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Review

LncRNAs: the missing link to senescence nuclear architecture

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During cellular senescence and organismal aging, cells display various molecular and morphological changes. Although many aging-related long noncoding RNAs (lncRNAs) are highly associated with senescence-associated secretory phenotype, the roles of lncRNAs in senescence-associated nuclear architecture and morphological changes are just starting to emerge. Here I review lncRNAs associated with nuclear structure establishment and maintenance, their aging-related changes, and then focus on the pervasive, yet underappreciated, role of RNA double-strand DNA triplexes for lncRNAs to recognize targeted genomic regions, making lncRNAs the nexus between DNA and proteins to regulate nuclear structural changes. Finally, I discuss the future of deciphering direct links of lncRNA changes to various nuclear morphology changes assisted by artificial intelligence and genetic perturbations.

Highlights

Nuclear morphology changes during senescence and may potentially underlie other molecular changes.

LncRNAs play architectural roles in the nucleus and change in abundance during aging and senescence.

RNA-dsDNA base pairing facilitates aging-associated lncRNAs in their interactions with nuclear proteins and DNAs.

A future direction is to combine artificial intelligence and CRISPR to directly fine-map lncRNAs' roles in nuclear architecture during aging.

Long noncoding RNAs (lncRNAs) in aging at a glance

It is now well established that cellular senescence is a causal event of organismal aging and that genetic, chemical, or immune clearance of senescent cells enhances overall health and extends lifespan in mice [1–6]. In addition to permanent cell cycle arrest, the most prominent features of cellular senescence are progressively and dramatically increased inflammatory cytokine secretion, termed **senescence-associated secretory phenotype** (**SASP**; see Glossary) [7–9], contributing to inflammaging at the organismal level [10–12]. The second most common but very early event is the formation of H3K9 trimethylation (H3K9me3) foci in the nucleus, which are called **senescence-associated heterochromatin foci** (**SAHF**) [13–15]. These two events are not isolated; rather, one enhances the other.

The majority of **lncRNAs** are transcribed at low levels and are less evolutionarily conserved than coding genes. However, those that are highly expressed are often evolutionarily conserved in sequence and potentially conserved in function; in particular, the aging-related expressed lncRNAs compared with non-aging-related lncRNAs are more evolutionarily conserved and potentially more functionally conserved [16]. A large fraction of the aging upregulated lncRNAs are regulated by inflammatory response, such that the NF-κB binding motif is a top enriched transcription factor binding motif on these lncRNAs, and a significant number of them also feed back to regulate NF-κB signaling and inflammaging [16–19]. Blocking these lncRNAs through dampening SASPs can serve as 'senomorphics' to mitigate the contagiousness of cellular senescence. These regulatory element-associated lncRNAs have relatively low abundance and tend to go up with stress and aging to promote SASP [16], whereas **architectural lncRNAs** are usually very long and, when induced or repressed with stress and aging, disrupt nuclear architectures and in turn lead to impaired cellular homeostasis and functions. In the following section, I summarize the changes of nuclear structures during aging and senescence, focusing on the lncRNAs reported to associate with these processes, followed by a specific and perhaps prevalent mechanism of lncRNAs in regulating one particular nuclear architecture, the RNA-DNA triplexes.

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LncRNAs associated with nuclear morphology and structural changes in aging and senescence

Compared with SASP, less well studied are the nuclear morphological changes during cellular senescence; yet, they are equally prominent and often start very early and incrementally progress during the course of senescence [20]. In fact, just like the human face [21,22] and brain morphology [23,24] are reliable biological age markers, nuclear morphology can be a reliable biomarker of aging and cellular senescence. Especially now with deep neural network technologies, the accuracy of recognizing a senescent cell by morphology can reach 100% [25] or near 100% [26], exceeding any other known senescence or aging biomarkers. This concurs with the notion that structure as the foundation, together with function and regulation, are the three essential and hierarchical components of a living system [27]. Structure or form enables function, which in turn enables regulation and fine-tuning. They are hierarchically established during development following that order and often deteriorate in the reverse order during degeneration and aging toward irreversibility. Below I describe different aging- and senescence-associated morphological changes of the nucleus and lncRNAs involved with these processes, if known.

