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The role of retrotransposable elements in aging and age-associated diseases

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Abstract

The genomes of virtually all organisms, including humans, contain repetitive sequences generated by the activity of transposable elements (transposons). Transposons are mobile genetic elements that can move from one genomic location to another; in this process, they amplify and increase their presence in genomes, sometimes to very high copy numbers. Transposons have been coevolving with their host genomes since the dawn of life. This relationship has been largely competitive, and transposons have earned epithets such as ‘junk DNA’ and ‘molecular parasites.’ Much of our knowledge of transposon evolution reflects their activity in the germline and is evident from genome sequence data. Recent research has revealed a wealth of information on the activity of transposons in somatic tissues during the lifetime of an individual, the underlying molecular mechanisms, and the manner in which these processes intersect with our own physiology, health and well-being. We discuss here new evidence and ideas that transposon activity influences and even promotes the process of aging and age-related diseases.

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Transposons belong to two main groups: those that move using a DNA intermediate ('DNA transposons') in a 'cut and paste' mechanism, and retrotransposable elements (retrotransposons) that move using a 'copy and paste' mechanism that involves an RNA intermediate[1]. Thirty five percent of the human genome is comprised of retrotransposon DNA sequences. Retrotransposons are further divided into Long Terminal Repeat (LTR) elements, derived from exogenous retrovirus infections, and more primitive and ancient non-LTR elements with an obligate intra-cellular life cycle (Fig. 1). Non-LTR retrotransposons consist of two main groups: the Long INnterspersed Elements (LINEs), which encode their own proteins necessary for retrotransposition, and the Short INnterspersed Elements (SINEs), which are short, non-coding RNAs that hijack the LINE protein machinery[2].

A retrotransposon onslaught can be profoundly deleterious and hence the germline is guarded with multiple defenses[3]. However, retrotransposons can also be agents of evolution[4]. In a process known variously as cooption, exaptation or preadaptation, over evolutionary time the hosts have 'borrowed' from retrotransposons and *vice versa*. Some examples of beneficial interactions are the establishment of chromatin states necessary for development in preimplantation mouse embryos by the transcriptional activation of distinct LTR and LINE elements[4], the maintenance of telomeres in *Drosophila* by specific non-LTR retrotransposons[5], and the use of LTR enhancers to regulate important genes in human innate immunity pathways[6]. The focus of this review is the unintended activation of retrotransposons in adult somatic tissues, in particular with advancing age, which appears to be deleterious[7].

Host defenses are highly effective, and hence the majority of retrotransposons in our genome are passive passengers, slowly accumulating mutations, deletions or other rearrangements[1]. While some older elements can still affect host function through cis-acting gene regulatory or recombinational mechanisms[8, 9], the deleterious effects of retrotransposons increasingly being linked with aging appear to be largely dependent on the activities of their encoded proteins, and fall into three general mechanisms: genetic and epigenetic effects associated with retrotransposition, DNA damage associated with active or abortive retrotransposition, and activation of immune pathways associated with detection of retrotransposon nucleic acids. The last topic has received considerable recent interest in the context of multiple age-related pathologies and diseases.

Retrotransposon life cycles

Different species harbor diverse retrotransposon portfolios whose activities evolve over time. The genomes of *Drosophila* and mice contain active LINE and LTR retrotransposons, whereas the human genome shows current evidence of only LINE-1 (and associated SINE) activity. LINE-1 (L1) comprises 17% of the human genome (~500,000 copies); however, only some 100–150 evolutionarily recent element copies are full-length and lack mutations that would be expected to affect function[10–12].

L1 consists of a 5'UTR with an internal promoter, two open reading frames, *ORF1* and *ORF2*, and a 3'UTR with a polyadenylation signal (Fig. 1). *ORF1* encodes an RNA chaperone and *ORF2* an enzyme with single-strand endonuclease and reverse transcriptase

activities. A small ORF of unknown function, *ORF0*, was recently discovered in the antisense orientation in the 5'UTR[13]. The L1 life cycle starts with its transcription by host RNA polymerase II. After the capped and polyadenylated bicistronic L1 mRNA is translated in the cytoplasm, the two encoded proteins interact *in cis* with the L1 mRNA forming ribonucleoprotein particles (RNPs)[14]. L1 RNPs consist of many ORF1 trimers[15] coating the mRNA and likely just one or two ORF2 molecules bound to the A-rich tail of the 3'UTR[16].

L1 RNPs gain access to the nucleus primarily during mitosis[17]. The endonuclease nicks the bottom strand of genomic DNA at A/T rich sites (TTTT/AA consensus) to initiate a process known as target-primed reverse transcription (TPRT) (Fig. 2)[18]. The polyA tail of the L1 mRNA then anneals to the thymidines adjacent to the cut site, enabling reverse transcription from the 3' end of the DNA flap. Subsequent steps are not well understood, but likely involve host factors to produce a double-stranded DNA (dsDNA) L1 element ligated to host DNA and flanked by target site duplications. These events occur during host DNA replication and take advantage of interactions with the replication fork[11, 12, 19, 20].

LTR retrotransposons move by a distinct process which is very similar to that used by retroviruses (Fig. 2). They encode Gag-like proteins, which assemble into capsid-like particles in the cytoplasm. Reverse transcription occurs inside these structures, typically using a host tRNA as a primer, generating a double-stranded cDNA product that, after entering the nucleus, is inserted into the genome by an integrase activity encoded by the element. Although LTR retrotransposons (including endogenized retroviruses) comprise approximately 8% of the human genome, all known sequences are mutated and no single element appears to be capable of retrotransposition. The human endogenous retrovirus K (HERV-K) appears to have become 'extinct' very recently, with the Gorilla genome still harboring some intact insertions[21]. However, several HERV elements in the human genome contain some intact ORFs, and expression of their envelope (Env) proteins in particular has been suggested to contribute to neurological diseases such as amyotrophic lateral sclerosis (ALS) and multiple sclerosis (MS)[22].

Mechanisms of retrotransposon silencing

Host defense mechanisms recognize retrotransposon DNA sequences in their genomes and induce heterochromatization at these locations very early in development[23, 24, 25] (Fig. 3). Heterochromatinization of retrotransposons requires the recruitment of chromatin remodeler enzymes and effector proteins to their sequences. These include histone methyltransferases (HMTs) such as SUV39H (suppressor of variegation 3–9 homolog) and SETDB1 (SET domain bifurcated 1), which are important for H3K9 (histone 3 lysine 9) methylation[26, 27] and recruitment of HP1 (heterochromatin protein 1) to constitutive heterochromatin[28]; EZH2 (enhancer of zeste 2), which mediates trimethylation of H3K27 (histone 3 lysine 27) and maintenance of the Polycomb complex associated with facultative heterochromatin[29]; and several other factors including the human silencing hub (HUSH) complex[19, 30] and retinoblastoma protein (RB1)[29]. TRIM28 (tripartite motif containing 28), also known as KAP1 (krüppel-associated box (KRAB) associated protein 1), acts as a co-repressor for multiple proteins that target retrotransposons, such as KRAB domain-containing zinc-finger proteins (KZFPs) and SIRT6 (silent mating type information regulator 2 (sirtuin) homolog

6), by recruiting SETDB1 and HP1[31, 32]. Loss of these factors reduces heterochromatin at retrotransposons and allows their transcriptional derepression[26, 29, 31, 33–35].

