iPSCs, differentiated cells, and mouse cells both *in vitro* and *in vivo*. In all cases, TTALE-based imaging was found to be comparable to 3D-FISH and better than the CRISPR/Cas9 system in imaging quality and transfection efficiency.

Theoretically, TTALEs can be used to track any region of the genome, but Ren *et al.* focused on three repeated regions all linked to aging in this initial study: telomeres, centromeres and ribosomal DNA (rDNA), as well as a specific gene locus, *MUC4*. Their success with this latter locus emphasizes the potential utility of the TTALE system for a wide range of experimental questions.

Several genomic loci have been linked to aging, including telomeres, centromeres and rDNA. Consistent with numerous reports of telomere shortening with aging, Ren *et al.* found that telomere TTALE signals were reduced in mesenchymal stem cells (MSCs) from three human cellular models of aging. Using one of these models, MSCs derived from Werner helicase (WRN)-deficient embryonic stem cells, the telomere TTALE signals were found to co-localize with γ -H2AX foci marking DNA damage, indicative of telomere-associated genome stress. Finally, telomere attrition was tracked successfully in multiple tissues from telomerase-deficient mice, showing that the TTALE system can be used successfully *in vivo*. WRN-deficient MSCs also

show indications of age-related changes at centromeres, with more dispersed TTALE signals correlated with aberrant transcriptional activation.

Whereas TTALE signals at telomeres and centromeres generally corroborate prior findings, the studies examining rDNA repeats and their residence, the nucleolar organizer region (NOR), break new ground, in part because this region of the genome had previously not been amenable to live-cell imaging. Interestingly in a variety of contexts, rDNA repeat signals diminished, including models of aged MSCs and peripheral blood cells isolated from aging humans. These findings indicate a high rate of genome instability at the rDNA with aging, and may mirror that reported in replicatively aging yeast cells [12]. Further study needs to be conducted in both yeast and mammals, but these initial findings open the possibility that the rDNA repeats may be a common source of genome instability and a driver of aging across diverse eukaryotes.

These findings emphasize the utility of TTALEs to understand genomic

events with aging, but the possibilities extend far beyond. For instance, TTALEs may provide a means to study pathologies with single nucleotide polymorphisms, such as autism-related disorders. Moreover, since TTALEs label gene loci in spite of dynamic chromatin changes during mitosis, they could also be invaluable in examining chromosomal and cell cycle aberrations in cancer.

TTALEs promise to provide a robust platform for imaging chromatin dynamics under physiological conditions, as well as a potential tool for genome editing in therapeutic approaches to various pathologies. While it remains to be determined whether TTALEs influence chromatin dynamics in real time or alter transcription states of target loci, the future seems bright for these new players in genome imaging.

Reshma Taneja¹,
Brian K Kennedy^{1, 2, 3}

¹Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117597; ²Department of Biochemis-

try, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117597; ³Buck Institute for Research on Aging, Novato, CA 94945, USA

Correspondence: Brian K Kennedy
E-mail: bkennedy@buckinstitute.org

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