Nuclear envelope

During aging and senescence, **nuclear envelope** ruffles and sheds nuclear DNA into cytoplasm (cytoplasmic chromatin fragments), all of which can be induced by the gradual loss of the lamin matrix in senescent cells [28]. XIST [29] and KCNQ1OT1 [30,31] are two of the lncRNAs known to be localized to nuclear periphery and are essential in tethering their target chromosomes or genomic regions to **nuclear lamina** (Figure 1). KCNQ1OT1 reduction induces senescence through activation of **transposon elements** (**TEs**) and likely their detachment of TEs from nuclear lamina [32].

Nucleolus

During aging and senescence, the **nucleolus** progressively increases in size and is a reliable biomarker for aging in many model organisms and humans and is even predictive of lifespan in *Caenorhabditis elegans* [33]. In human and mouse cells, overexpression and depletion of transcripts of **Alu**/B1 repeat elements increases and decreases nucleolus size, respectively [34]. Many nucleolus-localized, rDNA loci-generated lncRNAs have been shown to be upregulated by various but specific stresses and to change the rDNA loci in *cis* to a repressive state. These include the rDNA promoter-associated RNA (pRNA) [35], promoter and preRNA antisense (PAPAS) [36], and the intergenic spacer RNAs [37]. Additionally, the rDNA loci-associated snoRNA containing lncRNA LoNA, which inhibits ribosome biogenesis, is upregulated in an Alzheimer's disease mouse model and decreased by neuronal activity, and its reduction can rescue learning deficiency in these mice [38]. SLERT is another snoRNA containing lncRNA that regulates the open and closed state of nucleolus protein DDX21 and phase separation within the nucleolus, and thus the size of the nucleolus [39,40]. X-linked lncRNA Firre has also been shown to anchor the inactivated X chromosome to the nucleolus periphery [41], organize interchromosomal interactions, and form nuclear foci by binding to nuclear matrix protein hnRNPU in mouse embryonic stem cells [42]. However, whether SLERT or Firre changes or functions in stress response or aging has not been explored. Other than the lncRNAs that regulate ribosome biogenesis, as noted previously, lncRNA KCNQ1OT1 has also been shown to localize to the nucleolus periphery in addition to the nuclear periphery [30,31] (Figure 1).

Nuclear speckles

The abundant lncRNA MALAT1 forms the scaffold of **nuclear speckles**. A recent study using super-resolution imaging finds that the concatenated m^6A residues on MALAT1 form a scaffold to recruit m^6A reader YTH-domain-containing protein 1 (YTHDC1) to nuclear speckles and that

Glossary

Alu: a type of long interspersed repetitive DNA elements in the primate genome, similar to the B1 elements in rodents.
Architectural lncRNAs: lncRNAs involved in forming or maintaining nuclear structures.
ChIRP: chromatin isolation by RNA purification, a technique to detect double-strand chromatin DNAs that interact with an RNA.
Chromatin compartments and TADs: frequently contacting or interacting chromatin regions revealed chromosomal conformation capture techniques, such as Hi-C analysis. TADs are topologically associated domains and are relatively smaller and enclosed in much larger compartments, with A compartments in active chromatin state and B compartments in repressive states.
CLIP-seq: cross-linking immunoprecipitation followed by high-throughput sequencing is a method used to detect RNA targets of RNA-binding proteins.
Hi-C: high-throughput chromosome conformation capture technique to map long-distance interactions between chromosomal DNAs.
L1: a type of long interspersed repetitive DNA elements in mammalian genomes. Among them, the youngest subfamily still encodes transposases, enabling L1 and Alu elements to make new insertions in the genome.
Lamina-associated domains (LADs): large genomic domains identified by LMNB1 DNA adenine methyltransferase identification (DamID) or chromatin immunoprecipitation (ChIP).
Long noncoding RNA (lncRNA): refer to >200-bp long RNA that has no coding potential.
Nuclear envelope: the double membrane separating the nucleus from the cytoplasm disrupted by nuclear pores. The interior side of nuclear envelope is coated by the lamin matrix.
Nuclear lamina: the structural meshwork underlining the inner surface of nucleus. The major protein components of the nuclear lamina are intermediate filament proteins lamin A/C and B, among which lamin B1 (LMNB1) is exclusively localized to nuclear lamina.
Nuclear speckles: membraneless condensates in the nucleus formed by serine/arginine-*rich* (SR) splicing factors

