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tRNA-derived small noncoding RNAs: Roles in brain aging and neurodegenerative disorders

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ABSTRACT

Transfer ribonucleic acid–derived small ribonucleic acids (tsRNAs) are an emerging class of regulatory noncoding RNAs produced through the precise cleavage of mature or precursor tRNAs (pre-tRNAs). Once considered degradation byproducts, tsRNAs are now recognized as key modulators of gene expression, epigenetic regulation, and cellular stress responses. In recent years, growing evidence has implicated tsRNAs in the aging process of the brain and in the pathogenesis of age-related neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS). These small RNAs are involved in modulating synaptic function, neuronal survival, and neuroinflammation, and their expression profiles are dynamically altered in response to aging and disease-associated stressors. This review summarizes the biogenesis, classification, and molecular and cellular mechanisms of tsRNAs, with an emphasis on their subcellular locations and associated biological functions. We further explore their roles in brain aging and age-related neurodegenerative diseases and the emerging potential of tsRNAs as biomarkers and therapeutic targets for age-related neurological disorders while highlighting current challenges and future directions in this rapidly advancing field.

Keywords: tsRNA; Brain aging; Neurodegenerative disease; Diagnostic and therapeutic insight

INTRODUCTION

In the genome of eukaryotic cells, approximately 90% of the genes are transcriptionally active, but only a small fraction (approximately 1%–2%) of these genes are translated into proteins. Consequently, the greater part of the eukaryotic transcriptome comprises noncoding ribonucleic acids (ncRNAs), which do not encode proteins (Alexander et al., 2010; Esteller, 2011; Matera et al., 2007; Xu et al., 2019)[1]Alexander, Fang [1]. The ncRNAs were initially considered to be "junk" or mere byproducts of transcription; however, they are now recognized as key regulators of numerous physiological processes, including gene expression and epigenetic inheritance (Xiao et al., 2022).

Based on their length, ncRNAs are categorized into long noncoding RNAs (lncRNAs) and small noncoding RNAs (sncRNAs) (Chen et al., 2019; Esteller, 2011). The lncRNAs are typically longer than 200 nucleotides and play critical roles in epigenetic regulation, cell cycle control, and cell proliferation. The sncRNAs, which range in size from 18 to 200 nucleotides, include a variety of species, with transfer RNAs (tRNAs) being the most abundant (Chen et al., 2019; Cheng et al., 2020; Green et al., 2010; Kirchner & Ignatova, 2015; Li et al., 2020a). tRNAs have a canonical role involving the decoding of messenger RNA (mRNA) during protein

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translation; however, both mature tRNAs and their precursors can be enzymatically cleaved to generate tRNA-derived small RNAs (tsRNAs) (Aravin et al., 2003; Diallo et al., 2022; Jöchl et al., 2008). Emerging evidence now suggests that these heterogeneous tsRNA fragments also participate in various pathophysiological processes. Thus, these fragments could function as potential biomarkers and medical targets, particularly in aging, neurodegenerative disorders, and cancer (Bradshaw et al., 2017; Li et al., 2024; Li, 2007; Xiao et al., 2022). Notably, altered expression or mislocalization of tsRNAs is now considered to contribute to the functional declines associated with aging and neurodegeneration.

Aging-induced functional decline of the brain and age-related neurodegenerative diseases represent significant global public health challenges. Over 55 million people worldwide are currently suffering from the aging-related cognitive decline and dementia, with approximately 10 million new cases each year, imposing an enormous burden on global health care systems and economies (Li et al., 2025). By 2019, the economic cost of dementia had already reached 1.3 trillion USD (Wimo et al., 2023). The rapid growth of the aging population worldwide and the resulting socioeconomic burden underscore the urgent need for a deeper understanding of the pathophysiological roles of tsRNAs and to develop tsRNA-based therapies capable of preventing or even reversing age-related cognitive decline and neurodegeneration. The aim of this review is to summarize the current knowledge regarding the roles of tsRNAs in brain aging and neurodegenerative disorders and to explore the potential therapeutic use of these regulatory noncoding RNAs in aging and neurodegenerative diseases.

THE BIOGENESIS AND CLASSIFICATION OF tsRNAs

Function and structure of tRNAs

Understanding the biogenesis of tsRNAs first requires an overview of the formation and structural characteristics of tRNAs in cells. As shown in Figure 1, precursor tRNAs (pre-tRNAs) are transcribed by RNA polymerase III (Pol III) and then undergo several maturation steps to become functional tRNAs. These processing events include the following: (1) removal of the 5’ leader sequence by RNase P, (2) cleavage of the 3’ trailer sequence by RNase Z, (3) excision of introns from the anticodon arm, and (4) addition of a CCA trinucleotide to the 3’ terminus by tRNA nucleotidyl transferase (Kumar et al., 2016; Maraia & Lamichhane, 2011; Meynier