DNA cytosine methylation is an important mechanism of transcriptional silencing that exists in close relationship with histone modification-based mechanisms[36]. In mammals, retrotransposons are hypermethylated at CpG sites in adult somatic cells and in many (but not all) cases hypomethylation has been implicated in their de-repression[37, 38]. Deletion or inhibition of DNA methyltransferase (DNMT) enzymes leads to up-regulation of L1 elements[39, 40]. Conversely, SINEs are not de-repressed by *Dnmt1* deletion or treatment with DNA demethylating drugs and appear to be regulated primarily by histone-methylation[41]. Interestingly, in *Drosophila,* cytosine methylation is absent and while N6-adenine methylation is found it does not appear to play a major role in retrotransposon repression[42].

Histone and DNA methyltransferases are targeted to retrotransposon sequences through several pathways and effector proteins. KZFPs, of which there are over 400 in the human genome, co-evolve with retrotransposons as part of an ongoing ‘arms race’[25]. Many of these KZFPs bind to retrotransposon elements in the genome and promote their heterochromatinization via the recruitment of KAP1[43]. Retrotransposons mutate to avoid this surveillance, and the host organisms evolve variant KZFPs that can again repress the new generation of retrotransposons[24,44]. In mouse embryonic stem cells (ESCs), some LTR retrotransposons are repressed by KAP1-mediated recruitment of SETDB1 and not DNA methylation[38, 45]. Evolutionarily young L1s in human ESCs are bound by the transcription factor YY1 (yin-yang-1) at a site in their 5’ UTR; in neuronal cells loss of this site is associated with hypomethylation and L1 activation[46]. Older L1 families are bound by KAP1, and loss of DNMTs increases expression of younger L1s but not older ones[24]. The KAP1- and DNMT-dependent pathways in many cases thus play complementary roles specific to individual retrotransposon families.

Short interfering RNA (siRNA) and the PIWI (P-element induced wimpy testis)-interacting RNA (piRNA) pathways recruit chromatin repressive factors to retrotransposons by a mechanism based on base pairing between the small RNAs and their target transcripts. siRNAs are derived from nuclear double-stranded RNA (dsRNA) species arising from convergent transcription or annealing of expressed retrotransposon sequences, which are processed by RNase-III-like Dicer enzymes. siRNAs are then loaded onto Argonaute (AGO) effector proteins to form silencing complexes that bind to nascent retrotransposon transcripts to recruit heterochromatin factors[47]. In the piRNA system, PIWI protein binds to piRNAs derived from piRNA clusters, non-coding transcriptional units that contain embedded retrotransposon sequences. Both siRNAs and piRNAs also contribute to post-transcriptional silencing by mediating transcript degradation.

Many additional factors play roles in retrotransposon surveillance[48]: 1) RNA editing enzymes such as the cytidine deaminase APOBEC (apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like) family[49] or ADAR (adenosine deaminase acting on RNA)[50, 51]; 2) the RNA helicase MOV10 (Moloney leukemia virus 10 homolog)[48]; 3) homologous recombination repair factors such as BRCA1 (breast cancer gene 1)[20]; 4) the

nucleotide triphosphate hydrolase SAMHD1 (SAM domain and HD domain containing protein 1)[52]; and 5) the three-prime repair exonuclease 1 (TREX1)[53]. Some of these factors are defenses against exogenous viruses and act by diverse mechanisms such as editing of viral/retrotransposon genomes to mis-code the protein sequences (APOBEC), decreasing nucleotide triphosphate pools to limit viral/retrotransposon cDNA synthesis (SAMHD1) or degrading viral/retrotransposon nucleic acids (TREX1).

Loss of silencing with age

Aging is associated with extensive remodeling of the epigenome[54]. These changes are complex and generally cell-type specific, but a consistent trend is the loss of heterochromatin and an associated transcriptional de-repression at sites across the genome, which has been observed in species from yeast to humans[55–57]. Several studies have provided evidence that decreases of heterochromatin or heterochromatin-establishing factors contribute to elevated retrotransposon activity with age. In *Drosophila*, H3K9me3 and HP1 decline with age in pericentric regions and islands of heterochromatin[58]. Retrotransposons are enriched in these regions and later studies have confirmed their age-related activation[35, 59, 60]. Genetic interventions that promote heterochromatin formation and/or retrotransposon silencing attenuate age-related increases in retrotransposon expression and, remarkably, can increase life span[35].

Cellular senescence is an irreversible arrest of proliferation that can be elicited by a variety of stresses, in particular DNA damage[61]. While senescence has beneficial functions (such as tumor suppression), senescent cells accumulate in most tissues with age, and they are important components of the overall aging process[62]. In particular, due to their profound proinflammatory phenotype[63], senescent cells have been causally linked with many age-related pathologies and diseases[64]. Senescence is accompanied by complex changes and rearrangements of chromatin[65]. Relative decreases of heterochromatin have been mapped to retrotransposons and associated with their transcriptional derepression[66]. *RB1* expression and binding to L1 elements were reduced in senescent cells and were associated with loss of H3K9me3 and H3K27me3 at L1 loci[33]. *TREX1* was also downregulated, and the transcription factor *FOXA1* was upregulated and bound to the L1 5’ UTR. Knockdown of *RB1* and *TREX1* combined with *FOXA1* overexpression led to L1 derepression in human fibroblasts[33].

However, senescent cells comprise only a small fraction of cells in aged tissues[61] and are unlikely to be responsible for all of the retrotransposon activation that has been observed. Retrotransposon activation is found in diverse species, including nematodes[67] and *Drosophila*[35], which appear to be devoid of cell senescence. In mice, tissues with few senescent cells can show robust activation of retrotransposons with age (such as skeletal muscle)[33, 68]. One possible senescence-independent mechanism involves *SIRT6*, which promotes L1 silencing by binding to the L1 5’ UTR and mono-ADP ribosylating KAP1, which then recruits additional heterochromatin factors HP1a and MeCP2[31]. In aged mouse tissues, SIRT6 is lost from L1 elements, likely due to increased recruitment to sites of oxidative DNA damage, and its overexpression opposes age-related increases of L1 expression in diverse tissues[34]. A similar mechanism has been described for *SIRT1*

in mice[69], and the *SIRT1* homolog in *Drosophila* is also involved in the regulation of retrotransposons[35]. Importantly, the extent of retrotransposon activation in chronologically aged versus senescent cell types of complex mammalian tissues is not known.

