Molecules and Cells

Minireview

Non-Coding RNAs in *Caenorhabditis elegans* Aging

Sieun S. Kim[1] and Seung-Jae V. Lee[2,*]

[1]Department of Life Sciences, Pohang University of Science and Technology, Pohang 37673, Korea, [2]Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon 34141, Korea

*Correspondence: seungjaevlee@kaist.ac.kr
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Non-coding RNAs (ncRNAs) comprise various RNA species, including small ncRNAs and long ncRNAs (lncRNAs). ncRNAs regulate various cellular processes, including transcription and translation of target messenger RNAs. Recent studies also indicate that ncRNAs affect organismal aging and conversely aging influences ncRNA levels. In this review, we discuss our current understanding of the roles of ncRNAs in aging and longevity, focusing on recent advances using the roundworm *Caenorhabditis elegans*. Expression of various ncRNAs, including microRNA (miRNA), tRNA-derived small RNA (tsRNA), ribosomal RNA (rRNA), PIWI-interacting RNA (piRNA), circular RNA (circRNA), and lncRNA, is altered during aging in *C. elegans*. Genetic modulation of specific ncRNAs affects longevity and aging rates by modulating established aging-regulating protein factors. Because many aging-regulating mechanisms in *C. elegans* are evolutionarily conserved, these studies will provide key information regarding how ncRNAs modulate aging and lifespan in complex organisms, including mammals.

Keywords: aging, *Caenorhabditis elegans*, lifespan, non-coding RNA, small RNA

INTRODUCTION

Caenorhabditis elegans is an excellent model organism for studying aging (Park et al., 2017). Genetic studies using *C. elegans* have identified many genes that play key roles in organismal aging (Kenyon, 2010; Lee et al., 2015). For example, genetic inhibition of *daf-2*, which encodes a homolog of insulin/insulin-like growth factor 1 (IGF-1) receptor, dramatically increases lifespan and stress resistance. This is achieved by activating downstream longevity proteins, including the FOXO/DAF-16, heat shock factor 1 (HSF-1), and Nrf/SKN-1 transcription factors (Altintas et al., 2016). Other genetic and environmental factors, including reproductive systems, target of rapamycin, mitochondrial electron transport chain, and dietary restriction (DR), modulate lifespan and alter aging rates. Importantly, many of these pathways that regulate aging are evolutionarily conserved in complex organisms, including mammals.

The term "non-coding RNAs" (ncRNAs) refers to RNA species that do not encode proteins. Various ncRNAs are key regulators of gene expression and chromatin remodeling, by acting through binding to their targets (Cech and Steitz, 2014). MicroRNAs (miRNAs), endogenous small interfering RNAs (siRNAs), and PIWI-interacting RNAs (piRNAs) share certain features for their functions, and are associated with Argonaute proteins to modulate transcriptional or post-translational regulation (Hoogstrate et al., 2014). The exact functions of other small RNAs, such as circular RNAs (circRNAs) and transfer RNA (tRNA)-derived small RNAs (tsRNAs) remain largely unknown. Nevertheless, recent studies have suggested that several circRNAs and tsRNAs regulate gene expression at the transcriptional and translational levels (Li et al., 2018a; 2018b). Long non-coding RNAs (lncRNAs) are over 200 nucleotides in length and regulate transcription in

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a *cis* or a *trans* manner (Ulitsky and Bartel, 2013). Many cellular processes are regulated by ncRNAs and these processes, including small RNA-mediated silencing, are conserved in various species (Qu and Adelson, 2012).

ncRNAs regulate various physiological processes, including development, stress responses, tumorigenesis and immune responses (Fernandes et al., 2019; Oberbauer and Schaefer, 2018; Shin et al., 2018; Szczepanek et al., 2018). Interestingly, recent studies using various organisms, *C. elegans* in particular, have indicated that ncRNAs influence aging and longevity. Here, we discuss the roles of ncRNAs in the regulation of lifespan and aging in *C. elegans*. We also describe the findings regarding changes in the expression of these ncRNAs during the aging process. We then discuss how the changes in the expression of ncRNAs over the lifetime play roles in aging or anti-aging processes. Considering the evolutionarily conserved nature of many aging-regulating factors, understanding ncRNA-mediated regulatory roles in *C. elegans* aging will lead to insights into how mammalian health and age-associated diseases are influenced by ncRNAs.