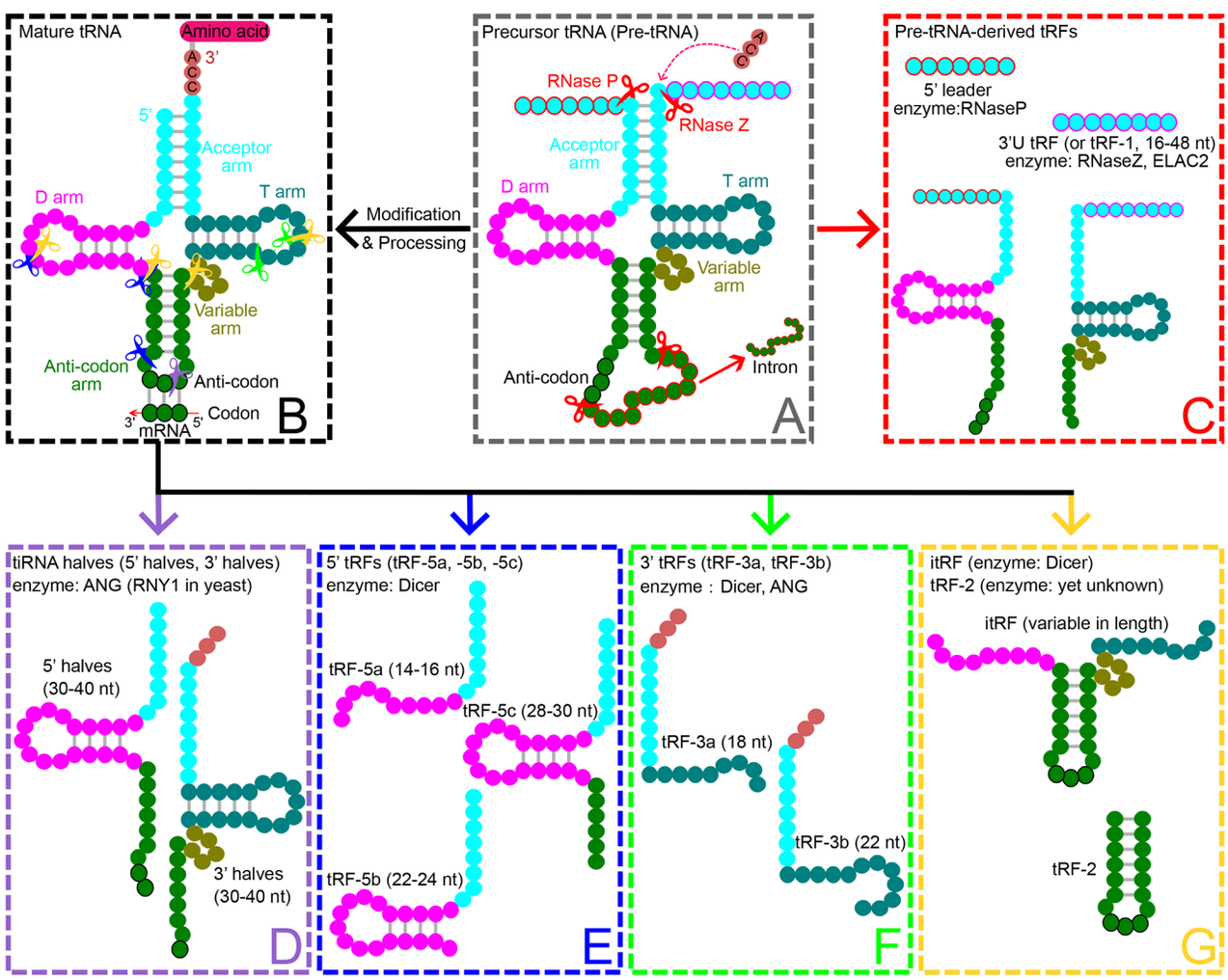

Figure 1 Schematic of the biogenesis and classification of tsRNAs

A, B: Distinct subtypes of tsRNAs derived from the processing of precursor tRNA and mature tRNAs; C–G: 3’ tRFs, tRF-1, 5’ tRFs, tRF-2 and itRFs, and tiRNAs. Abbreviations: D arm, Dihydrouridine arm; TΨC arm, arm that contains thymidine (T), pseudouridine (Ψ) and cytidine (C) residues; tRF, tRNA-derived fragments; ANG, angiogenin; itRF, internal tRF; nt, nucleotide.

et al., 2024; Phizicky & Hopper, 2010, 2023) (Figure 1A).

tRNAs, as one of the three major classes of RNA essential for protein biosynthesis, translate genetic information into functional proteins. Their primary function is to decode codons on mRNAs and deliver the corresponding amino acids to the ribosome for incorporation into the growing polypeptide chain. Structurally, tRNAs exhibit three hierarchical levels: 1. Primary structure: Each tRNA molecule is a linear chain of 74–95 ribonucleotides running from the 5' to 3' end. 2. Secondary structure: The single-stranded tRNA folds into a characteristic cloverleaf shape, composed of four major arms: the acceptor arm, dihydrouridine arm (D arm), anticodon arm, and the TΨC arm that contains thymidine (T), pseudouridine (Ψ) and cytidine (C) residues (Huang et al., 2024) (Figure 1B). 3. Tertiary structure: The tRNAs adopt an "L-shaped" three-dimensional conformation formed by stacking interactions between the TΨC arm and the acceptor stem on one side and the D arm and anticodon stem on the other. This compact and highly stable conformation is essential for the accurate positioning of tRNAs during translation (Biela et al., 2023).