Cytosine methylation tends to decrease slowly with aging over large, gene-poor regions of the genome that are enriched for heterochromatin and repetitive sequences, whereas methylation at gene-associated CpG islands can increase or decrease more dramatically, with pronounced tissue-specific effects[70, 71]. Some of these changes are conserved between individuals of the same age and subsets of sites can be used to devise 'methylation clocks' that are highly correlated with aging[72]. Senescent cells show similar overall, but even more pronounced changes, which interestingly resemble changes observed in cancer cell genomes[73]. Given the links between DNA methylation, heterochromatin, and retrotransposon expression, and reports that interventions that slow down aging also decrease the progression of methylation changes[74] and retrotransposon activation[75], it is tempting to speculate that these events are causally connected. However, DNA methylation changes are early events in senescence[73], whereas retrotransposon activation occurs only much later[33]. In cancer cells retrotransposon activation is quite sporadic between different cancer types as well as cases[7]. One possible explanation is that aging also increases methylome disorder[76], which could lead to a stochastic and progressive activation of retrotransposons.

Some evidence suggests that alterations in small RNA pathways occur with age[7, 77] and these changes may contribute to increased retrotransposon expression. Other than chromatin, however, most systems involved in retrotransposon regulation have not been well studied in the context of aging and thus provide interesting avenues for future exploration.

Consequences of retrotransposition

Over 120 L1-mediated germline insertions have been linked to cases of human genetic diseases[78]. Insertions of the human SINE *Alu* into the *NF1* (neurofibromatosis type I) gene have been linked to the development of neurofibromas[79]. The *NF1* gene appears to be a hotspot for *Alu* insertions, with 18 independent patients having been identified. However, retrotransposition events were estimated at ~0.4% of all *NF1* mutations, and hence are a small fraction of all mutational events affecting this gene. Retrotransposition during development or adulthood would lead to somatic mosaicism, and has been estimated to occur in adult neurons by qPCR or single-cell genomic studies at varying (but generally low) frequencies (events/cell): < 0.04–0.6[80], 13.7[81], 0.58–1[82], 0.2[83] and 0.63–1.66[84]. While retrotransposition frequencies in other tissues have not been investigated in detail, they appear to be significantly lower, and hence the adult human brain might be a particularly permissive site for L1 activation[84, 85].

Cancer is characterized by genomic instability and in many cases increased expression of L1 elements[86]. Subsequent efforts identified multiple *de novo* somatic insertions and associated rearrangements in human cancers, in some cases numbering in the hundreds for an individual tumor[86–89]. Some insertions into tumor suppressor genes such as *APC* (adenomatous polyposis coli) in colon cancer and *PTEN* (phosphatase and tensin homolog)

in endometrial carcinoma have been found and linked to the development of the cancer[87]. However, the majority of *de novo* insertions in cancers are located in non-coding sequences and do not show hotspots near tumor suppressor or oncogene loci[7, 90]. Thus, there is little current evidence that new L1 insertions play a major role in tumorigenesis[7].

Our knowledge is still limited on the consequences of insertions in intergenic regions, such as epigenetic effects (epimutations) on distal enhancers, long non-coding RNAs or architectural chromatin domains, especially over longer periods of time. It is plausible that such insertions could contribute to tumor evolution or development of therapy resistance. It would thus be interesting to test whether inhibition of retrotransposition might slow down relapse after therapy. However, in the absence of evidence to the contrary, we can tentatively conclude that the burden of disease attributable to L1 or *Alu* insertions, with the possible exception of some neurodegenerative diseases, is likely to be low.

Activation of innate immunity

Free DNA or dsRNA in the cytoplasm are generally perceived as invading pathogens. In vertebrates their presence triggers the interferon system, which then orchestrates immune responses. The signaling cascades start with a variety of sensors, such as RIG-I (retinoic acid-inducible gene I) and MDA5 (melanoma differentiation-associated protein 5) to detect cytoplasmic dsRNA, cGAS (cyclic GMP-AMP synthase) and AIM2 (absent in melanoma 2) to detect cytoplasmic DNA, and TLRs (toll-like receptors) to detect a variety of nucleic acids in the endosomal compartment[91]. These signals are propagated to the nucleus where they initiate the type-I interferon (IFN-I) response: a large number of factors that interfere with viral replication, expression of interferons α and β to alarm neighboring cells, a variety of cytokines and chemokines to communicate with the immune system, and in some cases, cell death pathways.

Increasing evidence indicates that retrotransposon DNA sequences accumulate in the cytoplasm under certain conditions. Pioneering work by Stetson et at.[53] found that retrotransposon DNA was present in the cytoplasmic fractions of hearts from *TREX1* mutant mice. Sequencing showed that L1, SINE, and LTR retrotransposon DNAs were significantly enriched compared to normal mice. Subsequent work found extra-chromosomal DNA in TREX1-deficient human neural cells, with L1 as the major source[92].

Recently, cytoplasmic L1 DNA was found in normal cells in association with cellular senescence and aging[33, 34]. Cytoplasmic DNA was also present in multiple tissues of *SIRT6* knockout mice[34] that display premature aging and derepression of L1 elements[31]. Importantly, L1 DNA was identified in association with the DNA sensor cGAS[34]. Upon binding to DNA, cGAS catalyzes a reaction of GTP with ATP to form cyclic GMP-AMP (cGAMP)[93]. cGAMP then binds to STING (stimulator of interferon genes), which subsequently triggers the phosphorylation of the transcription factor IRF3 (interferon regulatory factor 3) by the kinase TBK1 (TRAF family member-associated NF-kappa-B activator (TANK)-binding kinase 1). IRF3 then translocates to the nucleus where it activates the IFN-I response (Box 1).

In all conditions discussed above – *TREX1* or *SIRT6* deficiency, cellular senescence, or natural organismal aging – cytoplasmic L1 DNA was correlated with activation of an IFN-I response. Importantly, treatment with nucleoside reverse transcriptase inhibitor (NRTI) drugs that inhibit L1 reverse transcriptase, or a knockdown of L1 expression with shRNAs against active L1 families, decreased cytoplasmic L1 DNAs and ameliorated cGAS and IFN-I activation[33, 34, 92]. This finding indicates that cytoplasmic L1 cDNAs, produced by their reverse transcriptase activity, can be potent triggers of an IFN-I response.

The mechanisms by which cytoplasmic L1 cDNAs are produced are uncertain. In the TPRT model L1 reverse transcription occurs in the nucleus. One possibility is that cytoplasmic L1 DNA species are abortive TPRT products that are somehow exported from the nucleus. However, in postmitotic senescent cells one would expect L1 RNPs to be largely excluded from the nucleus. Retrotransposition is greatly diminished in non-dividing cells[94], although low levels could be detected in senescent cells[33] or neurons[92], and it is possible that abortive products of these processes could accumulate. Alternatively, reverse transcription could occur directly in the cytoplasm. Both ORF2 and L1 RNPs can reverse transcribe *in vitro* if provided with a primer[14, 18]. In senescent cells L1 RNPs could accumulate in the cytoplasm because of their inability to access the nucleus. Since reverse transcriptases can use both DNA and RNA primers and templates, the cytoplasmic RNPs could generate cDNA using as yet unidentified primers. *Alu* self-priming in the cytoplasm has recently been reported in age-related macular degeneration (AMD)[95].