miRNAs

Biogenesis of miRNAs

miRNAs are short ncRNAs that regulate various cellular processes, including transcription, translation, and gene silencing, which affect cellular growth, proliferation, and senescence (Ha and Kim, 2014; Szczepanek et al., 2018; Treiber et al., 2019). In particular, miRNAs mediate translational repression or messenger RNA (mRNA) silencing by binding to complementary mRNAs. miRNAs are first transcribed as pri-miRNAs by RNA polymerase II (Pol II) (Fig. 1A). Nuclear Drosha (*C. elegans* DRSH-1)-DGCR8 (*C. elegans* PASH-1) complex then cleaves pri-miRNAs for conversion to pre-miRNAs. Next, the exportin proteins translocate pre-miRNAs from the nucleus to the cytosol, which are then cleaved by Dicer (*C. elegans* DCR-1), a type III ribonuclease that generates short double-stranded RNAs. One of the strands then forms an miRNA-induced silencing complex (miRISC) with Argonaute proteins that unwind the RNA duplex. Recent studies using model organisms including *C. elegans* have indicated that the levels of many miRNAs are altered during aging and in turn affect organismal aging rates and lifespan. Several outstanding review papers discuss the relationship between miRNAs and *C. elegans* aging in detail (Garg and Cohen, 2014; Inukai and Slack, 2013; Kato and Slack, 2013). Here, we briefly summarize important findings and provide an update of the recent literature in the field.

The expression of several miRNAs changes during aging

Various miRNAs whose primary role is the down-regulation of target mRNA expression display age-dependent changes in their levels. Total miRNA levels decline during aging, but individual miRNAs display variable age-dependent changes in their expression (Kato et al., 2011) (Fig. 2). miR-71 generally decreases age-dependent miRNA expression by down-regulating *alg-1* (*C. elegans* Argonaute) expression (Inukai et al., 2018). Therefore, *mir-71* mutation increases the overall abundance of miRNAs during aging. Many individual miRNAs display age-dependent decreases in their expression shown in at least two out of three transcriptomic studies (de Lencastre et al., 2010; Kato et al., 2011; Lucanic et al., 2013). These include *let-7* miRNA, miR-70, miR-252, miR-81, miR-65, miR-237, miR-248, miR-1 and miR-235. In particular, all these three papers reported that *let-7* miRNA and miR-70 display age-dependent decreases in expression (de Lencastre et al., 2010; Kato et al., 2011; Lucanic et al., 2013). In contrast, the levels of miR-71, miR-239a, miR-34, miR-35, miR-37, miR-36, miR-39, and several members of the miR-35 family increase with age, shown in two out of the four reports (de Lencastre et al., 2010; Kato et al., 2011; Lucanic et al., 2013; Yang et al., 2013). These studies indicate that aging affects the levels of miRNAs in different ways and that miRNAs may influence mRNA expression during aging.