Classification of tRNA-derived small noncoding RNAs

The existence of tRNA-derived fragments was first reported in *Escherichia coli*, which showed cleavage of tRNAs into smaller fragments during bacteriophage infection (Levitz et al., 1990). The advent of high-throughput sequencing technologies has since identified an increasing number of distinct tsRNAs across both prokaryotic and eukaryotic transcriptomes (Aravin et al., 2003; Diallo et al., 2022; Huang et al., 2024; Jöchl et al., 2008). Based on the current understanding, tsRNAs can be broadly classified into two subgroups: tRNA halves (also known as tRNA-derived stress-induced RNAs [tiRNAs]) and tRNA-derived fragments (tRFs). The tRNA halves arise from the cleavage of mature tRNAs at anticodon arms under stress conditions, whereas the tRFs originate from either mature or precursor tRNAs but are generated independently of anticodon arm cleavage (Shen et al., 2018; Xie et al., 2020). tsRNAs are categorized into six subtypes based on the specific cleavage sites on their parental tRNAs and the enzymes involved in their biogenesis (Kumar et al., 2015; Liu et al., 2021; Sobala & Hutvagner, 2011). Five of these subtypes are derived from mature tRNAs: 1) 5'tRFs, which are further subclassified into tRF-5a, tRF-5b, and tRF-5c; 2) 3' tRFs, including tRF-3a and tRF-3b; 3) tRNA halves or tiRNAs, which are categorized as 5' tiRNAs and 3' tiRNAs; 4) tRF-2; and 5) internal tRFs (itRFs). 6) tRF-1s, which are derived from the 3' trailers of precursors during tRNA maturation (Shen et al., 2018) (Figure 1).

Biogenesis of tsRNAs

In vertebrates, the generation of tsRNAs from mature tRNAs primarily involves three key enzymes: RNase Z, Dicer, and Angiogenin (ANG) (Cole et al., 2009; Fu et al., 2009; Haussecker et al., 2010). In humans, pre-tRNAs can be cleaved near their 3' ends by RNase Z and ELAC2 to generate tRF-1 fragments (Babiarz et al., 2008; Lee et al., 2009; Liao et al., 2010; Wang et al., 2013) (Figure 1C). In mammals, ANG cleaves within the anticodon arms of mature tRNAs to produce 5' tiRNAs and 3' tiRNAs, each approximately 30–40 nucleotides in length (Figure 1D). In contrast, in yeast, RNY1, rather than ANG, is the corresponding nuclease that performs a similar cleavage (Fu et al., 2009; Yamasaki et al., 2009) (Figure 1D).

Dicer is involved in the generation of tRF-5 subtypes in species such as mice, *Drosophila melanogaster*, and *Schizosaccharomyces pombe*. These include tRF-5a (~14–16 nucleotides), tRF-5b (~22–24 nucleotides), and tRF-5c (~28–30 nucleotides), which are derived by cleavage at the D arm or the stem connecting the D arm and anticodon arm (Kumar et al., 2014, 2015) (Figure 1E). Similarly, tRF-3a (~18 nucleotides) and tRF-3b (~22 nucleotides) originate from cleavage at the T arm, with cleavage mediated by both ANG and Dicer (Kumar et al., 2014; Li et al., 2012; Qin et al., 2020; Zhou et al., 2017) (Figure 1F). The rapid development of high-throughput sequencing technologies has enabled the identification of additional tRNA-derived fragments (Shen et al., 2018); however, these less common fragments are typically two to three orders of magnitude less abundant than the canonical 5' tRFs, 3' tRFs, and tiRNAs (Kumar et al., 2015, 2016). A distinct class of tsRNAs, known as tRF-2s, has also been identified. These fragments are derived from the internal cleavage of specific tRNAs—such as tRNAGlu, tRNAAsp, tRNAGly, and tRNATyr—and uniquely retain an intact anticodon arm while lacking both 5' and 3' termini (Goodarzi et al., 2015; Kumar et al., 2015; Lee et al., 2009; Schaffer et al., 2014) (Figure 1G).

The tsRNA fragments generated from mature tRNAs by specific cleavage events are often triggered by stress or other cellular stimuli (Fu et al., 2009; Kumar et al., 2014, 2015; Li et al., 2012; Yamasaki et al., 2009; Zhou et al., 2017). Various stress conditions—including hypoxia, serum deprivation, and cancer cell invasion—activate the generation of a series of 5' tRNA-derived small RNAs (5' tsRNAs) from specific parental tRNAs, such as tRNAGlu, tRNAAsp, tRNAGly, tRNACys, tRNAAla, and tRNATyr (Goodarzi et al., 2015).

