Non-coding RNA contribution to aging and lifespan

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ABSTRACT

Aging is a multifactorial process characterized by an age-related decline in organismal fitness. This deterioration is the major risk factor for chronic diseases such as cardiovascular pathologies, neurodegeneration or cancer, and it represents one of the main challenges of modern society. Therefore, understanding why and how we age would be a fundamental pillar to design strategies to promote a healthy aging. In the last decades, the study of the molecular bases of disease has been revolutionized by the discovery of different types of non-coding RNAs (ncRNAs) with regulatory potential. In this work, we will review the implication of ncRNAs in aging, with the aim to provide a first approach to the different aging-associated ncRNAs, their mechanism of action and their potential relevance as therapeutic targets and disease biomarkers.

KEYWORDS

circRNAs, microRNAs, lncRNAs, piRNAs, rRNAs, snoRNAs

INTRODUCTION

Research on aging, the time-dependent decline of organism fitness, has gained much attention during the last decades. Clinical, social and technological advances have progressively extended the lifespan of modern human populations, but this phenomenon has not been accompanied by a proportional increase in healthspan (1). This situation has translated into a burden of chronic diseases and an increase in the period lived with disabilities, imposing profound socioeconomic costs. Closing this gap and promoting a healthy longevity is one of the major challenges of our society and requires a coordinate multidimensional strategy (2). Since aging is the major risk factor for chronic diseases and disabilities, understanding why and how we age is a fundamental pillar to achieve this goal. This question has puzzled geroscientists for more than a century and multiple theories of aging have been proposed, but a unified paradigm to explain this process has not been accomplished yet, probably as a reflection of the overwhelming complexity of aging. However, after decades of research scrutiny, we have a reasonable understanding of the alterations and the molecular, cellular and systemic processes that shape aging. Importantly, we know now that aging can be malleable in model organisms by interventions in certain molecular pathways that are highly conserved through evolution, opening the possibility of designing therapeutic strategies to delay aging in humans (3). All this collective knowledge has been recently conceptualized in the form of 12 interconnected hallmarks that integrate the process of aging (4). According to this framework, five primary hallmarks underlie the accumulation of damage that characterize aging: genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis and disabled macroautophagy. These eroding processes are counteracted up to a certain point by three antagonistic hallmarks that, nevertheless, can become deleterious at high levels: deregulated nutrient-sensing, mitochondrial dysfunction, and cellular senescence. Finally, four integrative hallmarks emerge from the interplay of these categories and explain the manifestations of aging: stem cell exhaustion, altered intercellular communication, chronic inflammation and dysbiosis.

Parallel to the expansion of our knowledge about aging, a major achievement of the first two decades of this century has been the discovery of non-coding RNA (ncRNA) as a widespread mechanism of gene expression regulation (5). Thus, our conception about RNA function transitioned from its mere consideration as housekeeping molecules and temporary messengers to their ample recognition as key regulatory molecules of cell biology. Inaugurated with the discovery of hundreds of microRNAs in the early 2000s, the list of regulatory ncRNAs has not stopped expanding since then (6) (Figure 1). Thus, soon after, the technological developments in genomics and bioinformatics led to the identification in the mammalian genomes of thousands of long (> 500 nucleotides) ncRNA molecules (lncRNAs) with regulatory potential originating from intergenic regions or overlapping protein-coding genes in sense or antisense direction (7). Later, a subtype of short-lived lncRNAs were identified in the vicinity of genomic enhancers (eRNAs), and circular RNAs (circRNAs) originated mainly from back-splicing of exons were also added to the list. Moreover, fragments derived from nucleolytic processing of known classes of ncRNAs, such as tRNAs and snoRNAs, were also found to play a regulatory role (8,9). The plethora of ncRNA functions is overwhelming and covers virtually every aspect of cell biology, although the function of many of these molecules remains unknown due to their frequent poor conservation and the technical limitations to study them in a high-throughput manner.

Besides the similar trajectories in aging and ncRNA knowledge, their interplay remains largely unexplored. However, given the large and expanding number of ncRNAs and their repertoire of regulatory cellular functions, it is highly plausible that these molecules play a leading role in the process of aging. In this article, we review the properties and functions of the main classes of ncRNAs and summarize the existing evidence regarding their contribution to aging (Figure 1). While there are available reviews focused on the role of specific classes of ncRNAs in aging, the goal of this work is to provide a global overview to researchers interested in the emerging field of ncRNA regulation in aging.

HOUSEKEEPING ncRNAs

Ribosomal RNAs

Ribosomal RNAs (rRNAs) are the most abundant ncRNA molecules in the cell, accounting for more than 80% of total RNA. These ncRNAs are fundamental constituents of the ribosome, the cellular machinery where the translation of the genetic code takes places to synthetize new proteins. The amino acids required for peptide elongation are transported to the ribosome by another abundant (around 15% of total RNA) class of ncRNAs, the transfer RNAs (tRNAs). tRNAs and the *5S* rRNA are transcribed by the RNA polymerase III (RPIII), whereas the *18S, 5.8S* and *28S* rRNAs are encoded in a *45S* rRNA precursor synthetized by the RNA polymerase I (RPI).

The most obvious aspect of rRNAs is their role in protein synthesis (Figure 2). rRNA transcription is the limiting step for ribosome biogenesis and therefore regulates the translation capacity of the cell (10). The rate of protein synthesis, in turn, is a primary modulator of proteostasis, and the loss of protein homeostasis, either because of a suboptimal translation rate or by an imbalance between synthesis and protein folding and degradation, is a primary hallmark of the aging process (11). It is widely recognized that the protein synthesis rate decays during aging in multiple organisms, from yeast to humans (12). Concomitantly, an age-related decrease in the rRNA levels has also been reported in different species. For example, reduced rRNA expression has been described in rat and human samples from older adults and in tissues from a mouse model of the human Hutchison-Gilford progeria syndrome (13–15). Strikingly, multiple studies have demonstrated that genetic inhibition of the translational machinery extends lifespan in different organisms (16). Thus, reductions in the levels of certain RPs or certain mutations in RPI and RPIII extend lifespan in yeast, worms and flies (17,18). Accordingly, a reduction in ribosome biogenesis and protein synthesis is also a common effect of different dietary or genetic interventions that delay aging (19). In eukaryotes, growth and lifespan are intimately connected by the nutrient sensing network, a system of interconnected pathways that regulate the balance between catabolic and anabolic metabolism in response to nutrient levels, growth factor signals, energy and oxygen availability, and different stress signals (20). These pathways are mechanistically involved in the anti-aging effects of caloric restriction (CR), and their genetic or pharmacological manipulation can extend lifespan and promote healthy aging in multiple model organisms. Conversely, age-associated alterations of this signaling network are recognized as a primary hallmark of aging (4). For example, inhibition of the target of rapamycin (TOR) and insulin/insulin-like growth factor (IIS) signaling pathways, which extends lifespan, downregulates rDNA transcription through the regulation of different RPI and RPIII

transcription factors (19). Similarly, the AMP-activated protein kinase (AMPK) signaling pathway receives inputs from the cellular energy status, and its activation –required for lifespan extension by CR or metformin treatment– represses rDNA transcription (21).