miRNAs that influence lifespan

One of the first breakthrough discoveries that demonstrated the roles of miRNAs in organismal longevity was reported by Frank Slack's group in 2005. They showed that loss-of-function mutations in an miRNA gene, *lin-4*, significantly shorten *C. elegans* lifespan by down-regulating FOXO/DAF-16 via up-regulating *lin-14* (Boehm and Slack, 2005). Since this seminal publication, many other lifespan-modulating miRNAs have been identified. Here, we focus on recent papers describing lifespan-changing miRNAs after the publication of previous review papers regarding miRNAs and *C. elegans* aging (Garg and Cohen, 2014; Inukai and Slack, 2013; Kato et al., 2011). Mutations in *mir-71*, *mir-238*, *mir-246*, *mir-81/82*, or *mir-58* shorten lifespan, whereas overexpression of *mir-71* or *mir-246* promotes longevity (de Lencastre et al., 2010; Zhang et al., 2018). Among these, miR-71 and miR-246 levels are also increased with age (de Lencastre et al., 2010; Kato et al., 2011), implying that the age-dependent increase in miRNAs plays a protective role against a short lifespan. In contrast, mutations in *mir-80*, *mir-83*, *mir-239a/b*, or *mir-228* extend lifespan (de Lencastre et al., 2010; Dzakah et al., 2018; Lucanic et al., 2013; Vora et al., 2013). An early study showed that two loss-of-function *mir-34* mutant alleles do not alter lifespan (de Lencastre et al., 2010), whereas another study reported that the same *mir-34* mutations significantly extend lifespan by enhancing autophagy (Yang et al., 2013). *mir-80* deletion mediates longevity conferred by DR via acting with FOXO/DAF-16 and HSF-1 transcription factors (Vora et al., 2013). As miR-80 down-regulates *cbp-1*, which encodes cAMP-response element (CREB)-binding protein 1, FOXO/DAF-16 and HSF-1 that are associated with CBP-1 fail to induce the expression of genes required for DR-induced longevity. *mir-83* loss-of-function mutation also increases lifespan through FOXO/DAF-16 (Dzakah et al., 2018). *mir-228* loss-of-function mutation extends lifespan via a DR pathway that requires FOXA/PHA-4 and Nrf/SKN-1 (Smith-Vikos et al., 2014). Thus, specific miRNAs play distinct roles in lifespan by modulating several aging-regulating factors, including FOXO/DAF-16, HSF-1, FOXA/PHA-4, and Nrf/SKN-1. Currently, the mechanisms by which gene expression changes caused by specific miRNAs affect lifespan remain poorly understood. Additionally, understanding how aging influences the expression of various miRNAs will be a key task for future research.