Evidence has also been presented that retrotransposons can be detected by RNA sensors. Retrotransposon dsRNA species are processed into siRNAs in the nucleus and normally do not reach the cytoplasm in sufficient numbers to overcome self-tolerance. In some circumstances, such as treatment of cancer cells with demethylating agents, retrotransposon upregulation overwhelms the system and is perceived by MDA5[96] or TLR3[97] sensors to trigger an IFN-I response. Major sources of dsRNA, inverted *Alu* repeats, are normally deaminated by ADAR to reduce their double-stranded character; decreased processing can be sensed by MDA5, resulting in IFN-I activation[50, 51, 98]. Overexpression of L1 can elicit an IFN-I response through either MDA5 or RIG-I[99, 100], possibly mediated by some secondary structure of the L1 mRNA.

The relative contributions of DNA and RNA sensing of retrotransposons to senescence, aging, and age-related diseases remain to be thoroughly investigated. Much work to date has relied on L1 overexpression, cell lines, or situations where mechanisms that restrict retrotransposons were compromised. Interestingly, in one report a robust L1-mediated IFN-I induction in a cell line that does not express cGAS-STING was abrogated by NRTI treatment[19]. This might be explained by activation of cytoplasmic RNA sensors by RNA:DNA hybrids[101], or the processing of L1 RNPs through lysosomes where L1 cDNA could activate TLR9[91]. Another possibility is the existence of self-tolerance thresholds to which both DNA and RNA sensors contribute: for example, TBK1, which is upstream of IRF3, can be activated by both cGAS-STING and RIG-I or MDA5[93, 101].

Promotion of DNA damage

DNA lesions, in particular dsDNA breaks (DSB), can arise as 'collateral damage' associated with retrotransposition[19, 20, 102]. In *Drosophila* this is likely to be of particular importance since this species lacks an interferon system. More primitive mechanisms based on RNA sensing though Dicer-2 and a TLR are present, as is the NF-kB pathway, which activates the production of antimicrobial peptides; however, DNA sensing and the cGAS-STING axis are absent[103]. Retrotransposon activation in *Drosophila* has been clearly linked with DNA damage, which is alleviated by interventions such as promotion of heterochromatin[35, 59, 60]. DNA damage-mediated cell death[104] might thus be a predominant cause of deleterious effects exerted by retrotransposons in flies. However, the basic innate immunity mechanisms of this organism remain to be examined in this context.

In mammals, retrotransposons, DNA damage, interferon and inflammation are very intricately intertwined. For example, retrotransposons can promote DNA damage, but DNA damage is also documented to activate retrotransposon expression[105]. Inflammation can drive DNA damage by diverse processes, but DNA damage also elicits inflammation, as in cell senescence. Retrotransposons trigger innate immune sensors, but interferon mechanisms can also regulate retrotransposons[106]. In many of the disease examples discussed below, inflammation and DNA damage coexist and have not been disentangled in sufficient detail. Given the current paucity of quantitative data, the frequency of DNA damage that can directly be attributed to retrotransposons is uncertain, and might be quite low, especially during natural aging.

Retrotransposons in aging

By increasing susceptibility to a wide range of diseases aging is the main cause of mortality and morbidity in the developed world. Could retrotransposons, as genomic parasites, be contributing to the process of aging (Fig. 4a)? Infestation with many parasites, such as nematodes or the plasmodium parasite that causes malaria, is clearly deleterious. The recent appreciation that retrotransposons are likely to be more active in somatic tissues than hitherto appreciated brings into focus the possibility that retrotransposon activity might have effects on the life span of a single individual.

Retrotransposons become reactivated in cell senescence and with age in both mammals and *Drosophila*[58, 60, 66, 68]. Remarkably, interventions that silence retrotransposons extended normal life span in flies, and treatments with NRTIs partially rescued the shortened lifespans of several fly mutations that de-repress retrotransposons[35, 59]. In mice, treatment with NRTIs doubled the lifespan of progeroid *SIRT6*-deficient mice, and improved overall health including bone density, muscle mass, intestinal function and exercise performance[34]. Treatment of middle-aged normal mice with NRTIs retarded the progression of aging biomarkers, in particular DNA methylation age and p16INK4A expression[34]. Collectively, these studies suggest that retrotransposons causally contribute to the aging process, and that interventions that oppose retrotransposon activity might improve healthy longevity.

Studies of long-lived animals have provided valuable insights into the mechanisms of aging[107]. Interestingly, the genome of the longest-lived rodent, the naked mole rat, contains fewer retrotransposons than other rodent genomes[108]; whereas the genomes of long-lived bats are replete with transposable elements[109]. Bats, however, have evolved dampened responses to cytoplasmic DNA[110]. Hence, there may not be a direct correlation between life span and transposon abundance but instead long-lived species may evolve better ways to respond to retrotransposons and associated inflammation.

Inflammation has emerged as a factor in multiple age-related pathologies, such as cancer, cardiovascular diseases or diabetes, and is a strong theme in many neurodegenerative disorders. Chronic low-level stimulation of the immune system is now viewed as a hallmark of aging[111] and is often referred to as 'sterile' inflammation, since occurs in the absence of obvious infection with pathogens. The causes of sterile inflammation are probably many and remain inadequately defined. We suggest that mis-recognition of retrotransposons activated during aging as invading pathogens is an important driver of sterile inflammation, which in turn contributes to many aging-associated diseases. As an example, epidemiological evidence suggests that NRTIs reduce the incidence of type 2 diabetes[112].

Diseases linked to retrotransposons

A pro-inflammatory role of retrotransposons has been noted in several autoimmune diseases (Fig. 4b). Elevated L1 transcripts were found in the synovial fluid of rheumatoid arthritis patients[113]. L1s are hypomethylated and their transcripts increased in patients with systemic lupus erythematosus (SLE) and Sjogren's syndrome[114], disorders also characterized by IFN-I induction. *TREX1* mutations have been identified in SLE[115] and familial chilblain lupus[116], implicating *TREX1* as a safeguard against autoimmune inflammation. Antibodies against DNA[117] are often detected in SLE[117] – could the production of such antibodies be triggered by L1 cDNA? A recent study identified antibodies to the L1 ORF1 protein in SLE patients with severe and active disease[118]. It remains to be determined whether L1 cDNA is an early trigger of disease or arises later during its course. It would thus be interesting to test whether suppression of L1 cytoplasmic DNA could prevent the onset of autoimmunity in genetically susceptible individuals or attenuate inflammation in established disease.