In addition to the well-recognized role of rRNA in protein translation, the relationship between rRNA and lifespan goes beyond ribosome assembly. A common denominator of aging is the accumulation of genetic damage throughout life, and rDNA is one of the most unstable regions of the genome (11). rDNA is highly transcribed and typically arranged as hundreds of tandemly repeated units, a combination that makes it especially susceptible to DNA breaks and unconventional recombination events that can lead to gains or losses of rDNA repeats (22) (Figure 2). In yeast, for example, the length of the rDNA array is associated with the production of toxic extrachromosomal circles (ERCs) and is the major determinant of replicative lifespan (23). In mouse cells, rDNA copy number increases with age, and in humans, replication stress in the rDNA has been linked to the functional decline of hematopoietic stem cells and to the pathogenesis of progeroid syndromes with defects in DNA damage repair genes (22,24). In eukaryotes, the stability of the rDNA arrays is largely influenced by chromatin organization. Notably, epigenetic alterations have also been proposed as an aging driver (11). Thus, despite the large number of rDNA repeats, ~50% of them are epigenetically silenced in most species to protect them from aberrant recombination (25). This process is conserved from yeast to mammals and involves members of the sirtuins, a family of NAD-dependent deacetylases that signal nutrient scarcity and elicit pro-longevity responses (26). In yeast, an extra copy of Sir2 sirtuin reduces rDNA recombination and ERC accumulation and extends lifespan. In mammals, Sirt1 takes part of the energy-dependent nucleolar silencing complex (eNoSC) that regulate rDNA silencing/transcription in response to the energy status, and Sirt7 has been involved in protecting cells from senescence induced by rDNA instability (25,27). In addition, epigenetic silencing through rDNA methylation is especially relevant in the repression of rDNA units in mammals (25). Notably, global and locus-specific alterations in DNA methylation have also been associated with aging and employed to predict biological aging (11,28). In particular, rDNA has been found hypermethylated during aging in tissues from mice, rats and humans, as well as in a mouse model of human progeria (13,14).

tRNAs

Transfer RNAs are the second most abundant class of ncRNA. While the role of tRNAs in lifespan and aging modulation has not been addressed specifically, tRNAs are essential factors for mRNA translation, and, as discussed above, protein translation plays a central role in growth and lifespan modulation (Figure 2). In yeast, nuclear retention of tRNAs due to mutations in *Los1* exportin (an ortholog of mammalian exportin-T) or *Nup100* nucleoporin, or by overexpression of the nuclear importer *Mrt10*, extends replicative lifespan (29,30). Notably, Los1 localization is regulated by dietary restrictions (DR) and responds to DNA damage and TOR signaling. Similarly, *RPIII* mutations, overexpression of the RPIII inhibitor *Maf1* or treatment with TOR inhibitors also reduce tRNA transcription and extend lifespan in yeast, worms and flies (18). Interestingly, this phenomenon has been linked in yeast to a reduced genome instability at the tRNA genes, similar to what has been observed at the rDNA arrays (31). In addition to their role in translation, mounting evidence indicates that tRNAs can be processed into shorter ncRNAs, named tRNA derived fragments (tRFs), with versatile functions associated with aging [reviewed in (8)].

snoRNAs

Small nucleolar RNAs (snoRNAs) constitute another abundant class of small ncRNAs with housekeeping functions. snoRNAs guide different RNA modifying protein complexes to complementary sequences in their targets (32). According to their sequence and binding partners, snoRNAs can be grossly classified as C/D and H/ACA snoRNAs. C/D snoRNAs RNPs (Ribonucleoproteins) contain the methyltransferase fibrillarin and mediate ribose 2'-O-methylation at specific positions. H/ACA RNPs associate with the isomerase dyskerin and catalyze the conversion of uridine to pseudouridine. snoRNAs are produced by RPII and are mainly encoded in introns of protein-coding and non-coding genes. The main known function of snoRNAs is to guide the modification of rRNAs, tRNAs and snRNAs (**Figure 2**). snoRNAs are also present in Archaea, but higher eukaryotes like humans are predicted to have >700 snoRNA genes (33). However, only a few snoRNAs display essential functions during the maturation of rRNA. For most of the snoRNAs, the function of the guided modification is unknown, with different studies pointing at a role in the fine-tuning of translation and splicing. For example, individual deletion of snoRNAs guiding modifications in the decoding center of the ribosome does not cause any obvious biological alteration, but combined mutations, global defects in snoRNA biogenesis or deletion of dyskerin and fibrillarin can impair translation fidelity and delay pre-rRNA processing (34–36). Collectively, these insights make snoRNAs strong candidates for aging modulators, since loss of proteostasis is a hallmark of aging and reduced translation rate or increased translation fidelity can extend lifespan (37). As a proof of concept, a recent work has found that the overexpression of a snoRNA named *jouvence* extends lifespan in flies (38). While the underlying mechanisms are not clear, this snoRNA is conserved in mice and humans and guides pseudouridylation of *18S* rRNA. In line with this, fibrillarin levels are regulated by genetic interventions that affect longevity and its knockdown reduced rRNA biogenesis and extended lifespan in worms (39). Likewise, age-related changes in snoRNAs have been reported in mouse brain and in human cartilage (40,41). Finally, it should be noted that many snoRNAs lack complementary sequences to canonical targets (orphan snoRNAs) and can participate in other processes, such as modifying mRNAs or long non-coding RNAs (lncRNAs), performing RNA-independent functions, or can be processed into smaller fragments (snoRNA-derived fragments) with different activities, although their contribution to aging has not been documented (9).