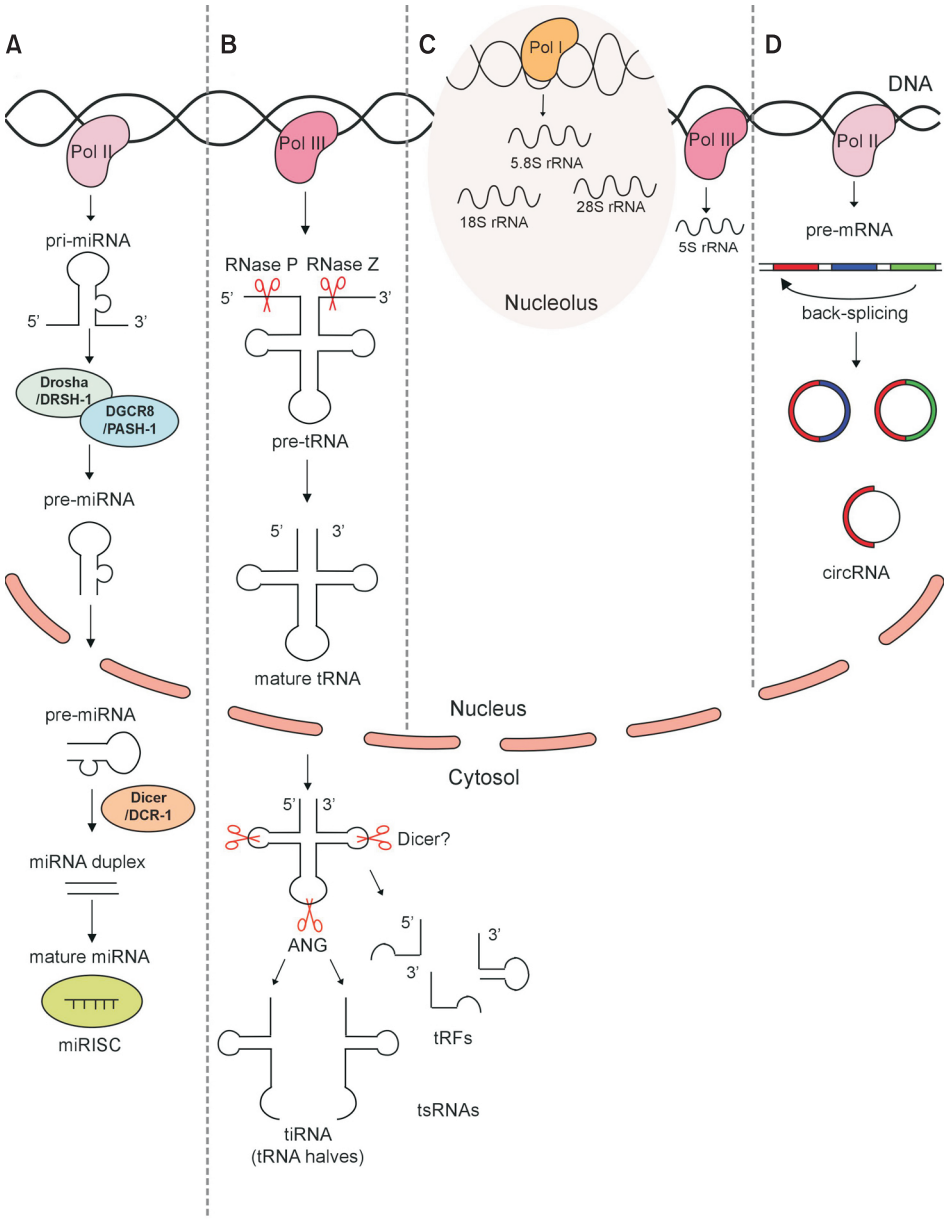

Fig. 1. Biogenesis of several types of non-coding RNAs: miRNA, tsRNA, rRNA and circRNA. (A) miRNAs are first transcribed to pri-miRNAs by RNA polymerase II (Pol II). Nuclear Drosha (*C. elegans* DRSH-1)-DGCR8 (*C. elegans* PASH-1) complex then cleaves pri-miRNAs for conversion to pre-miRNAs. Next, pre-miRNAs are translocated from the nucleus to the cytosol, for cleavage by Dicer (*C. elegans* DCR-1). One of the strands then forms miRNA-induced silencing complex (miRISC) with Argonaute proteins that unwind the RNA duplex. (B) tRNAs are transcribed by RNA polymerase III (Pol III) to precursor tRNA (pre-tRNA) transcripts, which have 5′-leader and 3′-trailer sequences. The 5′-leader sequence is cleaved by endoribonuclease P (RNase P) and the 3′-trailer sequence is removed by endonuclease Z (RNase Z) for generating mature tRNAs. Dicer may cleave the mature tRNAs to tRNA-derived fragments (tRFs). In mammals, angiogenin (ANG, ribonuclease A) cleaves tRNAs and generates tRNA-derived stress-induced RNAs (tiRNAs), also known as tRNA halves, tRNA half-types of tRNA-derived small RNAs (tsRNAs). (C) RNA polymerase I (Pol I) transcribes 18S, 5.8S, and 28S rRNAs in the nucleolus, whereas 5S rRNA is transcribed by Pol III in the nucleus. (D) Circular RNAs (circRNAs) are produced by back-splicing of precursor mRNAs that are transcribed by Pol II.

miRNA machinery regulates longevity in *C. elegans*

In addition to miRNAs, several miRNA machinery protein components influence longevity (Kogure et al., 2017). A recent study has shown that the mRNA levels of miRNA machinery component genes, *alg-1* and *drsh-1*, are up-regulated by fasting and that knockdown of these components suppresses longevity conferred by intermittent fasting, a paradigm for DR. Thus, miRNA machinery appears to mediate