SUBCELLULAR LOCALIZATION AND FUNCTIONS OF tsRNAs

The tsRNAs are a biologically versatile class of small noncoding RNAs that are tightly involved in distinct physiological processes and pathological outcomes, including the regulation of transcript stability and protein translation and the biosynthesis of rRNAs and ribosomes, as well as cell–cell communications (Li et al., 2024; Liu et al., 2021; Ruggero et al., 2014; Weng et al., 2022; Xiao et al., 2022; Yeung et al., 2009; Zhou et al., 2017; Zong et al., 2021). As with other biomolecules, the subcellular localizations of tsRNAs provide important clues to their physiological roles and potential pathological implications. Indeed, tsRNAs are distributed across multiple subcellular components, where they carry out distinct, spatially defined functions.

tsRNAs in the cytoplasm

In vertebrates, nuclear export receptors, such as exportin-T in *Xenopus laevis* (Xiong & Steitz, 2006), function as exporters of mature tRNAs from the nucleus to the cytoplasm. This nuclear-to-cytoplasmic transport requires the presence of both the 5' and 3' ends, as well as the nontemplated "CCA" sequence at the 3' terminus (Xiong & Steitz, 2006). The predominant cytoplasmic localization of mature tRNAs points to the reasonable inference that most of the identified tsRNAs are also primarily localized in the cytoplasm (Sobala & Hutvagner, 2011; Sun et al., 2020).

miRNA-like functions: tsRNAs range in size from ~18 to 40 nucleotides; thus, they share notable structural and size similarities with microRNAs (miRNAs), which are typically ~22 nucleotides in length. For example, certain 3' tRFs derived

from tRNALeu and tRNALys are identical in sequence to the 3' ends of miR-1280 and miR-1274a/b, respectively (Bidartondo, 2008; Huang et al., 2017; Kawaji et al., 2008; Xiao et al., 2022; Yang et al., 2024). Given this similarity, the finding that some tsRNAs exhibit miRNA-like regulatory functions is not surprising.

Several tsRNAs can bind to Argonaute (Ago) proteins to regulate RNA silencing. For example, the 3' tRFs derived from tRNAGly-GCC (Gly-3' tsRNA-GCC) can associate with Ago proteins to form the RNA-induced silencing complex-like (RISC-like) complexes. This binding is a well-characterized function of miRNAs, which bind to Ago proteins—particularly Ago2—to mediate post-transcriptional gene silencing. The RISC complexes guide sequence-specific binding of the tsRNAs to complementary sites within the 3' untranslated regions (3' UTRs) of target mRNAs, leading to translational repression and decreased protein expression (Maute et al., 2013) (Figure 2A).

Regulatory effects on ribosome biosynthesis: tsRNAs can also regulate ribosomal RNA (rRNA) synthesis and ribosome assembly. One well-characterized mechanism involves the nuclear import of Twi12, facilitated by specific 3' tRNA fragments. Twi12 is an essential component of a nuclear complex required for pre-rRNA processing, a critical step in ribosome biogenesis (Couvillion et al., 2012) (Figure 3A). The ribosome, as a fundamental organelle for protein synthesis, consists of a small 40S subunit and a large 60S subunit. Leu-3' tsRNA-CAG fragments can bind directly to the mRNAs of RPS28 and RPS15, two core proteins of the 40S subunit. This interaction enhances the translation of RPS28 and RPS15, subsequently facilitating pre-18S rRNA processing and increasing the production of 40S ribosomal subunits (Figure 2B).

Translation inhibition by promoting stress granule formation: In eukaryotic cells, environmental stress triggers the phosphorylation of the eukaryotic initiation factor eIF2 to promote the formation of stress granules (SGs) and inhibit translation. The 5' tsRNAs induce the formation of SGs through a mechanism that is independent of eIF2 phosphorylation (Lyons et al., 2016, 2017; Zhang et al., 2023). The 5' tiRNA-Ala contains a 5' terminal oligoguanine (TOG) motif that can assemble into an RNA G-quadruplex (RG4), which binds to the eukaryotic initiation factor 4F (eIF4F) complex. This binding promotes the dissociation of eIF4F from the m7GTP cap of mRNAs, thereby suppressing translation. The RG4 formed via 5' tiRNA-Ala can interact with Y-box binding protein 1 (YB1), which facilitates the formation of SGs (Figure 2C). Notably, this tiRNA-induced SG assembly is dependent on YB1, but it occurs independently of eIF2 phosphorylation (Ivanov et al., 2014; Zhang et al., 2023).