Aicardi–Goutières syndrome (AGS) is a childhood encephalopathy with similarities to SLE and strong IFN-I induction[119]. It is caused by recessive mutations in *TREX1*, *SAMHD1*, *ADAR* and *RNaseH2* or dominant mutations in *MDA5*, all of which are involved in nucleic acid processing or sensing, DNA repair, or retrotransposon restriction. TREX1 degrades cytoplasmic L1 DNA[53] and its loss initiates a cGAS/STING response. Treating *TREX*-deficient mice with a cocktail of NRTIs rescued their mortality[120]. SAMHD1 interacts with L1 in multiple ways[52] and might sequester ORF1 into stress granules[121]. RNaseH2 digests the RNA strands of RNA:DNA hybrids[122] and might reduce the levels of L1 nucleic acid species that interact with interferon sensors[123]. ADAR edits *Alu* RNA[51], and gain of function mutations in *MDA5* suggest that RNA-sensing mechanisms are involved in some AGS cases[119]. The extent to which the nucleic acids that activate interferon sensors are derived from chronic DNA damage or retrotransposons in not known; however, a clinical trial with NRTIs produced promising results[124].

The role of L1 activation in cancer is not well understood. Inflammation may promote cancer by inducing cell proliferation; alternatively, the L1-triggered interferon response or DNA damage could promote cell death. L1s are activated by loss of DNA methylation, which often occurs during premalignant hyperplasia[125]; the resulting IFN-I response could then trigger cell death pathways. The IFN-I response could also expose the tumor cells to surveillance by the immune system[96, 97]. Hence, L1s, and retrotransposons more generally, could play a tumor suppressor role. Chronic IFN signaling can also promote an immune suppressive environment[126].

Retrotransposons have also been implicated in several age-related neurodegenerative diseases[127]. AMD has been associated with the downregulation DICER1, and the consequent elevation of *Alu* RNA levels activates the NLRP3 (NOD, LRR and pyrin domain containing protein 3) inflammasome in retinal pigmented epithelial cells, leading to cytotoxicity and degeneration[128]. *Alu* RNA was reverse transcribed in the cytoplasm and the cDNA activated cGAS/STING signaling[95]. The ensuing IFN-I and inflammatory responses were mitigated by treatment with NRTIs[95, 129].

ALS and frontotemporal dementia (FTD) are characterized by cytoplasmic aggregates of TDP-43 (transactivation response DNA-binding protein of 43 kDa). TDP-43 is normally localized in the nucleus, and although its function is not fully understood, its cytoplasmic aggregation has been strongly linked with disease. In post-mortem human brain, nuclear TDP-43 loss has been associated with opening of chromatin regions enriched in retrotransposons[130]. The permissive chromatin environment was proposed to enable L1 and HERV expression, also noted by others[131, 132]. Elevated interferon and inflammatory markers have been noted in ALS patients and mouse models of ALS[132–134]. Overexpression of human TDP-43 in the *Drosophila* brain induced the derepression of retrotransposons, including the retrotransposition-competent LTR element Gypsy[135, 136]. Retrotransposons appear to mediate the ensuing DNA damage-associated cytotoxicity, since these effects were rescued with NRTIs.

Neurofibrillary tangles, formed by insoluble aggregates of MAPT (microtubule-associated protein tau) are a hallmark of Alzheimer's disease (AD) and other tauopathies. Postmortem AD brain samples exhibited increased markers of open chromatin[137] and elevated expression of retrotransposons[138, 139]. Expression of a disease-associated human Tau protein in *Drosophila* neurons elicited loss of H3K9me2, widespread retrotransposon activation, DNA damage, and neuronal cell death that could be rescued with NRTIs[139]. Retrotransposon expression was positively associated with tangle burden in human cortical samples, as well as enrichment of the open chromatin mark H3K9ac at some HERV loci[138]. Sporadic AD was recently associated with the insertion of processed pseudogenes of *APP* (amyloid precursor protein), some possibly expressing disease-associated variants, into adult neuron genomes[140] in a process that required reverse transcriptase activity.

Cytoplasmic retrotransposon DNAs have been detected in brain tissue of ataxia-telangiectasia (A-T) patients[141], a neurodegenerative and cancer-prone syndrome caused by mutation of the *ATM* (A-T mutated) gene, which encodes a protein kinase that regulates cell cycle checkpoints, DNA repair and apoptosis in response to DSB. ATM kinase

inhibition in glial cells of *Drosophila* activated an innate immune response and elicited neurodegeneration[142]. Loss of *ATM* was associated with increased L1 retrotransposition in human neural progenitor cells[141]. Loss of *ATM* in microglia led to accumulation of cytoplasmic DNA, activation of the cGAS-STING axis and production of neurotoxic cytokines[143]. The extent to which these responses are driven by retrotransposons or nuclear DNA fragments emanating from compromised DNA repair remains to be determined.

Therapeutic opportunities

The implication of retrotransposons in aging and age-related diseases provides an opportunity to develop novel treatments. Autoimmune disorders are typically treated with anti-inflammatory drugs, such as TNF antagonists, which target more distal aspects of the response. Small molecule inhibitors of STING have recently been reported[144] and found to ameliorate STING-mediated inflammatory conditions in mouse models. cGAS inhibitors are being developed[145]. Interestingly, some old drugs such as aspirin[146] and antimalarials quinacrine and chloroquine[147] inhibit cGAS, further suggesting that downregulating this pathway might confer health benefits. The disadvantage of treatments that overly target immunity mechanisms is that they might increase susceptibility to infections.

One consistent feature in multiple models and situations is the observed efficacy of NRTIs to alleviate the effects of retrotransposon activation. This finding is intriguing given that some aspects of the retrotransposon lifecycle, such as transcription of the elements or stimulation of RNA sensors, should not be directly affected by NRTIs. One explanation might be downstream cross-talk between RNA and DNA sensing pathways and the existence of overall self-tolerance thresholds. Another possibility is the reported anti-inflammatory property of NRTIs to alleviate activation of the NLRP3 inflammasome without engaging reverse transcriptase[129]. Many NRTI drugs have been FDA approved for the treatment of HIV (human immunodeficiency virus) infections and are generally well-tolerated. However, NRTIs are not without side effects, as they can be recognized by cellular polymerases such as mitochondrial DNA polymerase or telomerase.

The most proximal approach to inhibiting retrotransposons would be to strengthen the epigenetic mechanisms responsible for their silencing, especially those that become compromised with age. One such epigenetic regulator is *SIRT6*[31]. Male mice overexpressing *SIRT6* show increased life span[148], which may be due in part to more efficient silencing of L1 elements and reduced inflammation. SIRT6 is involved in multiple processes which include DNA repair, telomere maintenance and metabolism. Small molecule activators of SIRT6 are being developed and may provide an array of health benefits, including improved retrotransposon silencing.

Recent discoveries reviewed here show a convergent theme in which advancing age weakens host mechanisms that control retrotransposons, leading to their derepression and eventual activation of innate immune pathways, toxicity and degeneration. The initiating events are diverse and include genetic predisposition, environmental influences, and the natural aging process itself. Activation of interferon pathways has only recently emerged as a major downstream mechanism of retrotransposon activation and provides an exciting

new framework for the relationship of retrotransposons with their hosts. Activation of innate immune signaling by retrotransposons can take place in all somatic cells, including postmitotic cells, without the need for actual retrotransposition events. The promotion of inflammatory processes resonates well with our emerging understanding of the central role inflammation plays in aging and many age-related diseases.