Other housekeeping ncRNAs

In addition to *5S* rRNA and tRNAs, RPIII also synthetizes other abundant and evolutionary conserved ncRNAs that, although not directly linked to aging, might participate in the modulation of lifespan by this RNA polymerase (Figure 2). For example, *H1* RNA is the ncRNA component of RNAse P, an essential RNP that participates in the maturation of precursor tRNAs from bacteria to humans. Interestingly, RNAse P has also been found enriched in the chromatin of *5S* and tRNA genes, where it plays a role in the regulation of transcription mediated by RPIII (42). Closely related to *H1*, the ncRNA *RMRP* is the RNA component of the mitochondrial RNA processing (MRP) RNAse, an RNP identified by virtue of its endoribonuclease activity against an RNA primer during mitochondrial replication. However, multiple studies have shown that mutations in *RMRP* cause growth defects in humans linked to defective cleavage of *5.8S* rRNA and cyclin mRNAs (43,44). The *7SK* is another RPIII-transcribed nuclear ncRNA that takes part in an RNP complex that regulates RPII transcriptional rate by controlling the activity of the transcription elongation factor P-TEFb (45). Interestingly, an age-related increase in RPII elongation speed has been described in multiples species and associates with

changes in splicing, while decelerating RPII increases lifespan in nematodes and flies (46). In addition, several studies have linked *7SK* with transcription regulation upon DNA damage, and a recent report has shown in mice that aging and DNA damage-related reduction of Larp7, a core *7SK* RNP, accelerates cellular senescence and aging by reducing Sirt1 activity (47).

Spliceosomal small nuclear RNAs (snRNAs) are other well-known housekeeping ncRNAs that participate in the fundamental process of intron excision and exon joining during RNA maturation (48). These molecules include the *U1, U2, U4, U5* and *U6*, and the alternative, less used, *U11, U12, U4atac and U6atac* snRNAs. All of them are transcribed by RPII, except for *U6* and *U6atac*, which have RPIII promoters. Splicing is a highly dynamic and regulated process that adds complexity to higher eukaryotes by virtue of its ability to produce alternative mRNA isoforms with different functions. Global age-related alterations in spliceosomal snRNAs have not been reported, but aberrant maturation of specific sets of mRNAs has been described during aging and in response to lifespan modulation in multiple species [reviewed in (49)]. These alterations are generally associated with age-related changes in splicing factors and show high tissue specificity, but they are enriched in aging-associated biological processes, such as DNA repair or mitochondrial function (50).

REGULATORY ncRNAs

microRNAs

miRNAs constitute the most intensively studied subset of regulatory ncRNAs. They are characterized by their small size (~22 nucleotides) and are usually encoded inside other genes (protein-coding or lincRNAs), although they can also be controlled by their own promoters (6). After transcription as a long (often multicistronic) primary transcript, a series of processing steps lead to the generation of mature miRNAs, which associate in the cytoplasm with Argonaute (AGO) proteins to form the RNA-induced silencing complex (RISC). miRNAs act as molecular guides that direct this complex to complementary sequences usually present in the 3' untranslated region (3'UTR) of target mRNAs. This interaction between the RISC and the mRNA leads to translation inhibition and gene expression silencing (Figure 3).

Since their discovery, miRNAs have stood as important regulators of pathophysiology, and as such they are implicated in processes like cancer or aging. The first miRNA found to modulate aging was *lin-4*, a regulator of *Caenorhabditis elegans* development. This miRNA suppresses translation of *lin-14*, a component of the IIS pathway. *lin-4* mutants displayed a shortened lifespan and accelerated tissue aging, whilst its overexpression or the decrease of *lin-14* activity extended lifespan (51). This pathway is also conserved in *Drosophila melanogaster*, where *lin-4* orthologue, miR-125, also modulates lifespan and its depletion results in a short-lived phenotype (52). In *C. elegans*, *miR-71* and *miR-239* also control longevity by regulating the **nutrient-sensing** pathways (53). Conversely, *miR-34* depletion extends lifespan by increasing Atg9 and therefore autophagy levels (54). In mammals, however, there is less evidence of miRNAs capable of modulating longevity by themselves. In mice, *miR-17* overexpression extends lifespan by inhibiting cellular senescence by targeting of different components of the IIS pathway. (55). Conversely, *miR-1* upregulation has been linked to deleterious reduction of the IIS pathway in progeroid mice, and preliminary findings show that the overexpression of miR-29 in mice provokes a premature aging phenotype, with an altered phenotype in autophagy, extracellular matrix and immune response (56,57).

Consistently with their widespread regulatory function, it is not surprising that miRNAs are implicated in the modulation of other hallmarks of aging. Their role is well established in the regulation of **senescence**, controlling multiple signaling pathways such as p53/p21 and p16/Rb, as well as participating in the senescence-associated secretory phenotype (SASP) (58). Senescence is also relevant in **stem cell** aging, and the depletion of these cells is also controlled by miRNAs. As an example, *miR-141* upregulation during human mesenchymal stem cells (hMSCs) aging represses the protease ZMPSTE24 and leads to the accumulation of toxic prelamin A, cell damage and senescence. Conversely, the downregulation of this miRNA with anti-miRs increased hMSCs survival (59). miRNAs can also modulate cellular environment during aging by controlling inflammation. The systemic chronic inflammation associated with aging, known as "**inflammaging**", is influenced by miRNAs through their regulation of innate immune elements such as toll-like receptors (TLRs) (60). In fact, *miR-146a* and *miR-21* have been described as key "inflammamiRs", due to their regulation of multiple components of the NF-κB pathway (61).

Besides altering the extracellular environment, miRNAs also regulate **intercellular communication**. Their transport through exosomal vesicles is an important mechanism of communication and is altered with age (62). In line with this, a recent study that characterized the changes in miRNA levels during mouse aging has found a set of global age-related miRNAs (*miR-29, miR-184 and miR-1895*) that are also present in circulation inside exosomes. Interestingly, the aging-associated increase in *miR-29*, which has also been described in a mouse model of premature aging, can be partially reversed through heterochronic parabiosis, pointing to an important role of exosomal communication in the regulation of aging-associated miRNA levels (63,64). Another important player in intercellular communication is the extracellular matrix. Fibrosis, which consists in the excessive accumulation of extracellular matrix components and the formation of scar tissue, also alters this communication. This also occurs during aging and is partially mediated by miRNAs (65).

Loss of proteostasis and **disabled macroautophagy** are other important hallmarks of aging that dampen organismal fitness and subsequently contribute to aging. As previously mentioned, aging-associated *miR-34* is a regulator of autophagy, but it also modulates other proteostasis-related genes, such as *Lst8*, a subunit of TORC1 complex (66). Also, another previously mentioned miRNA, *miR-71*, controls lifespan in *C. elegans* by regulating ubiquitin-dependent proteolysis in a food odor-stimulated mechanism (67). In addition to protein homeostasis, mitochondrial homeostasis is also controlled by miRNAs. A subset of miRNAs, known as **mitomiRs**, control important mitochondrial components, both in the cytoplasm and inside the mitochondrial directly interacting with mitochondrial mRNAs. These mitomiRs have been described in several pathological contexts related with aging; moreover, their deregulation has been observed in aged tissues (68,69).