tsRNAs in mitochondria

Mitochondria possess their own genome (mtDNA), which

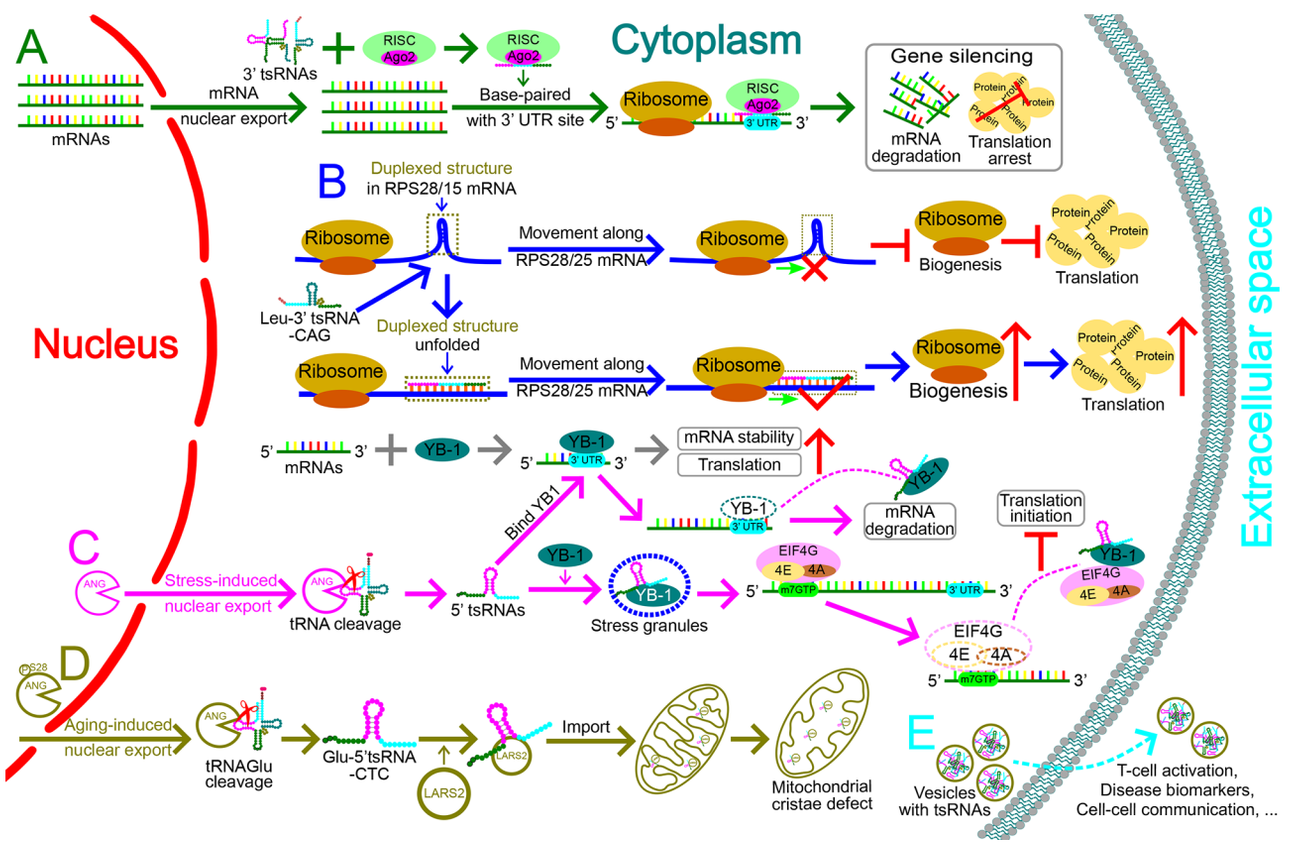

Figure 2 Biological functions of cytoplasmic tsRNAs

In the cytoplasm, tsRNAs play regulatory roles in various pathophysiological processes, including A: Compromising the ultrastructural integrity of mitochondrial cristae through LARS2-mediated import into mitochondria; B: Inducing gene silencing via base-pairing with the 3' UTR of target mRNAs; C: Promoting stress granule formation and inhibiting translation initiation and mRNA degradation by displacing YB1 from the 3' UTR; D: Enhancing ribosome biogenesis by unfolding duplexed structures of RPS28/25 mRNAs; and E: Regulating T cell activation and intercellular communication, as well as serving as potential disease biomarkers when secreted within lipid bilayer-enclosed extracellular vesicles. Abbreviations: 3' UTR, 3' untranslated region; RISC, RNA-induced silencing complex; Ago2, Argonaute 2; YB1, Y-box binding protein 1.