Outlook

Much work remains to be done on the basic biology side to understand the mechanisms and consequences of retrotransposon activation in adult somatic tissues. Heterochromatin has emerged as an important mechanism of retrotransposon repression. The loss and redistribution of heterochromatin that occur during aging thus warrant further investigation. Other pathways of retrotransposon suppression (such as siRNAs and piRNAs) need to be more fully investigated, especially in mammals. The upstream stimuli that initiate compromise of surveillance systems are beginning to come into view (for example, SIRT6 relocalization to sites of DNA damage[31], or L1 chromatin opening due to TDP-43 relocalization into the cytoplasm[130]), but our understanding of these mechanisms is still very primitive. What is especially needed is a more holistic view of how aging mechanisms contribute to disease and *vice versa*.

Retrotransposon activation in senescent cells is well established, but the extent and mechanism of activation in postmitotic cells (such as neurons or myocytes) is not known. It appears that L1 has alternative lifecycles in proliferating and senescent/postmitotic cells, but the latter are not understood at all. Once retrotransposons are derepressed, is interferon activation the major aging- and disease-driving consequence, and what are the relative roles of DNA damage, responses to it, and cell death? For the pro-inflammatory effects, what are the relative roles DNA-sensing and RNA-sensing pathways? L1 has emerged as an important driver of IFN-I activation, but the genesis of the cytoplasmic L1 cDNAs that stimulate cGAS/STING signaling is not yet known. What are the relative contributions of L1s and HERVs to human diseases? It is already apparent that many species-, cell type- and disease-specific components exist, and disentangling which retrotransposons are being activated and how they are being sensed in different situations will have to be a major effort.

On the side of translation, an important consideration will be to identify patients in whom retrotransposon activation is triggering disease. For this it will be necessary to develop biomarkers that can reliably detect retrotransposon activation for patient stratification purposes. In terms of potential therapy, NRTIs have showed early promise[124]. Given the good safety record of recent NRTIs in HIV treatment, repurposing these drugs for a variety of indications is feasible and would generate a wealth of new information. Better-tolerated drugs will be desirable for long-term treatments of age-related pathologies, especially in geriatric populations. One strategy would be to develop new, high efficacy NRTIs specifically targeted to retrotransposon reverse transcriptases. Non-NRTI inhibitors that bind to non-catalytic sites on the HIV reverse transcriptase have been quite successful, and the same strategy can be pursued with retrotransposons. Drugs that target other functions, such as L1 endonuclease, or that destabilize RNP complexes can be contemplated.

Finally, combination therapies that include drugs that target multiple pathways engaged by retrotransposons, such as DNA damage, are worth investigating.

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Box 1

Diverse sources of cytoplasmic ‘self’ DNA.

cGAS-STING signaling can be activated by a variety of DNA species that leak into the cytoplasm, such as DNA fragments arising during DNA damage repair, fragments of telomeric DNA, ruptured micronuclei, and cytoplasmic chromatin fragments (CCF) formed by a nuclear autophagic process in senescent cells[149, 150]. Another source of cytoplasmic DNA is compromised mitochondria that can leak their DNA and prime innate antiviral responses[151]. Mitochondrial dysfunction was required for the progression of cellular senescence and the induction of its proinflammatory phenotype[152]. Another report found that mitochondrial dysfunction was required for the formation of CCF and induction of inflammatory signaling in senescent cells[153]. How these diverse cytoplasmic DNA species are functionally interrelated in triggering cGAS-STING signaling, in which cell types and under what conditions, is not well understood.

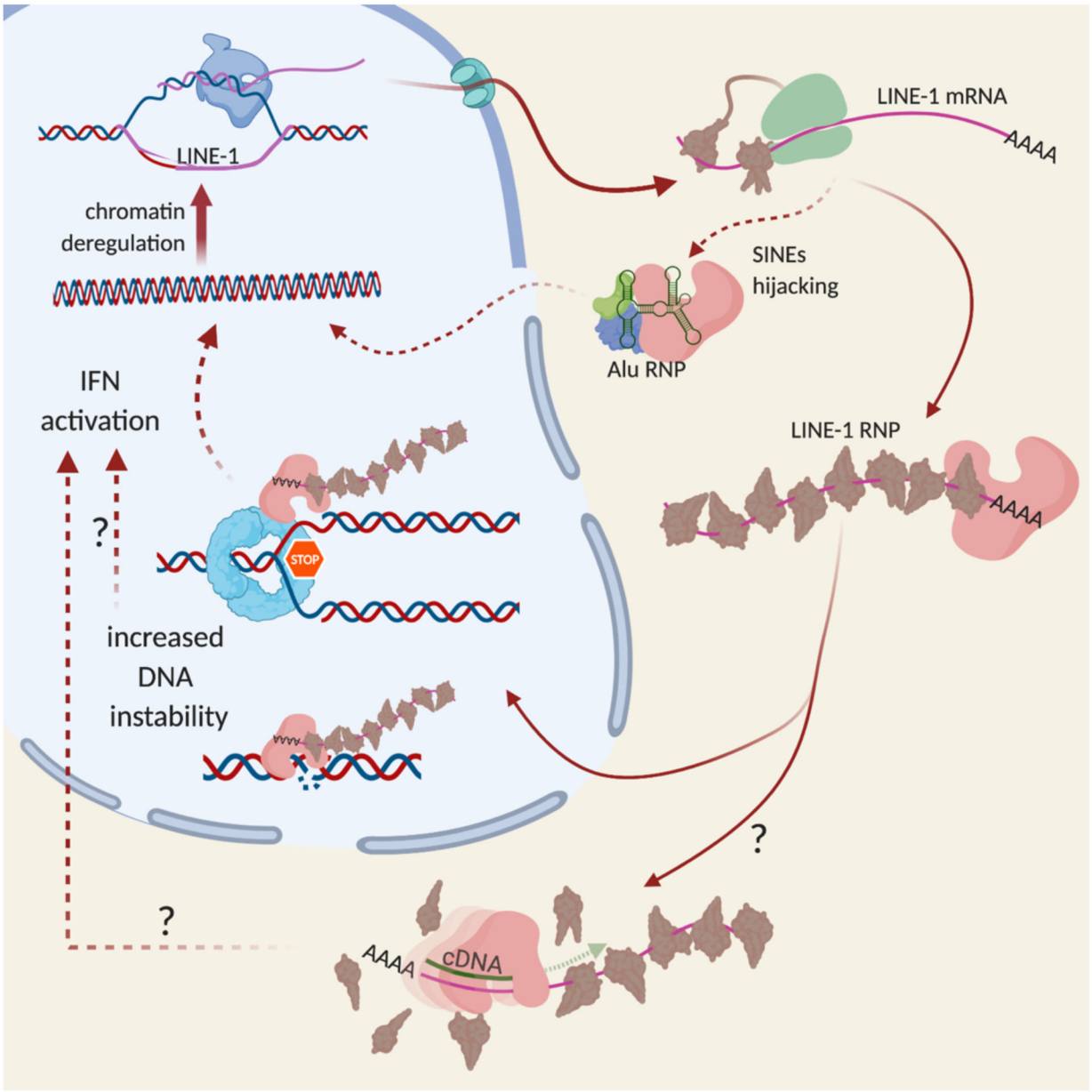

Fig. 1 |. L1 life cycle.