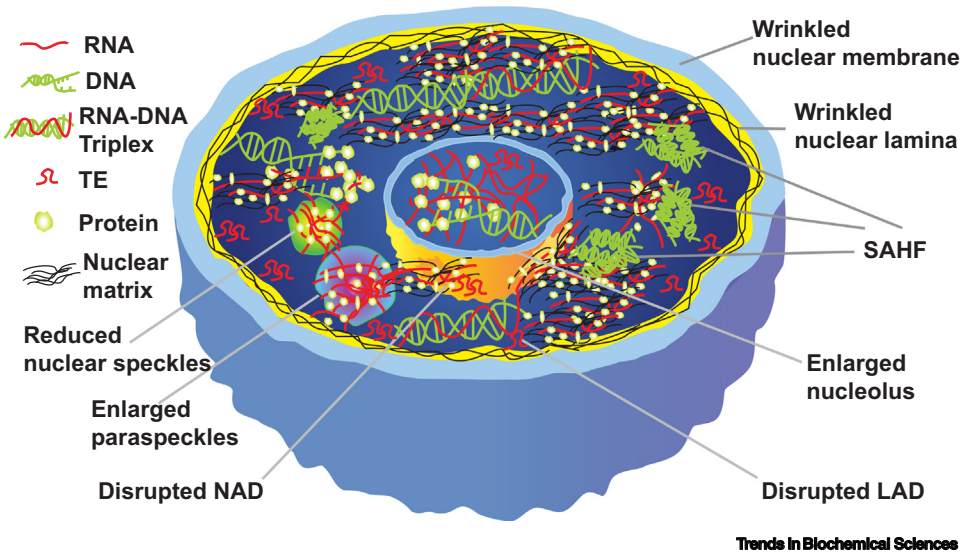

Figure 1. Typical nuclear morphology changes during aging and cellular senescence are often associated with changes in architectural long noncoding RNAs (lncRNAs). These include the increase of NEAT1 in paraspeckles; the decrease of MALAT1 in nuclear speckles; the increase in various rDNA triplex-forming lncRNAs in the nucleolus upon stress; the loss of KCNQ1OT1, which results in the detachment of its triplex forming transposon element (TE) DNAs from heterochromatic lamin-associated domains (LADs) and nucleolus-associated domains (NADs); and derepression of evolutionarily young transposon elements. No lncRNA has yet been shown to localize to the senescence-associated heterochromatic foci (SAHF). Aging-upregulated lncRNAs are mostly regulatory lncRNAs, regulated by and regulate senescence-associated secretory phenotypes (SASPs). In senescent cells, the TE transcripts increase dramatically, and overexpression of Alu elements results in increased nucleolus size. Although the precise subnuclear localization of the TE transcripts are largely unknown, KCNQ1OT1 that binds and suppresses TEs is associated with nuclear actin filament proteins.

the recognition of MALAT1-m^{6}A by YTHDC1 is essential to maintain the genomic binding sites of nuclear speckles [43]. Consistent with its role in splicing, MALAT1 has been shown to bind pervasively to active chromatin [44]. MALAT1 expression is known to decrease during WI-38 fibroblast senescence, and artificially reducing its level induces senescence [45] (Figure 1). Interestingly, MALAT1 translocates to a distinct nuclear body named the heat shock-inducible noncoding RNA-containing nuclear body in mammalian cells upon heat shock stress [46].

Paraspeckles

Similar to MALAT1, as the backbone of **paraspeckles**, NEAT1 also directly binds active chromatin [44]. NEAT1 paraspeckles are upregulated in neuronal stress response [47,48] and upon virus infection [49]. NEAT1 RNA increases during brain aging and compromises memory and other brain functions [50] (Figure 1) and has been found to be a marker of frailty at the single-cell level in human blood [51]. NEAT1 has also been reported to be required for the formation of stress-induced TDP43 nuclear bodies and phase separation [52].