Genomic instability and **epigenetic alterations** are also influenced by miRNA action. Some miRNAs, such as *miR-421*, directly regulate key DNA damage repair proteins ATM and ATR (70). On the other hand, other miRNAs control epigenetic signatures such as DNA methylation or histone modification (71). That is the case of *miR-290*, a cluster of miRNAs that control the levels of DNA methylation enzymes by targeting retinoblastoma-like 2 (Rbl2) (72). Conversely, the expression of the aforementioned *miR-34* is also essential for a healthy brain aging in *D. melanogaster* through the regulation of the histone methylation complex PRC2 (73). Furthermore, **telomere maintenance** is also controlled by miRNAs. *miR-23a* was identified in a genomic screen as a direct regulator of Telomeric repeat binding factor 2 (TRF2). Its overexpression resulted in accelerated senescence of

human fibroblasts, and it could be rescued by TRF2 ectopic expression (74). Another important telomere component, Protection of telomeres 1 (POT1), is regulated by *miR-185* and its overexpression had a similar effect, accelerating senescence and increasing telomere dysfunction-induced foci (75).

Finally, gut **dysbiosis** has recently emerged as an important hallmark of aging. Gut microbiome diversity decreases during aging and accelerated aging pathologies, and its reversion by fecal microbial transplantation, or administration of certain bacteria, increases lifespan and healthspan in animal models (76). Host miRNAs also exert their effect over gut microbiota, as miRNAs secreted by the gut travel inside extracellular vesicles and enter bacteria, regulating gene expression. Accordingly, depletion of these miRNAs causes dysbiosis and colitis (77). This host miRNA-gut interaction has potential therapeutical interest. In multiple sclerosis patients and in an experimental autoimmune encephalomyelitis (EAE) mouse model, miR-30d was found to be upregulated. Oral delivery of this miRNA ameliorated EAE mouse phenotype by targeting a lactase of *Akkermansia muciniphila*, a well-known prolongevity bacteria, causing its increase in the gut (78,79).

lncRNAs

LncRNAs represent an abundant and well-known class of ncRNAs characterized by their length, currently stablished in over 500 nucleotides (7). They can be transcribed from several different locations on the genome, including introns and exons of protein-coding genes, intergenic regions and enhancers. Their location is primarily nuclear, but they can also be found in other cellular compartments. LncRNAs are highly heterogenous in terms of sequence and structure, and are able to interact with DNA, RNA and proteins to exert a large variety of regulatory and structural functions. Multiple lncRNAs regulate gene expression by affecting chromatin architecture through different mechanisms, including the formation of DNA-RNA hybrids or by recruiting or repressing different chromatin modifying complexes (80). Similarly, a subgroup of lncRNAs transcribed from gene enhancers (eRNAs or elncRNAs depending on their size) can actively promote chromatin looping and bring together enhancers and target genes, regulating their expression. LncRNAs can also regulate gene expression post-transcriptionally through interactions with the splicing machinery or with different proteins involved in the degradation, localization or translation of mRNAs. Moreover, some lncRNAs harbour multiple miRNA binding sites and function as competing endogenous RNAs (ceRNAs) by diverting the corresponding miRNAs from their targets (7,80).

Many lncRNAs play an important role in the context of aging (Figure 4). Several recent studies have reported the deregulation of hundreds of lncRNAs during aging in several species, including mice and human. Even though the vast majority of these lncRNAs were highly tissue-specific, the pathways regulated by these aging-related lncRNAs are commonly shared among tissues (81). This suggests that lncRNAs in general are involved in fine-tuning mechanisms of the aging process, in a tissue-specific manner. Age-related lncRNAs appear to be mostly involved in the regulation of immune response pathways, including the NF-κB signaling pathway, suggesting that both inflammaging and immunosenescence are partially related to regulation by lncRNAs (82). This points towards a generalized deregulation of the immune response pathways, which has already been described to occur in aging (11). Similar results were obtained by another study that analyzed the aging-associated expression levels of lncRNAs in multiple tissues from mice (83). Interestingly, they reported that white adipose tissue (WAT) displays the most profound age-related changes in lncRNA

and found a strong correlation with the expression of protein-coding genes involved in immune response pathways, suggesting that WAT might play a leading role in mouse aging.

Besides this generalized effect, several individual lncRNAs have stood out in the study of aging due to their relevance in regulating several pathways (**Figure 4**). *MALAT1*, for example, is downregulated in skeletal muscle during mouse aging and has been described to act as a ceRNA for *miR-34a*, contributing to its age-related upregulation and the concomitant repression of its target gene Sirt1 (84). The low levels of this deacetylase, in turn, promote senescence and fibrosis through the increased expression of several age-related proinflammatory factors such as TGF-β1, normally repressed by Sirt1. Another lncRNA implicated in aging is *Sarrah*, expressed in mouse cardiomyocytes and downregulated with age (85). This lncRNA has anti-apoptotic effects and its expression promotes cardiomyocyte recovery from acute myocardial infarction. Another lncRNA, *CDKN2B-AS1* (also known as *ANRIL*) is involved in the silencing of the tumor suppressor *INK4B*, and thus is significantly downregulated in senescent cells (86). The pseudogene-derived lncRNA *Lethe* is part of a negative feedback loop with NF-κB and its levels are decreased with age in mice (87). The lncRNA *NORAD* has been shown to play an important role in the aging process. *Norad*-deficient mice display an increased activity of Pumilio proteins, genomic instability and phenocopy accelerated aging (88). In another example, the lncRNA *Gadd7* interferes with the binding between the protein Tdp-43 and *Cdk6* mRNA in response to genetic damage, thus promoting *Cdk6* mRNA decay and inducing cell cycle arrest (89). Besides this, the lncRNAs *ES1, ES2, ES3* and *linc-RoR* have been described as essential for the maintenance of stem cells, whose depletion is a well-described hallmark of aging (90,91).