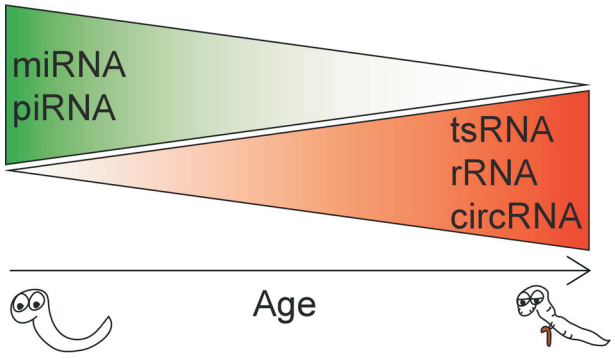

Fig. 2. Age-dependent expression changes in *C. elegans* non-coding RNAs. Total microRNA (miRNA) and PIWI-interacting RNA (piRNA) levels are gradually decreased during *C. elegans* aging. In contrast, tRNA-derived small RNA (tsRNA), ribosomal RNA (rRNA) and circular RNA (circRNA) levels generally display age-dependent increases.

the effect of DR on long lifespan.

In contrast, another study has shown that the two *C. elegans* Argonaute proteins, ALG-1 and ALG-2, have distinct expression patterns and exert differential effects on lifespan; a loss-of-function mutation in *alg-1* decreases lifespan, whereas a loss-of-function mutation in *alg-2* extends lifespan (Aalto et al., 2018). These seemingly contradictory results may have originated from substantial sequence homology between *alg-1* and *alg-2*. Therefore, RNAi knockdown of either *alg-1* or *alg-2* appears to decrease the expression of both *alg-1* and *alg-2*, whereas DNA mutations are specific to individual *alg-1* and *alg-2* genes. Overall, miRNA biogenesis mediated by the miRNA machinery complexes appears to affect aging and lifespan in *C. elegans* by modulating the expression of genes that encode factors in various aging-regulating pathways, including insulin/IGF-1 signaling and DR effectors.

tsRNAs

Definition of tsRNAs

tsRNAs (also known as tRNA-derived fragments [tRFs]) are fragments derived from tRNAs (Kumar et al., 2016). tsRNA can be categorized based on the position of cleavage in tRNA transcripts. tRNAs are transcribed by RNA polymerase III (Pol III) (Fig. 1B). Precursor tRNA (pre-tRNA) transcripts exist as cloverleaf shapes and have 5′-leader and 3′-trailer sequences. The 5′-leader sequence is cleaved by endoribonuclease P (RNase P) and the 3′-trailer sequence is removed by endonuclease Z (RNase Z). Nucleotidyltransferases then mediate the addition of a 3′-CCA tail. Dicer may cleave these mature tRNAs to tsRNAs that are loaded onto Argonautes. In mammals, various stresses appear to induce a mammalian ribonuclease A angiogenin (ANG) that cleaves tRNAs and generates tRNA half-types of tsRNAs, also known as tRNA-derived stress-induced RNAs (tiRNAs). tsRNAs regulate various biological processes, including gene expression, interference of translation, intergenerational inheritance, apoptosis, and viral infection (Oberbauer and Schaefer, 2018).

The levels of tsRNAs increase during aging

While the total levels of miRNAs gradually decrease during aging, the levels of tsRNAs generally increase during *C. elegans* aging (Kato et al., 2011), perhaps in response to stresses induced by aging (Fig. 2). Interestingly, the levels of 3′-tiRNAs generally increase in aged rat brain (Karaiskos and Grigoriev, 2016). Therefore, age-dependent increases in tsRNA levels appear to have common features between *C. elegans* and mammals. A recent study has also shown that the inhibition of Pol III extends lifespan by reducing protein synthesis and decreasing pre-tRNA levels (Filer et al., 2017). Because pre-tRNAs can be processed to tsRNAs, this study raises the possibility that changes in tsRNA levels contribute to longevity and healthspan.