Chromatin compartments

Chromatin is organized into A/B compartmentalization and **topologically associating domains** (**TADs**) hierarchically at multimegabase and hundreds of kilobases scales, respectively [53,54]. During cellular senescence, the 3D genome is rearranged with both TAD fusion and fission and stronger long-distance interactions [13,55–57]. However, during late senescence, there is a gain of short-distance interactions and a loss of long-distance interactions [58]. The loss of HMGB2 very early in senescence induces CTCF clustering and rearrangement of TAD boundaries [57].

attached to the abundant lncRNA MALAT1.

Nucleolus: the ribosomal RNAs and several lncRNAs targeting the ribosomal DNAs together with the RNA binding proteins form the basis of this membraneless organelle within the nucleus, which is the site of ribosome biogenesis.

Nucleolus-associated domains (NADs): genomic domains identified by nucleolus fractionation followed by sequencing, composed of the centromeric satellite repetitive sequences in constitutive heterochromatic state.

Paraspeckles: membraneless condensates in the nucleus formed by transcripts of the lncRNA NEAT1 (nuclear paraspeckle assembly transcript 1), which sequester more than 40 paraspeckles proteins, including DBHS (*Drosophila* behavior human splicing) family of nuclear factors, NONO, SFPQ, and PSPC1.

RNA-dsDNA Hoogsteen base pairing: a type of noncanonical base pairing that occurs between an RNA and dsDNA, which involves the formation of hydrogen bonds between the purine base (adenine or guanine) of one nucleotide and the Watson–Crick face of another purine base.

Senescence-associated heterochromatin foci (SAHF): H3K9 trimethylation (H3K9me3) foci in the nucleus early in senescence.

Senescence-associated secretory phenotype (SASP): increased inflammatory cytokine secretion by senescent cells that contribute to inflammaging.

Transposon elements (TEs): mobile repetitive elements in the genome.

TADs also allow the segregation of senescence activated and inactivated enhancers to be separately targeted by bZIP (e.g., C/EBP) transcription factors and basic helix-loop-helix (e.g., TCF21) transcription factors, respectively [55]. During senescence, the B-to-A compartment switch enriches for SASP gene enhancers [55], consistent with the frequent aging upregulation of SASP enhancer RNAs [16].

Lamina-associated domains (LADs)

LADs are often in B compartments and largely marked by repressive chromatin marks, in particular H3K9me2/3 [59]. Artificial depletion of LMNB1 in mouse embryonic stem cells induces LAD disassociation from the nuclear periphery [60,61]. During fibroblast cell senescence, LMNB1 globally decreases, and LMNB1 binding to LAD is reduced, whereas knocking down LMNB1 cooperatively with overexpression of HMGA1/2 induces the formation heterochromatin foci in the nucleus [62] (Figure 1).

Senescence-associated heterochromatin foci (SAHF)

SAHF often contain DNA sequences from both coding regions and noncoding regions and bear heterochromatin marks such as H3K9me3 and HP1 proteins [13–15] (Figure 1). The high-mobility group A (HMGA) proteins, such as HMGA1/2, accumulate on the chromatin of senescent fibroblasts and as essential structural components of SAHFs and cooperate with p16 to promote SAHF formation [62,63]. Similar to lamin B1 knockdown [62], knocking down lncRNA KCNQ1OT1 induces the formation of SAHFs [32]. However, so far, it is not known whether lncRNAs exist inside SAHFs.

Nucleolus-associated domains (NADs)

NADs are composed of centromeric satellite repetitive sequences in constitutive heterochromatic state. Unlike LADs, whose tethering mechanisms to the nuclear lamina are well studied, little is known about the tethering mechanism of NADs to the nucleolus periphery [64]. During cellular senescence, it seems to be the first change at the chromatin level, starting with early opening and becoming continuously more open and exposed with the progression of senescence [65,66]. Consistently, distension of satellite heterochromatins occurs earlier and more commonly than SAHF [67].

Nuclear actin skeleton

Like the cytoplasmic actin network being responsible for the shape of the cell, the nuclear actin skeleton is at least partially responsible for the shape of the nucleus. During mitotic exit, inhibiting nuclear actin polymerization impairs the cell size increase and chromatin decondensation and renders unstructured nuclear surface morphology independent of actin-nucleating ARP2/3 complex [68]. Nuclear actin has been shown to be required for efficient Pol II transcription by promoting phase-separated transcription factories. In this case, the nascent transcripts are part of these phase-separated droplets [69]. Although during double-strand DNA break the nuclear ARP2/3 drives the clustering of the damaged DNA for homology-directed repair [70]. Actins also associate with heterochromatins and relocalize damaged heterochromatic DNA breaks for repair [71,72]. Consistent with these observations, RNA pulldown followed by mass spectrometry analyses found that the KCNQ1OT1 lncRNA, a guardian against transposon derepression and genome instability, associates with actin filament proteins and DNA repair complexes through its 5′ and 3′ halves, respectively [32].