A case which deserves special attention in the context of aging-related lncRNAs are those involved in telomere maintenance and telomerase activity. There are mainly two lncRNAs associated with telomerase activity: *TERC* (Telomerase RNA Component) and *TERRA* (Telomeric Repeat Containing RNA). *TERC* is an essential part of the telomerase complex, acting as a template for the retrotranscriptase TERT during the addition of telomeric repeats (92). On the other hand, *TERRA* is a heterogenous group of RPII-transcribed lncRNAs produced from the telomeric regions. Although the exact function of *TERRA* lncRNAs is unknown, they play a role in telomere homeostasis through interactions with different proteins that assist in the maintenance of the heterochromatic state of the telomere, and through the regulation of telomerase activity (93). Since telomere attrition has been extensively linked to aging and senescence and *Terc*$^{-/-}$ mice show premature aging and genomic instability, the relevance of both *TERC* and *TERRA* in aging is unequivocal (11,92). For further information, the role of many other lncRNAs in the aging process has been extensively reviewed elsewhere (94).

CircRNAs

A special case among lncRNAs are circRNAs, another type of non-coding RNAs that have recently gained popularity. They consist of exceptionally stable, covalently closed single-stranded RNA molecules that often contain complete exons from protein-coding and non-coding genes, although they can also derive from introns or contain both types of elements (95). Like linear lncRNAs, circRNAs can regulate gene expression through multiple mechanisms, being able to function as miRNA sponges, as transporters, decoys and scaffolds of RNPs, or in the *cis* regulation of the production of their linear counterparts. In addition, circRNAs have been found in exosomal vesicles,

suggesting that they might participate in intercellular communication, although it could also represent a clearance mechanism (96). Moreover, besides their function as noncoding RNAs, circRNAs can also be translated, producing several small peptides with diverse functions, mainly on neuronal synapsis (95).

circRNAs have been found to accumulate during the aging process, an accumulation that is particularly evident in brain tissue, especially in neurons (Figure 5) (97). This finding led circRNAs to be initially linked with neuronal degenerative diseases (98). However, brain-related genes are highly enriched in circRNAs-producing exons and neurons display the highest rate of alternative splicing among all cell types in the human body (99), which could partially explain the accumulation of circRNAs on this tissue. Furthermore, neurons do not divide, and it has been shown that the amount of circRNAs expressed in proliferative cells is much smaller than the one on post-mitotic cells (100), in part due to the long mean life of circRNAs. This is consistent with the fact that circRNAs are strongly and dynamically regulated during embryonic development (101), suggesting that circRNAs as a whole tend to play a role on cell proliferation and differentiation. Some notable examples of this are *circHIPK3*, which regulates cell growth via its interaction with several miRNAs (102), or *circFOXO3*, which is also involved in cell cycle regulation (103). Even though current knowledge seems to indicate that the increased amount of circRNAs with age is mostly due to accumulation and not to age-specific regulation, the fact that several studies find a strong correlation cannot be ignored. A significant increase has been found in *Drosophila* and mouse brains with age (104,105), and in *C. elegans* whole-body studies (106). However, it is important to point out that the majority of adult cells in worms are post-mitotic (107). All in all, circRNAs tend to show significant correlation to aged neuronal tissue, but further research is needed to determine whether they actively participate in the regulation of the aging process or are a passive marker of the age-related decline of their degradation mechanisms.

piRNAs

piRNAs (PIWI-interacting RNAs) are a recently described subtype of regulatory small ncRNAs. They differ from miRNAs, as they are slightly longer (24-31 nucleotides), are 2-O-methylated at their 3' end, and associate with PIWI AGO-clade proteins (108). piRNAs are mostly produced from specific loci in the genome called piRNA clusters, which have their evolutionary origin on transposable elements inserted backwards. However, piRNAs can be also produced from the 3'-UTR of mRNAs, from exonic regions of lncRNAs and from processed forms of other ncRNAs, such as tRNAs and snoRNAs. Although transcriptional or post-transcriptional silencing of transposons in the germline is considered the ancestral function of piRNAs, multiple studies have demonstrated that these molecules are also expressed in somatic cells and can participate in gene expression regulation of protein-coding genes (through epigenetic silencing or in a miRNA-like fashion), play a role in the viral defense, or regulate global and transcript-specific m^6A RNA methylation (109) (Figure 5).

Even though piRNAs have been recently described, some evidence of their implication in aging already exists. Most piRNAs originate from transposon elements and are involved in their silencing, and interestingly, several retrotransposons have been linked to the aging process, with their derepression promoting different age-related phenotypes, such as inflammaging (110). Similarly, related to their function in m^6A methylation, a reduction in the levels of this RNA modification have been linked to aging as well (111). Likewise, piRNA-mediated DNA methylation and histone

modifications might underline the epigenetic alterations linked to aging (11). When talking about specific piRNAs involved in the aging process we can find several examples. *piR_02887, piR_000580, piR_025007* and *piR_025576* are known to be downregulated in cellular senescence and the expression of *piR_025578* has been found to inhibit this process in human chondrocytes (112). Besides this, several PIWI proteins such as Mili are known to have an anti-senescence effect (113). In summary, piRNAs seem to be implicated in the aging process but further research is needed to assess the exact mechanisms of action.

CLINICAL EPIDEMIOLOGY OF ncRNAS IN AGING

Direct evidence of ncRNA contribution to human aging is very scarce, but different studies have revealed important associations between ncRNAs and age-related phenotypes and diseases. Genome-wide association studies (GWAS) have proven to be efficient tools to identify genetic associations of protein-coding genes to complex traits, such as aging, and a growing number of studies are exploring the implication of ncRNAs residing in disease-associated loci. An outstanding example is the previously discussed lncRNA *CDKN2B-AS1*. Single nucleotide polymorphisms (SNPs) in this lncRNA have been associated in different GWAS studies with paternal lifespan, coronary artery disease, type 2 diabetes, and cancer; and functional experiments show a mechanistic relationship with these traits (114,115). Other examples of functionally-validated disease-associated lncRNAs are *H19* and *MIAT*, associated with coronary artery disease and myocardial infarction, respectively (114). Interestingly, *MIAT* is also expressed in retinal cells, and genetic variants in this lncRNA and others, such as *TUG1*, have been associated with increased risk to develop diabetic retinopathy (116). Genetic variants in miRNAs have also been linked to age-related diseases in GWAS studies. For example, a SNP associated with increased risk of Alzheimer´s disease affects the *pre-miR-1299* sequence and results in elevated levels of *miR-1299-3p*. Moreover, variants associated with Parkinson disease reduce the levels of mature *miR-584at-5p* and *miR-4519* due to alterations in their pre-miRNA structures (117,118). Similarly, a polymorphism in *pre-miR-499* reduces the expression of the cardiac-abundant *miR-499-5p* and confers increased susceptibility to coronary heart disease (119). Another remarkable example is the 14q32 locus, which includes a cluster of snoRNAs, two clusters of miRNAs and two lncRNAs, and has been genetically linked to cardiovascular diseases and associated with stem cell pluripotency and cancer. (120).