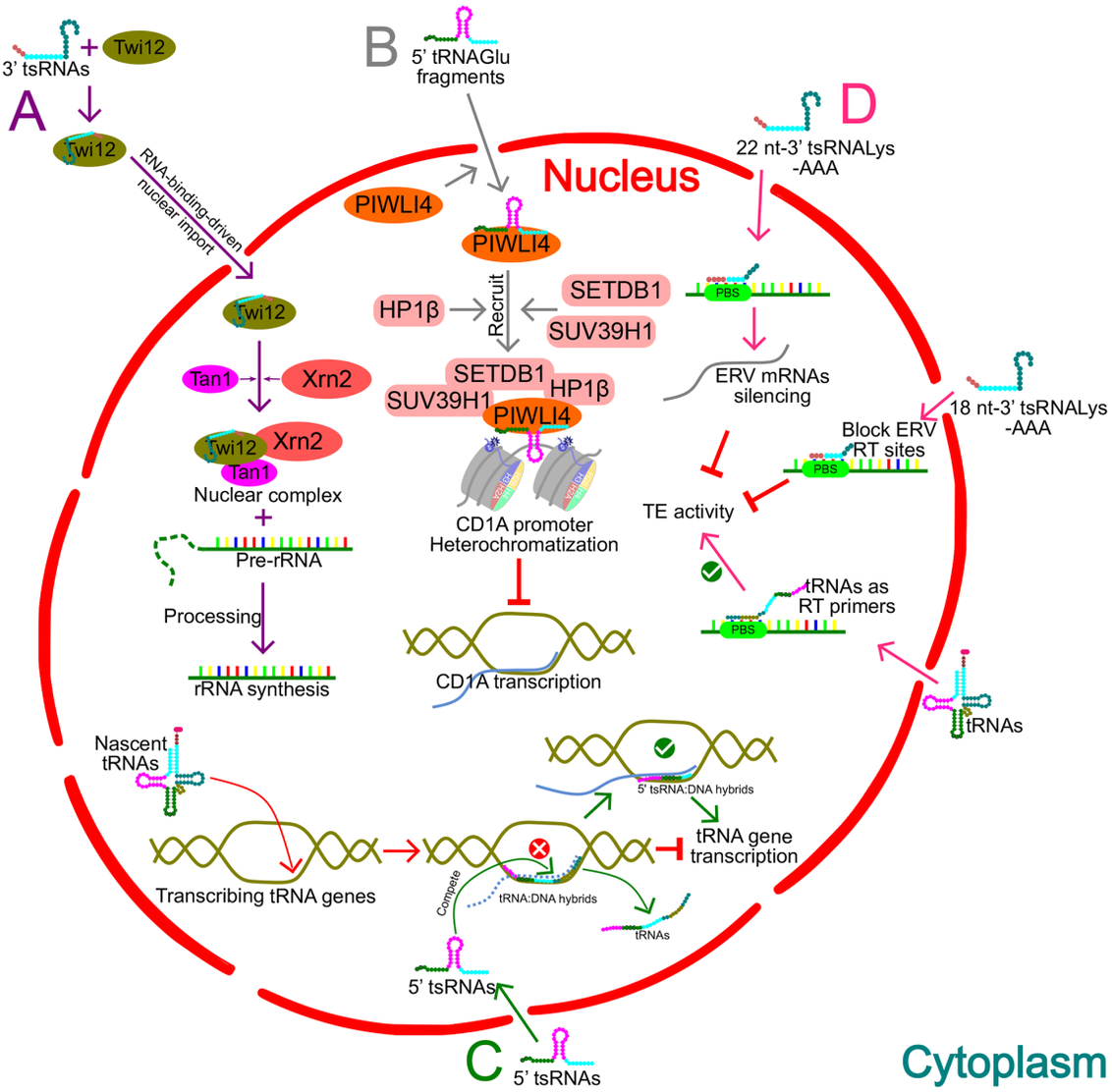

Figure 3 Biological functions of nuclear tsRNAs

tsRNAs have regulatory roles in the nucleus, including A: Promoting the nuclear import of Twi12 to form a complex that facilitates rRNA biosynthesis; B: Binding to PIWLI4, leading to transcriptional repression by recruiting SETDB1, SUV39H1, and HP1β to the CD1A promoter; C: Facilitating tRNA transcription by displacing nascent tRNAs from transcribing loci; and D: Blocking ERV reverse transcription sites or silencing newly produced ERV mRNAs to inhibit TE activity. Abbreviations: ERV, endogenous retrovirus; TE, transposable element; RT, reverse transcription.

encodes 13 essential proteins involved in the assembly of the electron transport chain (ETC) and oxidative phosphorylation (OXPHOS), as well as coding for 2 mitochondrial ribosomal RNAs (mt-rRNAs) and 22 mitochondrial tRNAs (mt-tRNAs) required for organelle-specific protein synthesis (Suzuki & Suzuki, 2014). ANG—a key ribonuclease used in cytoplasmic tRNA cleavage—is absent in mitochondria; however, the presence of other RNA-processing enzymes, such as Dicer and Ago2 (both central components of the miRNA pathway), has been confirmed in this organelle (Li et al., 2024; Meseguer, 2021; Ro et al., 2013). The detection of Dicer and Ago2 in mitochondria supports the hypothesis that mt-tRNAs can be enzymatically processed into smaller tsRNA fragments, analogous to their cytoplasmic counterparts. This notion has been substantiated by recent studies demonstrating the existence of mitochondrial tsRNAs (Karousi et al., 2020; Londin et al., 2020; Meseguer, 2021; Pliatsika et al., 2018). Athough ANG itself is undetectable in mitochondria, its cytoplasmic cleavage product can translocate into mitochondria, where it disrupts mitochondrial ultrastructure and ultimately impairs synaptic and memory function (Li et al., 2024).

tsRNAs in the nucleus

In addition to their predominant localization in the cytoplasm, tsRNAs have also been detected in the nucleus (Chen et al., 2021; Di Fazio et al., 2022; Li et al., 2024; Meseguer, 2021; Pliatsika et al., 2018; Zhang et al., 2016). The maturation of tRNAs begins in the nucleus, where pre-tRNAs undergo several processing steps, including removal of the 5' leader sequence, cleavage of the 3' trailer sequence, and addition of the non-templated "CCA" trinucleotide at the 3' end (a step essential for amino acid attachment) (Meynier et al., 2024; Phizicky & Hopper, 2010, 2023). The nuclear localization of pre-tRNAs means that the products of RNA processing, such as tsRNA species like tRF-1 fragments (derived from the 3' trailer) and 5' leader-derived fragments, can be detected within the nucleus. This reflects the origin of the tsRNAs during the early stages of tRNA processing, prior to the export of the fully processed tRNA to the cytoplasm. In addition,

tsRNA species derived from mature tRNAs are also present in the nucleus and regulate various nuclear biological processes, extending their role beyond the promotion of ribosome biogenesis (as discussed in section "**Regulatory effects on ribosome biosynthesis**").