Nuclear matrix and ribonucleoprotein network

Nuclear matrices are filamentous meshes consisting of actin, matrins, and heterogeneous nuclear ribonucleoproteins (hnRNPs), lncRNAs, repeat elements, and unspliced transcripts

[73]. Several nuclear matrix components have been functionally delineated [74]. A naturally occurring, dominant negative point mutation in human PIT1(R271W) results in the loss of association to the matrin-3-rich network and targets gene activation, which can be rescued by retethering of the mutant Pit1 protein to the matrin-3 network, demonstrating an essential role of the matrin-3-rich network in gene regulation [75]. The repeat-rich C0T-1 hnRNAs (predominantly consisting of 5′ truncated **L1** repeat RNAs) tightly associate with euchromatins, dependent on functionally intact hnRNP-U/SAF-A matrix protein, and loss of C0T-1 hnRNAs results in chromatin condensation [76]. However, the changes of nuclear matrix and nuclear actin skeleton during aging and senescence have not been studied.

Architectural and regulatory lncRNAs with triplex-forming capabilities in aging

As described in the preceding text, lncRNAs are essential components, even the backbone of many nuclear structures, such as nuclear speckles and paraspeckles, or organize the 3D genome; thus, these lncRNAs are also called 'architectural lncRNAs' [77]. Given the flexibility and multivalency of lncRNAs, they, together with RNA binding proteins (RBPs), often promote the formation of nuclear condensates and membrane-less structures through phase separation [78,79]. For example, among many aging-related lncRNAs, MALAT1 and NEAT1, together with their binding partners, condense into nuclear speckles and paraspeckles, respectively [80], and NEAT1 also serves as a sponge to soak up nuclear protein factors [81]. A variety of age-relatedly changed RNAs also nucleate and form other nuclear bodies [82], including nuclear stress bodies [83]. Additionally, dysregulation of several lncRNAs contribute to yet other nuclear foci formation directly or indirectly [84–86], although their relevance to aging and senescence is unclear, except for KCNQOT1 to SAHF [32]. LncRNAs may modulate the chromatin structures and phase separation not only by interacting with RBPs but also through forming dynamic RNA-DNA triplexes, and these RNAs can sometimes be regarded as regulatory lncRNAs because of their sequence-specific targeting and potentially reversible interactions with DNAs, and, if changed during the aging process, they can trigger a downstream effect on their DNA targets.

RNA-DNA Hoogsteen base pairing triplex target lncRNA to chromatin

LncRNA can sequence-specifically form a triplex with double-strand DNA (dsDNA) through **Hoogsteen base pairing**, which occurs at C:GC, U:AT, and G:GC triads parallelly and A:AT, G:GC, and U:AT antiparallelly over a stretch of nucleotides (often >18) [87,88] (Figure 2A). **RNA-dsDNA triplex** formation has been known for a few lncRNAs, such as telomere interacting TERRA, which is generated from the reverse telomeric sequence, rDNA interacting pRNA, PAPAS, and the centromere transcribed MajSat [89], which all induce heterochromatic epigenetic states. Most of them, including the aforementioned transcription-activating NFkBMARL-1 [16], act *in cis* on the chromatin state of the region from whence they are transcribed [90] or *in trans* to one or two specific gene promoters nearby [91].