Apart from these genetic approaches, transcriptomics studies have also revealed striking associations between aging and age-related diseases and the expression patterns of certain ncRNAs. Amongst the different types of ncRNAs, miRNAs are the most studied in this regard. Multiple studies have proposed a strong association with age in the profiles of blood miRNAs during healthy aging and its disruption during disease, highlighting their potential as disease biomarkers (121–123). Looking at specific examples, several miRNAs have been proposed as biomarkers for cardiovascular disease, such as *miR-197*, which is associated with myocardial infarction, *miR-150* and *miR-191*, which are associated with atherosclerosis, and *miR-126* and *miR-223*, which are associated with both diseases (124). Others such as *miR-122* are associated with metabolic alterations like diabetes type 2 (124), and several serum miRNA signatures have been linked to Alzheimer´s disease (125). Although less explored, changes in the expression profiles of lncRNAs have also been linked to human aging and age-related diseases. Thus, gene expression studies on human tissue samples identified several lncRNAs associated with degenerative neurological diseases, such as *BACE1-AS* and *17A* in Alzheimer´s disease, *naPINK1* and *lnc-FRG1-3* in Parkinson's disease, and *HTTAS_V1* in Huntington´s

disease (126). Altered expression of lncRNAs has also been identified in other aging human tissues. For example, the expression of lncRNA *Meg3* (located in the above-mentioned 14q32 locus) is increased in the aged human heart, and is proposed to regulate the process of angiogenesis (127). Apart from regulatory ncRNAs, the role of housekeeping RNAs in the context of aging diseases is also relevant. Most neurodegenerative diseases are caused by abnormal protein homeostasis; therefore, it is not surprising that the levels of rRNA are altered in diseases such as Alzheimer´s and Parkinson (128). In summary, ncRNAs have greatly expanded the repertoire of new players in aging and multiple studies highlight their remarkable potential as therapeutic targets and/or biomarkers for age-related diseases, which has translated in a growing number of clinical trials trying to make use of their medical potential (e.g ClinicalTrials.gov accession IDs NCT04509271, NCT06213493).

CONCLUSIONS AND PERSPECTIVES

Aging might be inexorable, but it is now clear that longevity is plastic and largely determined by the genetic background and its crosstalk with the environment. Short-lived invertebrate model organisms have been instrumental to identify conserved molecular pathways that also operate during mammalian aging, but they miss a large part of the biologic and genomic complexity of higher eukaryotes. While the number of protein-coding genes has not changed significantly during the evolution of metazoan, there is a clear correlation between biological complexity and the proportion of the non-coding genome. Not surprisingly, most longevity genes described so far are protein-coding, while only 1-2% of mammalian genomes code for these molecules. Over the last decades it has become clear that the non-coding part of the genome is transcriptionally active and produces thousands of ncRNAs deeply involved in the regulation of all aspects of cell biology. While our knowledge about the role of these molecules in aging is still very limited, the collective evidence summarized in this work shows that the study of these molecules could unlock new venues for designing anti-aging drugs to cope with the burden of age-related diseases in our societies. Compared to protein-coding genes, ncRNAs generally perform fine-tuning functions and display a higher tissue specificity, properties that make them excellent therapeutic targets. Moreover, they are promising candidates for RNA-based therapeutics, a rapidly expanding drug category that has gained great momentum since the success of the mRNA-based COVID-19 vaccines. Anti-miRNAs and miRNA-mimics have yielded favorable results in animal studies and some of them have already entered clinical trials, and antisense oligonucleotide technology can potentially be applied to any ncRNA. Moreover, given their ease to be detected with standard techniques in tissue samples and body fluids, many clinical trials are exploring their utility as biomarkers for age-related diseases. Hopefully, the improvements in sequencing and genome editing technologies and the generation of new mouse models will help imputing the function of the growing number of ncRNAs and expand the repertoire of potential anti-aging ncRNAs. However, given the generally poorer inter-species conservation of ncRNAs, specially lncRNAs, advances in the development of *in vitro* 3D models that recreate human tissues will be pivotal for the evaluation of the efficiency of candidate ncRNA as anti-aging interventions.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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Table 1: List of aging-associated ncRNAs discussed in this work

ncRNA	Species	Function	Ref.
Housekeeping RNAs			
rRNA	Multiple	Protein synthesis. Effect on nutrient sensing pathways. Accumulation of DNA damage during aging in rRNA gene arrays.	(12) (19) (23)
tRNA	Multiple	Protein synthesis. Effect on nutrient sensing pathways. Accumulation of DNA damage during aging in tRNA gene arrays. tRNA-derived fragments with roles in aging.	(29,30) (31) (8)
snoRNA *jouvence*	Fly	Guides 18S rRNA pseudouridylation. Its overexpression extends lifespan.	(38)
H1	Human	ncRNA component of RNAse P. Regulation of RPIII transcription.	(42)
RMRP	Human, Mouse	ncRNA component of MRP RNAse. Implicated in ribosome synthesis and cell cycle regulation.	(43,44)
7SK	Mouse	Part of an RNP complex that regulates RPII transcription rate by modulating P-TEFb	(45) (47)
snRNA	Multiple	Mediate mRNA splicing. Altered during aging.	(49)
miRNAs			
lin-4	Nematode	Regulates *lin-14*. Mutants have shortened lifespan	(51)
miR-71, miR-239	Nematode	Regulate nutrient-sensing pathways.	(53)
miR-34	Nematode, Fly	Modulates autophagy and proteostasis by targeting *Atg9, Lst8*, and others. Modulates histone methylation through targeting PRC2.	(54)
miR-17	Mouse	Inhibits cellular senescence and targets components of IIS pathway. Its overexpression extends lifespan.	(55)
miR-1	Mouse	Reduction of IIS pathway activity in premature aging models.	(57)
miR-29	Mouse	Increased in circulation and multiple tissues during aging. Its depletion provokes premature aging.	(63)
miR-141	Human	Regulates lamin A processing by targeting *ZMPSTE24*. Its overexpression in hMSCs increases cell damage and senescence.	(59)
miR-146a, miR-21	Human, Mouse	Proposed as key *inflammamiRs*.	(61)
miR-184, miR-1895	Mouse	miRNAs altered in multiple tissues during aging	(63)
miR-421	Human	Represses the DNA damage repair protein ATM.	(70)
miR-290	Mouse	Cluster of miRNAs regulating DNA methylation by targeting *Rbl2*.	(72)
miR-23a	Human	Direct regulator of telomericTRF2. Its overexpression results in accelerated senescence.	(74)
miR-185	Human	Represses telomeric *POT1*. Its overexpression accelerates senescence.	(75)
miR-30d	Mouse	Targets a lactase mRNA of *Akkermansia*	(79)