Regulatory roles of tsRNAs on gene transcription: The 5' tsRNAs derived from tRNAGlu in the nucleus can form functional complexes with Piwi-like RNA-mediated gene silencing 4 protein (PIWIL4). This complex subsequently recruits H3K9 methyltransferases, including SETDB1 and SUV39H1, as well as heterochromatin protein 1β (HP1β), to the promoter region of *CD1A*. This recruitment leads to heterochromatin formation at the promoter, thereby significantly repressing *CD1A* transcription (Zhang et al., 2016) (Figure 3B).

In developing zebrafish (*X. laevis*) embryos, 5' tsRNAs also enter the nucleus to enhance the transcription of their corresponding tRNA genes. Nascent tRNA transcripts normally tend to hybridize with their DNA templates to form tRNA-DNA hybrids that hinder efficient transcription. However, 5' tsRNAs can compete with full-length tRNAs for binding to these DNA templates, thereby relieving transcriptional inhibition and promoting tRNA gene expression (Chen et al., 2021) (Figure 3C).

In addition to their effects on transcription, tsRNAs can regulate gene expression through post-transcriptional mechanisms within the nucleus. Certain tsRNAs processed by Dicer are able to bind complementarily to nascent mRNA transcripts, resulting in nascent RNA silencing (NRS) of the targeted genes (Di Fazio et al., 2022).

Effects of tsRNAs on retrotransposon control: Transposable elements (TEs) play critical roles in maintaining genomic stability and are closely linked to new gene formation, chromosome abnormalities, and biological evolution (Zhang et al., 2017). Among the known TEs, retrotransposons are known to hijack the 3' ends of mature tRNAs to facilitate their transposition. For instance, endogenous retroviruses (ERVs) utilize 3' tRNAs as reverse transcription (RT) primers by binding to a highly conserved primer binding site (PBS) to initiate reverse transcription. However, 18-nt and 22-nt 3' tsRNALys-AAA molecules can competitively bind to the PBS to prevent tRNA binding and effectively block ERV transcription. Notably, 18nt 3' tsRNALys-AAA molecules compete with tRNAs for PBS binding and the ERV reverse transcription (RT) sites, while 22nt alternatives act by competing with tRNAs for PBS binding and silencing nascent ERV mRNAs (Schorn et al., 2017; Slotkin & Martienssen, 2007) (Figure 3D).

Translocation of tsRNAs within cells

tsRNAs have been identified in multiple subcellular compartments, including the nucleus, cytoplasm, and mitochondria. However, many of these tsRNAs do not remain confined to their sites of origin, as they are capable of dynamic translocation between compartments. For instance, tRF-1 fragments, which are initially generated in the nucleus, are subsequently translocated to the cytoplasm, although the exact mechanism governing this export remains unclear (Kumar et al., 2014; Lee et al., 2009; Liao et al., 2010; Singh et al., 2018). Conversely, 3' tRFs, which are initially processed in the cytoplasm, can bind to Twi12, a *Tetrahymena thermophila* Argonaute/Piwi protein, and facilitate its nuclear import in the form of a 3' tRF–Twi12 complex (Couvillion et al., 2012). Similarly, in HeLa cells, 5' tRFs can localize predominantly in the nucleus, suggesting that cytoplasmic tsRNAs can be imported into the nucleus via specific regulatory mechanisms (Kumar et al., 2014, 2015). Furthermore, the detection of tsRNAs of cytoplasmic origin in mitochondria (Wang et al., 2012) raises the possibility that tsRNAs can be translocated into mitochondria. This possibility is supported by recent experimental evidence (Li et al., 2024). The export of mitochondrial tRNAs (mt-tRNAs) in the opposite direction—from the mitochondria into the cytoplasm—has also been documented (Maniataki & Mourelatos, 2005; Traube & Carell, 2017).

A recent study has provided direct insight into the mitochondrial import of cytoplasmic tsRNAs by demonstrating that Glu-5' tsRNA-CTC, derived from nuclear-encoded tRNAGlu, showed an age-dependent increase in importation into the mitochondria of glutamatergic neurons. This pathological accumulation was linked to a disruption of mitochondrial translation and crista integrity. The import of Glu-5' tsRNA-CTC into mitochondria was mediated by its physical association with leucyl-tRNA synthetase 2 (LARS2), a protein synthesized in the cytoplasm and subsequently imported into mitochondria (Li et al., 2024; Riley et al., 2016) (Figure 2D). Notably, mitochondrial accumulation of Glu-5' tsRNA-CTC interfered with the aminoacylation of mitochondrial tRNALeu, ultimately impairing the translation of all 13 of the mitochondrially encoded proteins (Li et al., 2024). This finding provides one of the first mechanistic insights into the directed translocation of cytoplasmic tsRNAs into mitochondria and highlights their potential pathological consequences.