In contrast, the fairly abundant nuclear lncRNA KCNQ1OT1 is able to induce DNA methylation and transposon repression *in trans* at thousands of sequence-specific evolutionarily young repetitive DNA elements genome-wide [32]. This *trans* targeting is guided by an RNA-dsDNA Hoogsteen base-pairing triplex formed between the repeat-rich region of KCNQ1OT1 and the target DNA and simultaneous binding to HP1 . The Hoogsteen base-pairing sequences in KCNQ1OT1 are mostly encoded by the L1 repeats in the KCNQ1OT1 gene, which is 91 kb long and contains a nearly 50-kb repeat-rich region at its 3′ half (Figure 2B). Deleting the repeat-rich region of KCNQ1OT1 alone leads to activation of KCNQ1OT1 targeted Alu, L1, and satellite repeat elements and L1 retrotransposition, and repression of KCNQ1OT1 results in spontaneous cellular senescence [32]. Thus, the RNA-dsDNA triplex-forming sequence in KCNQ1OT1 serves as a mammalian guiding RNA mechanism to achieve sequence specificity

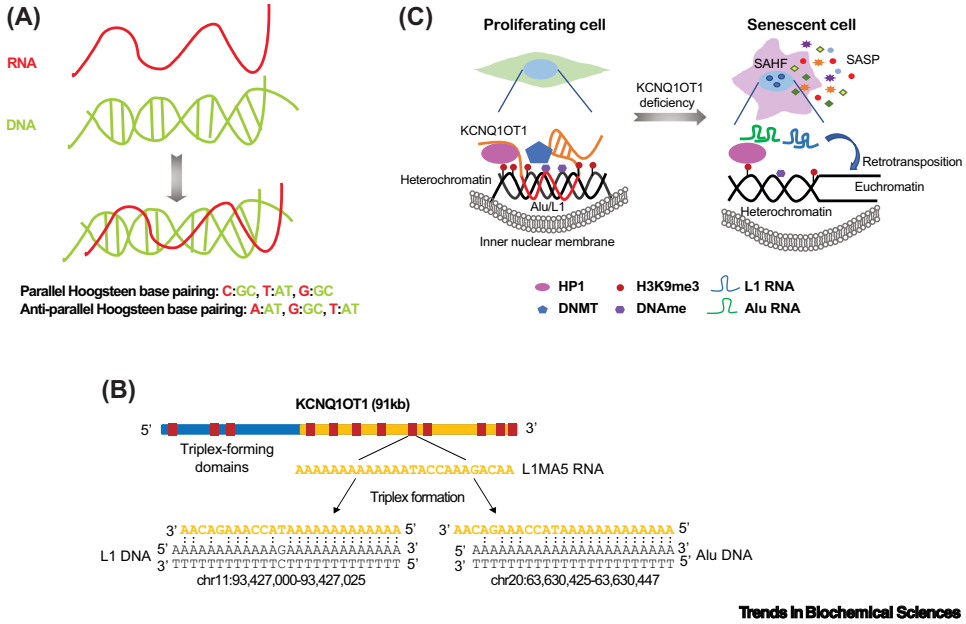

Figure 2. RNA-dsDNA triplexes and their guiding and targeting functions. (A) Long noncoding RNAs (lncRNAs) can form parallel Hoogsteen base pairing with dsDNA at C:GC, U:AT, and G:GC triads and antiparallel pairing at A:AT, G:GC, and U:AT triads. (B) Human lncRNA KCNQ1OT1 can form a triplex with evolutionarily young L1 and Alu elements at many sites encoded by repeat elements within KCNQ1OT1. Red blocks are triplex-forming domains. Blue and yellow mark the conserved non-repeat-rich region and the species-specific repeat-rich region in KCNQ1OT1, respectively. Hoogsteen base pairing is denoted by ':'. (C) Deficiency of KCNQ1OT induces cellular senescence-associated events, including heterochromatin decompaction and TE's detachment from nuclear membrane, loss of H3K9me3 and DNA methylation, retrotransposition, formation of SAHF, and increased SA- β-gal activity and SASP. (B) and (C) are reproduced, with permission, from Zhang *et al.* [32]. Abbreviation: DNMT, DNA methyltransferase.

in transposon silencing and heterochromatin stabilization (Figure 2). This solves the long-standing mystery of sequence specificity of transposon silencing and makes it the first mammalian endogenous guiding sequence-based Cas9-like epigenome editing system. Even a randomly selected genomic region can be ectopically silenced by incorporating an artificially designed guiding RNA-dsDNA triplex sequence inserted in the KCNQ1OT1 gene, demonstrating the sufficiency of triplex formation in the guiding process [32].