		muciniphila, increasing its abundance in the gut. Its oral delivery ameliorates the phenotype of a EAE mouse model.	
lncRNAs			
Malat1	Mouse	Acts as a ceRNA, decreasing miR-34a levels. Downregulated in skeletal muscle aging.	(84)
Sarrah	Mouse	Expressed in cardiomyocytes. It has antiapoptotic effects and promotes recovery from myocardial infarction. Downregulated during aging.	(85)
ANRIL	Human	Represses tumor suppressor *INK4B*. Downregulated in senescent cells.	(86)
Lethe	Mouse	Conforms a negative feedback loop with NF- κB. Decreased in aging.	(87)
Norad	Mouse	*Norad*-deficient mice phenocopy premature aging, with increased genomic instability and increased activity of Pumilio proteins.	(88)
Gadd7	Chinese Hamster	Interferes with Tdp-43/*Cdk6* binding in the context of DNA damage, promoting *Cdk6* decay and cell cycle arrest.	(89)
ES1, ES2, ES3, linc-RoR	Human	Essential for stem cell maintenance.	(90,91)
TERC	Multiple	Template for TERT retrotranscriptase. Essential for telomere maintenance.	(92)
TERRA	Multiple	Group of RPII-transcribed lncRNAs from telomeric regions. Important for telomere homeostasis.	(93)
circHIPK3	Human	Modulates cell growth by interacting with several miRNAs	(102)
circFoxo3	Mouse	Involved in cell cycle regulation	(103)
piRNAs			
piR_02887, piR_000580, piR_025007, piR_025576	Human	Downregulated in cellular senescence	(112)
piR_025578	Human	Its expression inhibits cellular senescence in chondrocytes.	(112)

FIGURE LEGENDS

Figure 1: main classes of ncRNAs according to their functions and properties. RP, RNA polymerase; RBP, RNA binding protein.

Figure 2: contribution of housekeeping ncRNAs to aging.

Figure 3: miRNA biogenesis and their role in organismal aging. Green arrows indicate miRNAs whose overexpression has been linked to longevity extension and/or whose depletion reduces lifespan. Red arrows depict miRNAs whose overexpression has been linked to longevity reduction and/or whose inhibition extends longevity.

Figure 4: lncRNA mechanisms of actions and their implication in aging. Green arrows indicate miRNAs whose overexpression extends longevity and/or whose depletion reduces lifespan.

Figure 5: Mechanisms of action of circRNAs and piRNAs and their implication in aging.