L1s are transcribed by host RNA polymerase II. The mRNAs are translated (green ribosome) into ORF1 (brown) and ORF2 (pink) proteins. Multiple trimers of ORF1 and one ORF2 bind the mRNA in *cis* to form RNPs. SINEs (Alu) hijack ORF2 in *trans* to form their own RNPs. RNPs interact with the DNA replication machinery to retrotranspose into the genome. How L1 cDNA is produced in non-replicating cells is not clear.

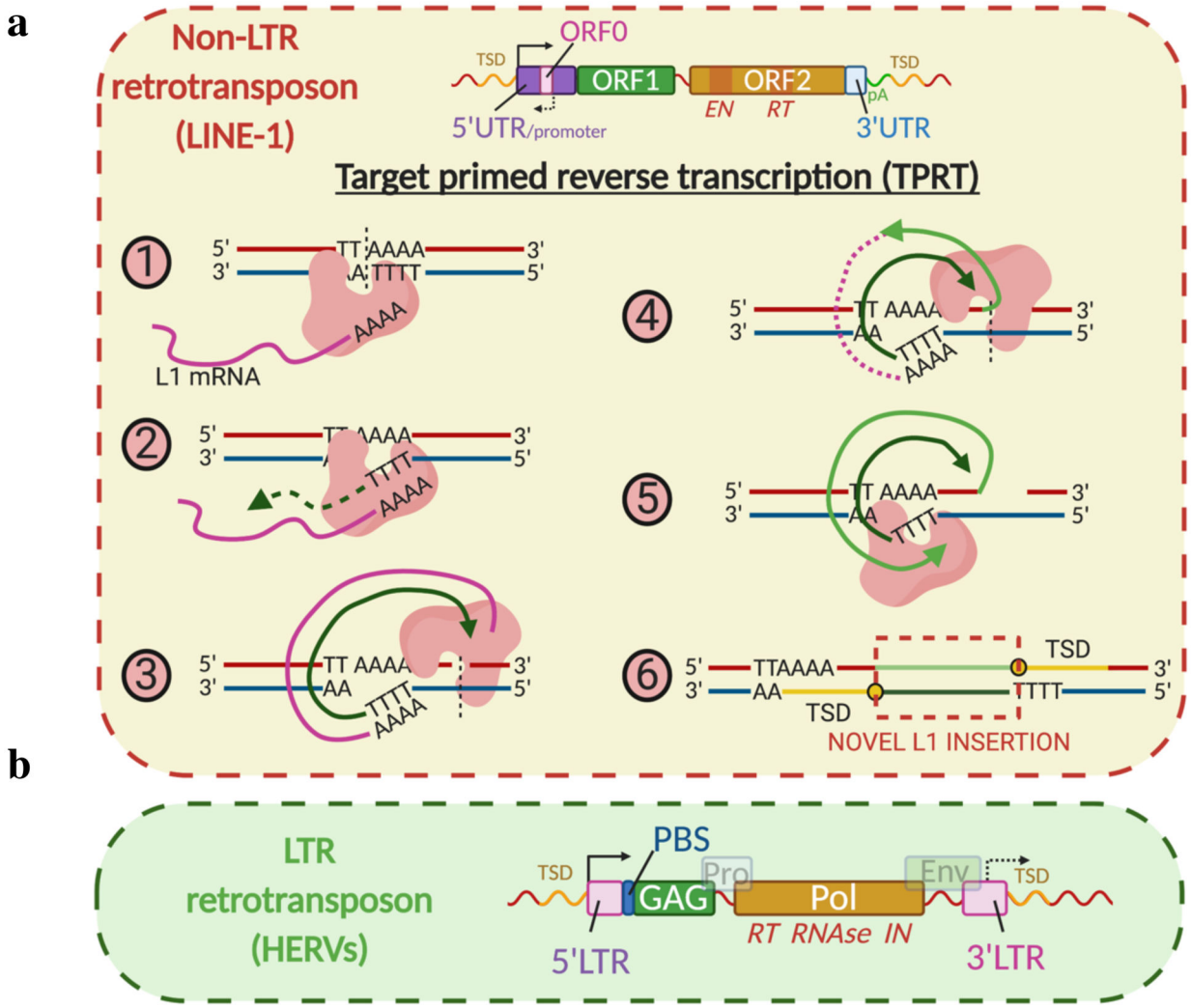

Fig. 2 |. Retrotransposition mechanisms.

a, Non-LTR elements. L1 structure: 5'UTR with internal promoters, purple; ORF0, pink; ORF1, green; ORF2, orange; EN, endonuclease domain of ORF2; RT, reverse transcriptase domain of ORF2; 3'UTR with polyadenylation signals (pA), blue; TSD, target site duplications. The steps (1–6) of TPRT are illustrated. **b,** LTR elements. HERV structure: LTRs with internal promoters, pink; PBS, tRNA primer binding site; Gag (capsid protein), green; Pro (protease), grey-blue; Pol (polymerase), brown; Env (envelope protein), grey-green. Many HERVs lack Env. Pol has domains with RT, ribonuclease H (RNase) and integrase (IN) activities.