rRNAs

Ribosomal RNAs (rRNAs) are the RNA components of the large and small subunits of the ribosome, which is essential for protein synthesis (Lambert et al., 2019). In eukaryotes, the large subunit contains 5S, 5.8S, and 28S rRNAs, and the small subunit contains 18S rRNAs. Among them, 18S, 5.8S, and 28S rRNAs are transcribed by RNA polymerase I (Pol I) in the nucleolus, whereas 5S rRNA is transcribed by Pol III in the nucleus (Fig. 1C). In *C. elegans*, the levels of 28S and 18S rRNAs do not show any substantial differences between young and old animals in a temperature-sensitive sterile mutant [*spe-9*(*hc88*); *fer-15*(*b26*)] background, or by longevity-promoting phosphoinositide 3-kinase (*PI3K*)/*age-1* mutations (Fabian and Johnson, 1995). However, a recent study reported that the total levels of rRNAs gradually increase during aging in another sterile mutant [*spe-9*(*hc88*)] background (Kato et al., 2011) (Fig. 2). Therefore, whether rRNA levels influence aging and longevity requires further investigation.

piRNAs

piRNAs are small ncRNAs that form an RNA–protein complex through binding to the PIWI Argonaute (Ozata et al., 2019). The piRNA–PIWI Argonaute complex maintains the genome stability of the germline, and suppresses transposition in many animals, including *C. elegans* (Weick and Miska, 2014). The PRG-1 (a *C. elegans* PIWI Argonaute)–piRNA complex interacts with target mRNAs that are partially complementary to the piRNAs, and is important for gene silencing in germ cells. This is evidenced by many findings, including one showing that mutations in *prg-1* result in decreased fertility because of defects in germline integrity (Batista et al., 2008). Importantly, this fertility defect is suppressed by increasing the activity of longevity transcription factor FOXO/DAF-16 in insulin/IGF-1 receptor/*daf-2* mutants (Simon et al., 2014). In addition, the total levels of piRNAs decline during aging in *C. elegans* (Kato et al., 2011) (Fig. 2). In *Drosophila*, PIWI is necessary and sufficient for reducing age-related intestinal stem cell dysfunction and apoptosis (Sousa-Victor et al., 2017). In addition, transposable element expression and transposition are increased during aging in *Drosophila*. All of these findings are consistent with the possibility that PIWI and piRNAs play anti-aging roles at least in these two invertebrate models.

However, the direct relationship between piRNAs and lifespan remains unknown and should be tested experimentally in future work.

circRNAs

circRNAs are mostly ncRNAs produced by the back-splicing of precursor mRNAs of diverse genes in eukaryotes (Li et al., 2018b) (Fig. 1D). Another type of non-coding circRNA can be produced during metazoan tRNA splicing, which is called tRNA intronic circular RNAs (tricRNAs) (Lu et al., 2015). circRNAs are highly stable because the lack of free 5′- and 3′-ends confers resistance to exonucleases. circRNAs were discovered over two decades ago (Cocquerelle et al., 1993). Dramatic advances in RNA sequencing (RNA-seq) technology that has enabled a global analysis of non-polyadenylated transcriptomes have recently boosted interest in circRNAs. Although circRNAs were once thought to be by-products of mis-splicing and their exact role remains poorly understood, emerging evidence indicates that circRNAs affect various biological pathways. For example, specific circRNAs regulate gene expression by titrating miRNAs (Hansen et al., 2013) or by interacting with RNA Pol II (Li et al., 2015). A circRNA participates in cell cycle regulation by binding to p21 and CDK2 (Du et al., 2016). RNA-seq results indicate that circRNA isoforms are much more diverse than linear RNAs from the same loci, suggesting that circRNAs have various functional roles (Salzman et al., 2013; Zhang et al., 2016).