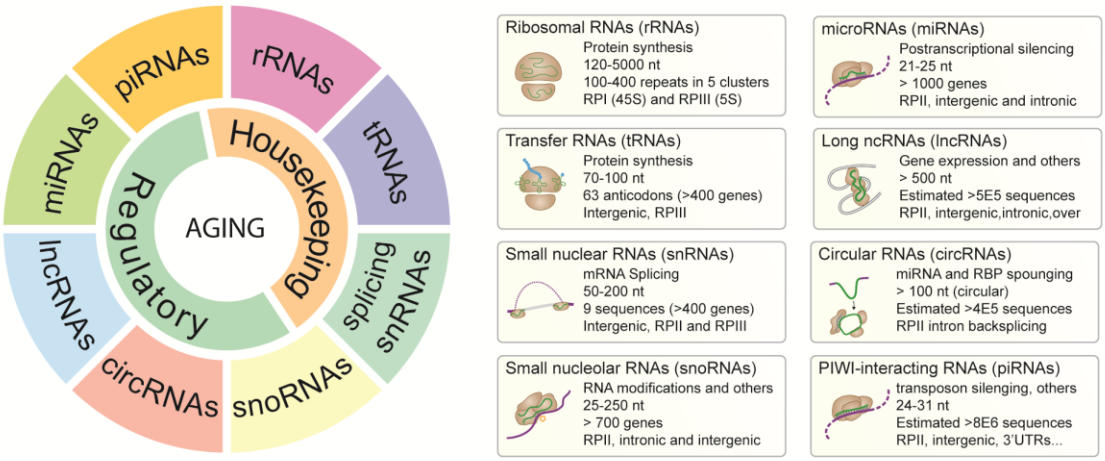

Figure 1

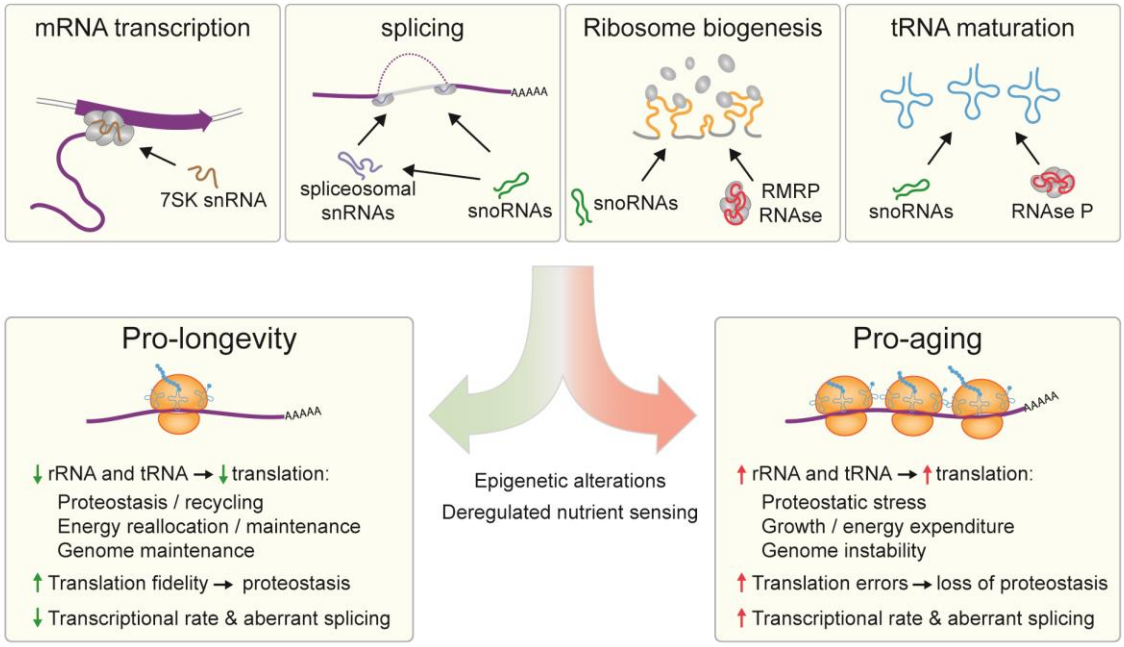

Figure 2

Figure 3

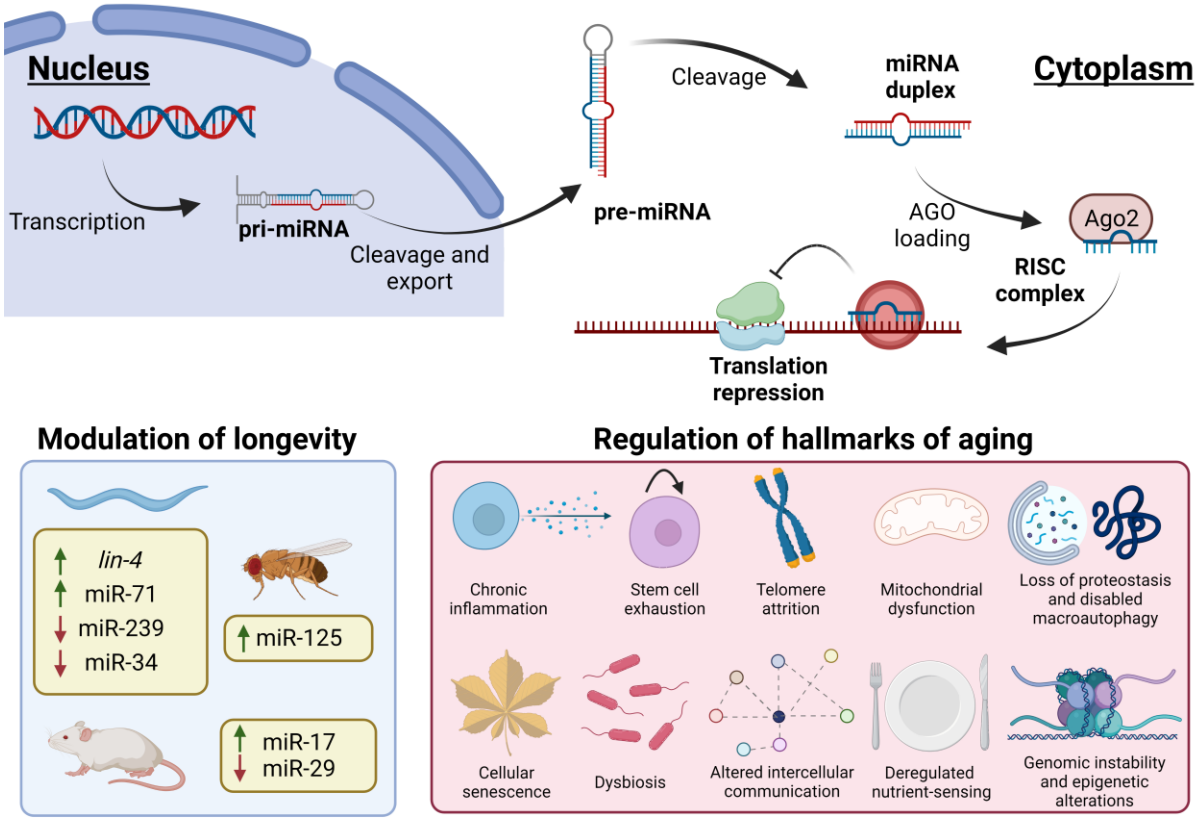

Figure 4

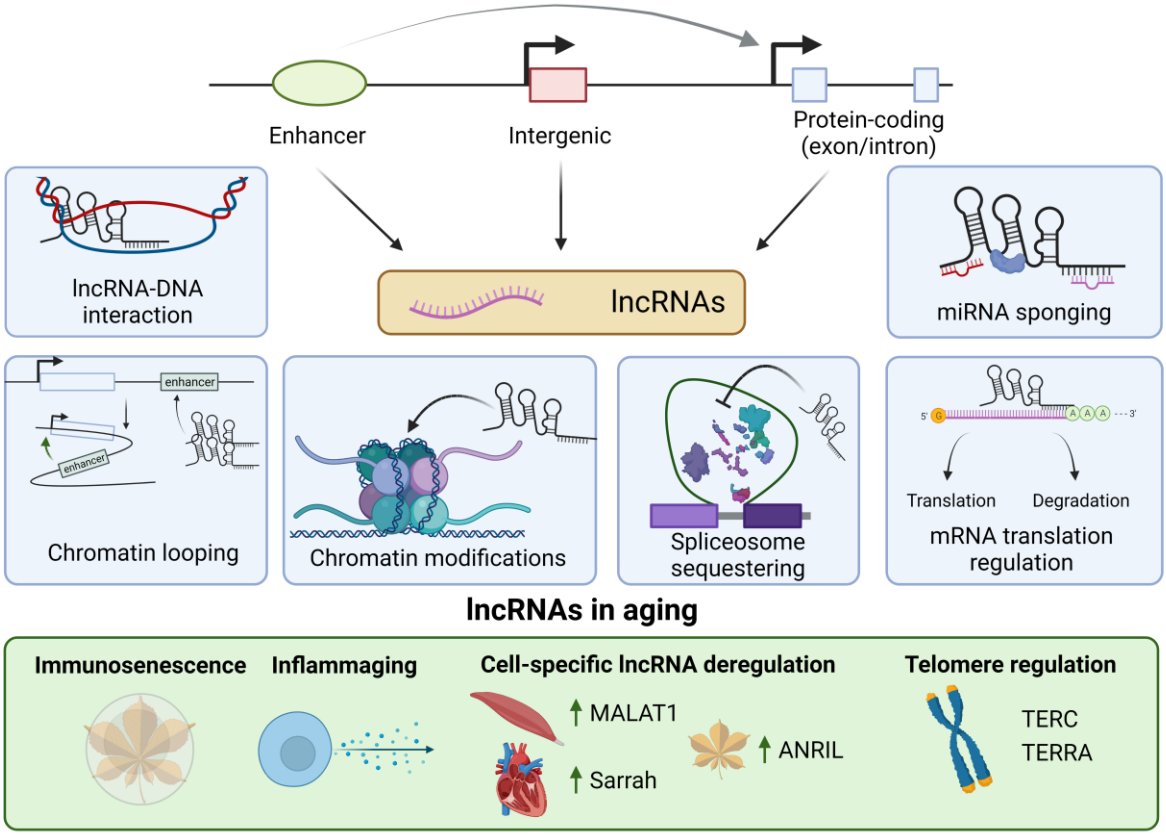

Figure 5