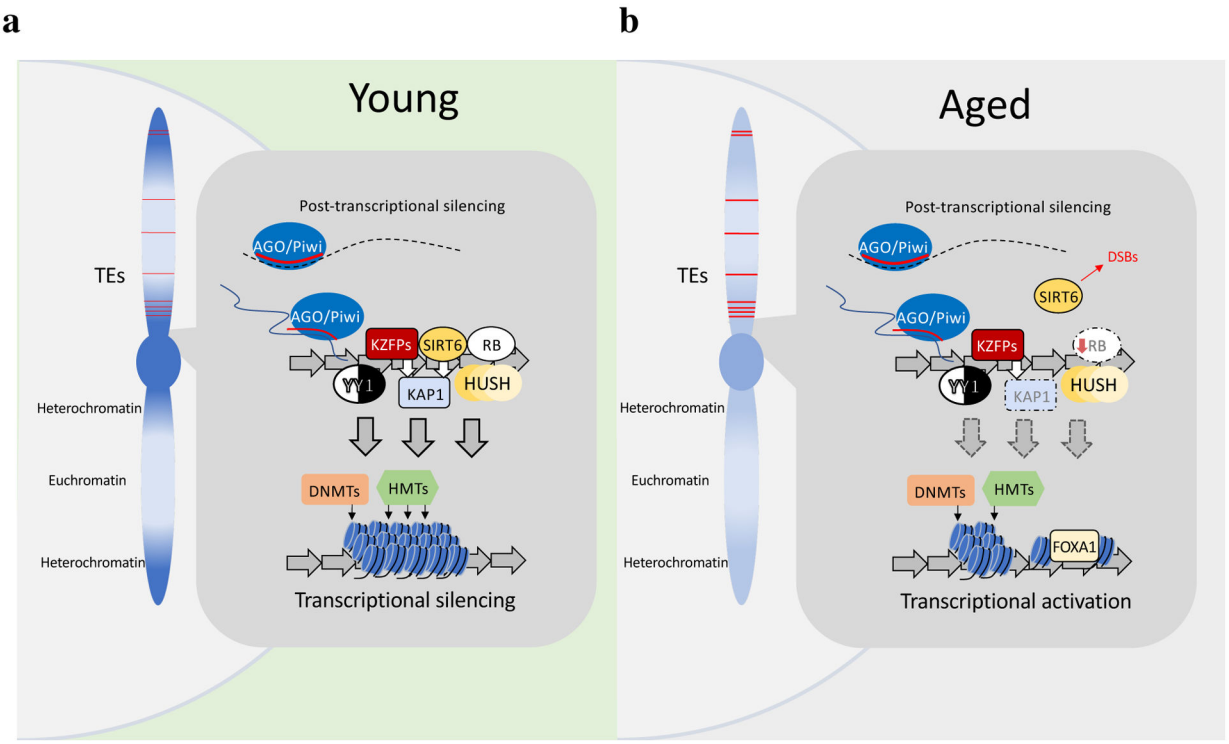

Fig. 3 |. Surveillance of retrotransposons.

Heterochromatin, dark blue; euchromatin, light blue; retrotransposons, red lines. **a,** Young condition. Transcriptional regulators (KZFPs, RB, SIRT6, HUSH, YY1) target retrotransposon elements (repeating arrows) in the genome. SIRT6 and KZFPs both recruit (white arrows) the co-repressor KAP1. AGO and PIWI are targeted to nascent retrotransposon transcripts by siRNAs or piRNAs (red lines). Collectively these effectors recruit DNMTs and repressive HMTs to promote heterochromatin formation. Retrotransposon transcripts (dashed black lines) are also degraded by argonaute slicer complexes. **b,** Aged condition. Surveillance by regulators such as RB and SIRT6 is diminished, resulting in decreased KAP1 recruitment and reduced DNA methylation and repressive heterochromatin modification at retrotransposons. This allows the binding of transcriptional activators (FOXA1).

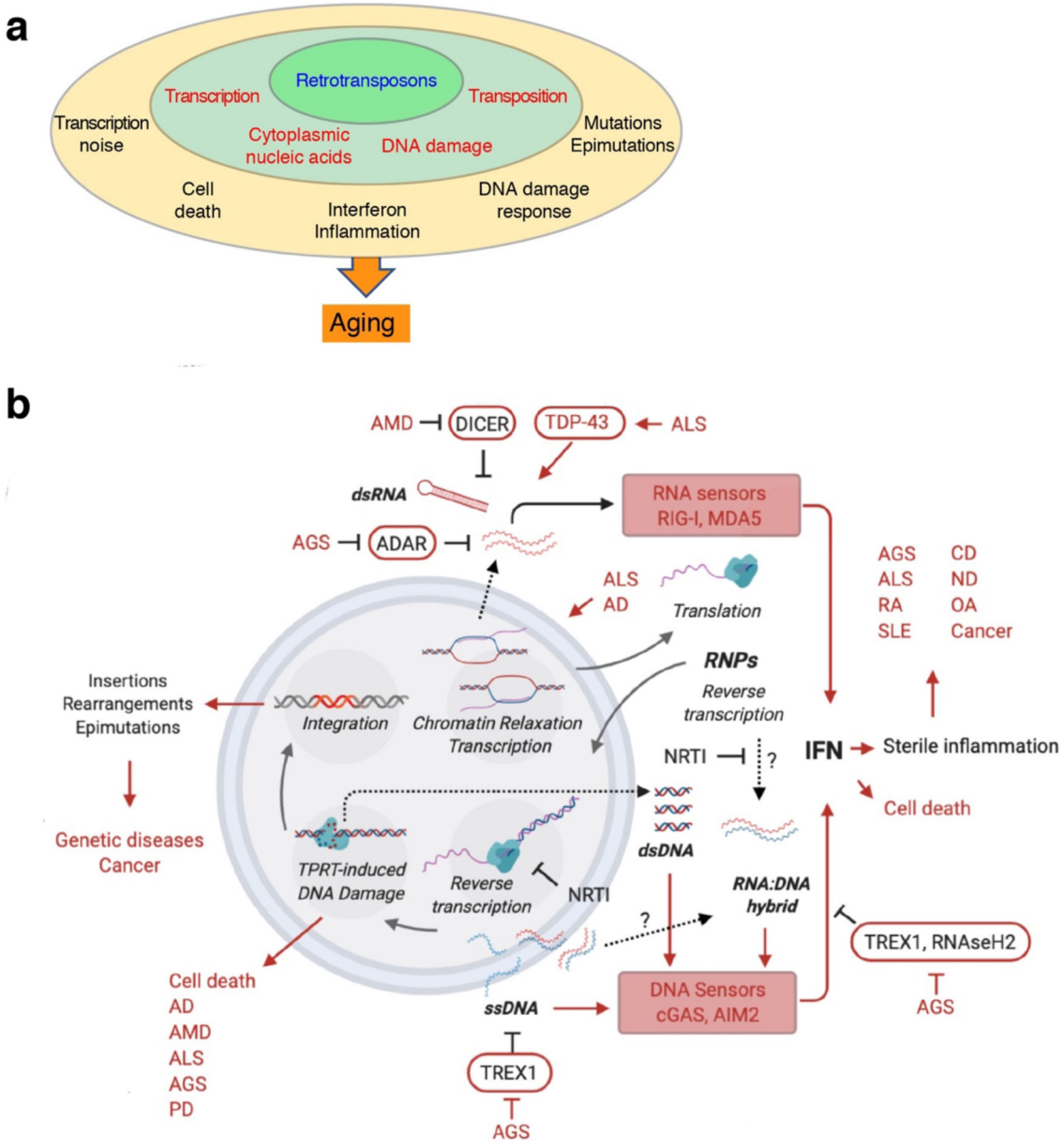

Fig. 4 |. Retrotransposons as agents of aging and disease.

a, Overview of retrotransposon–aging interactions. Defense mechanisms are weakened with age, allowing the deprepression of retrotransposons (inner layer). The direct molecular consequences of retrotransposon activity (middle layer) drive several cellular and tissue-level processes (outer layer) which collectively promote aging. **b,** Specific retrotransposon–disease interactions. L1 transcription and integration are shown inside the nucleus (double circle), translation in cytoplasm, and reverse transcription in both compartments. DNA strands are shown in blue and RNA strands in red. Diseases are abbreviated in capital red letters (terms not defined in text: CD, cardiovascular diseases; ND, neurodegenerative diseases; OA, osteoarthritis; PD, Parkinson's disease; RA, rheumatoid arthritis). Arrows:

activating effects; lines ending in bars: inhibitory interactions; dashed arrows/question marks: hypothetical or uncertain processes.