RNA-dsDNA triplex regulate chromatin modifications and structure

Different from the guide RNA-DNA interactions in the Cas9 system or other forms of known RNA-DNA interactions, such as R-loop and RNA-DNA hybrid [92], which need the DNA damage repair system to remove the structures, Hoogsteen base pairing of RNA to dsDNA does not disrupt the dsDNA structure and thus is likely to be reversible. It is also much weaker than other RNA-DNA interactions. Thus, RNA-dsDNA triplexes formed by Hoogsteen base pairing are ideal means for reversible regulation of the genome architecture. A recent study found that computationally predicted DNA-DNA interactions mediated by dsDNA-lncRNA triplex hotspots facilitate reconstitution of experimentally observed **high-throughput chromosome conformation capture (Hi-C)** interactions, suggesting a potential role of lncRNA in organizing the 3D genome structure and in a sequence-directed manner [93].

Meanwhile, L1 and Alu elements are found to localize mainly to B and A compartments, respectively, and when L1 transcripts are knocked down, the chromatin compartmentalization is dysregulated

[94]. However, how the lncRNAs through triplex formation affect 3D genome organization, how transposons such as L1 and Alu help organize the 3D genome, and whether these two are linked as regulators or targets are unclear. KCNQ1OT1 lncRNA forming triplex with evolutionarily young L1 and Alu subfamilies provides the first example of such chromatin organization by lncRNA-repeat elements interactions [32]. A deep learning model on 12 chromatin isolation by RNA purification (**ChIRP**)-sequencing datasets indeed shows that the majority of the RNA binding DNA motifs inferred by the model match the Hoogsteen base pairing triplexes, suggesting the triplex formation is a pervasive mechanism bringing lncRNAs to their target DNA sites [95].

RNA-dsDNA triplex affinity is additive and copy number dependent

Although a single copy of the RNA-dsDNA triplex is sufficient to induce ectopic binding of KCNQ1OT1 to an H3K9me3 marked site as inferred from a Bayesian network analysis [32], in addition to H3K9me3/HP1 binding, the induction of DNA methylation is also dependent on the number of RNA-dsDNA triplexes on L1 and Alu elements. That is, KCNQ1OT's affinity to these targets is correlated with the number of Hoogsteen base-pairing triplexes at the target region. With an increasing number of triplexes on L1 and Alu elements, their probability of being targeted KCNQ1OT1 increases, peaking at six and four triplexes for L1 and Alu, respectively [32], and the dwindling in binding probability after these optimum numbers perhaps indicates a spatial or stereo constraint competing among different triplex sites. Additionally, as shown by **CLIP-seq** and RNA pulldown, KCNQ1OT1 also directly binds to HP1, which recognizes and binds H3K9me3 on the chromatin and can further recruit DNA methyltransferases to catalyze DNA methylation (Figure 2C). Bayesian network analysis also infers that the triplex formation and H3K9me3 on the TE elements are both required for the DNA methylation induced by KCNQ1OT1 binding and downstream tight transcription repression of the TE elements, suggestive of an essential role of KCNQ1OT1 to convert transient repressive mark H3K9me3 to the stable repressive mark DNA methylation for tight repression of the target DNA (Figure 2C) [32].

It would be interesting to see whether the presence, number, and affinity of triplexes can predict lncRNA target genes and their expression, given the expression level of the lncRNA and whether it is a repressor or an activator. Furthermore, the significance of lncRNAs and RNA-dsDNA triplexes during cell aging might be estimated by the gain and loss of RNA-dsDNA triplexes according to aging differentially expressed lncRNAs. The aging-related changes in lncRNA-dsDNA may further elicit changes in chromatin phase separation, epigenomic states, and 3D genomic structure and subnuclear structure attachment that change the local and/or global nuclear morphology. For example, downregulation of KCNQOT1 alone is sufficient to lead to the derepression of its target transposons and their detachment from the repressive chromatin-associated nuclear lamina and further induces the formation of nuclear heterochromatin foci and senescent cell morphology [32] (Figure 2C).

Evolution of DNA-RNA triplex targeting

Not all TEs are repressed in young and healthy cells, and not all TEs are derepressed in aging and cellular senescence. In fact, many of the TEs serve as enhancers, and some as promoters, in many cell types [96], and many are activated and functional during development, aging, and disease [15,97–100]. Transposon repression and activation are often sequence specific; for example, although the evolutionarily young AluY subfamily has repressive chromatin marks, older Alu subfamilies have an active enhancer-like chromatin state in human immune cells [101]. Consistently, the KCNQ1OT Hoogsteen base-pairing triplexes are only enriched for evolutionarily youngest Alu and L1 subfamilies, increasing in triplex number with decreased evolutionary age and depleted in the old subfamilies of Alu and L1 [32].