Interestingly, circRNAs accumulate during aging in *Drosophila* brain (Westholm et al., 2014) and in *C. elegans* (Cortes-Lopez et al., 2018) (Fig. 2). In *C. elegans*, circRNAs are also differentially expressed during development (Memczak et al., 2013). These results raise the possibility that circRNAs participate in modulating development and aging. In addition, circRNAs play important roles in the senescence of mammalian cells (Du et al., 2017; Wang et al., 2015). Mammalian *Foxo3* circRNA promotes cardiac senescence by inhibiting the translocation of several transcription factors that regulate heart aging (Du et al., 2017). Moreover, circRNA 100783 may play a role in the immunosenescence of CD28-related CD8(+) T cells (Wang et al., 2015). Therefore, specific and general circRNAs may act as biomarkers of aging and/or may serve as anti-aging therapeutic tools.

lncRNAs

lncRNAs are ncRNAs that are longer than 200 nucleotides (Quinn and Chang, 2016). Most lncRNAs are derived from loci that do not overlap with protein-coding exons or are from opposite strands of protein-coding genes (Grammatikakis et al., 2014). While the functions of most lncRNAs remain poorly understood, some lncRNAs' roles in gene regulation have been extensively investigated, as exemplified by mammalian *Xist* (X-inactive specific transcript), an effector of X-chromosome inactivation (Sahakyan et al., 2018). In *C. elegans*, 170 potential lncRNAs were initially identified (Nam and Bartel, 2012), and a recent study extended the potential number of lncRNAs in this species to 3,397 (Akay et al., 2019). lncRNAs affect various cellular processes such as senescence, proliferation, differentiation, and age-associated diseases (Kour and Rath, 2016). For example, MIAT and TUG1 lncRNAs are essential for brain development and neurogenesis (Briggs et al., 2015; Kour and Rath, 2016). Because declines in neurogenesis in aged brain are potential causes of brain aging, these lncRNAs may contribute to the age-dependent declines in brain function. Several studies have also shown that lncRNAs can influence organismal aging and lifespan (Grammatikakis et al., 2014). For example, lncRNA *tts-1* in *C. elegans* is required for longevity conferred by mutations in the insulin/IGF-1 receptor gene, *daf-2* (Essers et al., 2015). Therefore, it is likely that other lncRNAs also modulate lifespan by affecting known or novel longevity signaling pathways.

CONCLUSIONS

A large fraction of mammalian genomes consist of non-protein-coding sequences, as exemplified by the finding that only 1.5% of the human genome is transcribed to protein-coding mRNAs. Thanks to newly developed RNA-seq technologies and bioinformatics approaches, RNA fragments that were once regarded as by-products of RNA degradation have now become focal points as interesting bio-molecules that have important and specific roles. For example, tsRNAs play inhibitory roles in translation in response to stress, and circRNAs appear to act as regulatory factors that interact with miRNAs or Pol II. Other ncRNAs, including miRNAs, rRNAs, piRNAs, and lncRNAs regulate gene expression. In this review paper, we have discussed the roles of various ncRNAs in aging and lifespan, focusing on the findings obtained using *C. elegans*. However, the mechanisms and functions of most ncRNAs in aging and lifespan remain elusive. For example, it is unclear how specific ncRNAs play different roles in lifespan, either as pro- or anti-longevity factors. As many ncRNAs modulate target gene expression, it will be crucial to identify and characterize target genes that influence aging and lifespan. *C. elegans* is a genetically amenable metazoan with a short lifespan; it is, therefore, suitable as a key model for unraveling the mechanisms and functions of ncRNAs in aging regulation.

Mutations in several miRNAs and miRNA machinery factors affect the lifespan of *C. elegans*. In addition, the expression levels of many miRNAs, tsRNAs, piRNAs, and circRNAs change during aging. Interestingly, it has been shown that many ncRNA levels are altered in patients with age-associated diseases (Chalbatani et al., 2019; Dimmeler and Nicotera, 2013; Kour and Rath, 2016; Yang et al., 2018). Therefore, these ncRNAs have potential as biomarkers of aging and aging-related diseases. In addition, several ncRNAs have the potential for use as therapeutic targets for aging-associated diseases. Further studies of aging and RNA biology in *C. elegans* will eventually enhance our understanding of human aging and age-related diseases.

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ORCID

Seung-Jae V. Lee https://orcid.org/0000-0002-6103-156X

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