Intriguingly, in contrast to the highly species-specific repeat-rich region, the nonrepeat region of KCNQ1OT1 is highly conserved across mammals, interacting with actin and nuclear matrix proteins [32], suggestive of its association with the nuclear matrix and a potential role contributing to the nuclear morphology and its change during aging and senescence.

Studies on the evolution of KRAB transcription factors have shown that they preferentially bind to nonactive but rapidly evolving TE that encode regulatory elements (not the evolutionarily youngest) and evolve with the TEs in an arms race manner [102,103]. It would be equally interesting to elucidate the evolution of the guiding sequence in KCNQ1OT1 against its young TE targets across different species and in general lncRNAs against DNA targets.

How artificial intelligence (AI) may help to fine-map lncRNAs in aging nuclear architecture

Some changes in nuclear architecture could represent a late change in aging, appearing more as a consequence of aging, whereas some might be an initiator of aging. For example, NAD defects [65,66] and SAHF formation [13–15] are very early events during senescence that precede most senescence hallmarks. Deficiency of KCNQ1OT alone induces heterochromatin decompaction and detachment from nuclear lamina of TEs, SAHFs, and other senescent phenotypes (Figure 2C) [32]. More senescence initiation lncRNA changes await to be discovered in large scale, preferably genome-wide screens, which can be facilitated by AI.

Toward this, AI can already recognize senescent cells and nuclei with high accuracy [25,26]. Coupled with genome-wide CRISPR screens, lncRNA perturbations that render cells and nuclei more senescent-like can be scored by AI models as senescent probabilities. Then detailed nuclear structures and shapes can be further learned using supervised approaches when large numbers of labeled training images are available or using Autoencoder or Transformer models to identify the structures/areas of attention (large weight) to the AI classifiers. For example, a nucleolus defect can be identified through either a specific AI model recognizing normal nucleoli or by first identifying the most import area for the AI classifier and then retrospectively labeling it as a nucleolus defect by nucleolus markers. AI can also help automate image segmentation and feature extraction, such as separating the nucleolus, from the background and other structures and quantifying its size, shape, and texture. These AI applications not only will speed up the nuclear structural and morphological analyses but also will make the analyses more objective, quantitative, accurate, and consistent.

Concluding remarks

It appears most known lncRNAs' architectural roles have been investigated with their protein-interacting partners, and, given that many lncRNAs are also computationally capable of forming extensive RNA-dsDNA triplexes, they are likely the nexus between nuclear proteins and DNA, making lncRNAs an equally important yet so far less well studied missing piece to fully decipher the senescence-associated nuclear architectural and morphological changes.

Although accumulating evidence has now established that many lncRNAs are architectural backbones or regulators of various nuclear structures and that these lncRNAs drastically change in abundance during aging, a direct link of lncRNA changes to nuclear morphology changes has not been established in most cases. This is perhaps due to the lack of global assessment of nuclear morphology in most experimental settings as different nuclear structures need different probes to visualize. Now AI is able to distinguish senescence-associated nuclear morphological changes at low and high resolutions [25,26]. This, together with CRISPR-enabled genetic perturbations of lncRNAs, especially the aging-related lncRNAs, will help map and establish their direct

Outstanding questions

What lncRNAs regulate what aging nuclear structure? How many of them involve dsDNA-RNA interactions?

Are different nuclear structural changes during aging coordinated, sequential, or hierarchical? What lncRNAs are the master architects?

Can dsDNA-RNA triplexes accurately predict lncRNA targeting? Can target gene expression changes be predicted from lncRNA changes?

How did RNA-dsRNA triplex evolve through evolution? Do TEs play a key role in this process?

Can AI accurately map nuclear morphology changes to nuclear structures and even underlying lncRNA changes?

involvement in the fundamental structural changes in the nucleus and their impact on cellular senescence and aging (see Outstanding questions).

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Declaration of interests

The author has no interests to declare.

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