The relocalization of tsRNAs from their original compartments appears to be a common phenomenon in both normal and diseased states. For example, Li et al. reported that the translocation of the Glu-5' tsRNA-CTC from the cytoplasm to mitochondria in neurons contributes to brain aging and AD pathology (Li et al., 2024), highlighting the multifunctional nature of tsRNA fragments. However, the underlying mechanisms and full consequences of their mislocalization remain incompletely understood. Nevertheless, the identification of an increasing number of functional roles associated with tsRNAs continues (Figures 2, 3).

Translocation of tsRNAs across cells

The secretion of tsRNAs into the extracellular space extends their roles beyond processes occurring at the individual cell level and enables their participation in communication between different cells. This communication role is primarily mediated by the encapsulation of tsRNAs in exosomes, a major subtype of extracellular vesicle (EV) (Kalluri & Lebleu, 2020; Li et al., 2020b; Pegtel & Gould, 2019; Weng et al., 2022). For example, during T-cell activation, 5' tRFs are selectively packaged into exosomes and secreted from the cell. This process appears to function as a protective mechanism that prevents the tsRNA-mediated suppression of gene expression programs essential for T-cell activation (Chiou et al., 2018). Currently, EV-associated tsRNAs are emerging as promising diagnostic biomarkers; for example, tRF-Lys-TTT has been detected in EVs derived from patients with breast cancer, while 5' tsRNAs corresponding to Glu, Gly, and Val tRNAs have been identified in EVs from individuals with liver cancer (Koi et al., 2020; Zhu et al., 2019). These findings highlight the disease-specific profiles of EV-

associated tsRNAs (Figure 2E).

Once the tsRNA-containing EVs are secreted into the extracellular environment, they can enter the bloodstream. From there, they can be transported to and taken up by distant recipient cells, thereby enabling the transfer of regulatory tsRNAs from donor to recipient cells. This process can mediate intercellular communication and potentially influence the physiological or pathological states of targeted recipient cells (Weng et al., 2022).

THE EFFECTS OF tsRNAs ON AGING AND AGE-RELATED NEURODEGENERATIVE DISORDERS

Functionally, tsRNAs have emerged as key regulatory molecules in a variety of physiological and pathological processes. Abnormal expression and tissue-specific enrichment of tsRNAs have been associated with cancer, metabolic diseases, neurodegenerative conditions, and aging-related memory decline (Goodarzi et al., 2015; Li et al., 2024; Shen et al., 2018; Zhang et al., 2014).

tsRNAs in aging

Aging is a natural biological process characterized by a progressive decline in physiological function over time (Boa Sorte Silva et al., 2024; Huang et al., 2025; Jin et al., 2024; Kirkwood & Austad, 2000; Zhang & Liu, 2023). Accumulating evidence from various model organisms suggests that the expression profiles of tsRNAs undergo dynamic changes with age. For example, in *Caenorhabditis elegans*, global levels of tiRNAs increase with aging, and bioinformatics analyses reveal that 5'tiRNAs, particularly those derived from high-copy-number tRNA genes (e.g., 5'-Asp-GTC, 5'-Gln-TTG, 5'-Ser-CGA), are generally upregulated. Conversely, 3' tiRNAs display more variable expression patterns, showing both upregulation (e.g., 3'-Lys-CTT, 3'-Ser-CGA) and downregulation (e.g., 3'-tRNA fragments with the terminal CCA sequences) (Shin et al., 2021).

Similarly, in drosophila, tsRNAs also exhibit age-dependent expression changes, with notable aging-associated decreases in the loading of specific tsRNAs, such as GluCTC, onto Ago1, whereas loading onto Ago2 increases with aging. This age-related redistribution of tsRNAs between Ago1 and Ago2 suggests the operation of a coordinated mechanism by which tsRNAs may influence age-related regulatory pathways via Ago-containing RISC (Karaiskos et al., 2015) (Figure 4B).

Deep sequencing of small RNAs in mouse serum has also identified a population of circulating 5' tRNA halves derived from various tRNA species, but 3' tRNA halves are largely undetectable. Interestingly, the serum levels of specific 5' tRNA halves exhibit significant age-related changes, with some subtypes increasing (e.g., His-5' tsRNA-GTG) and others decreasing (e.g., Arg-5' tsRNA-CCG, Cys-5' tsRNA-GCA, Gly-5' tsRNA-GCC, Lys-5' tsRNA-CTT, Val-5' tsRNA-AAC) with age. (Figure 4D). Notably, these alterations in expression can be attenuated by calorie restriction, suggesting a potential regulatory link between metabolism, aging, and tsRNA dynamics (Dhahbi et al., 2013). Nevertheless, the mechanisms by which circulating tsRNAs influence the aging process remain unclear and warrant further investigation.

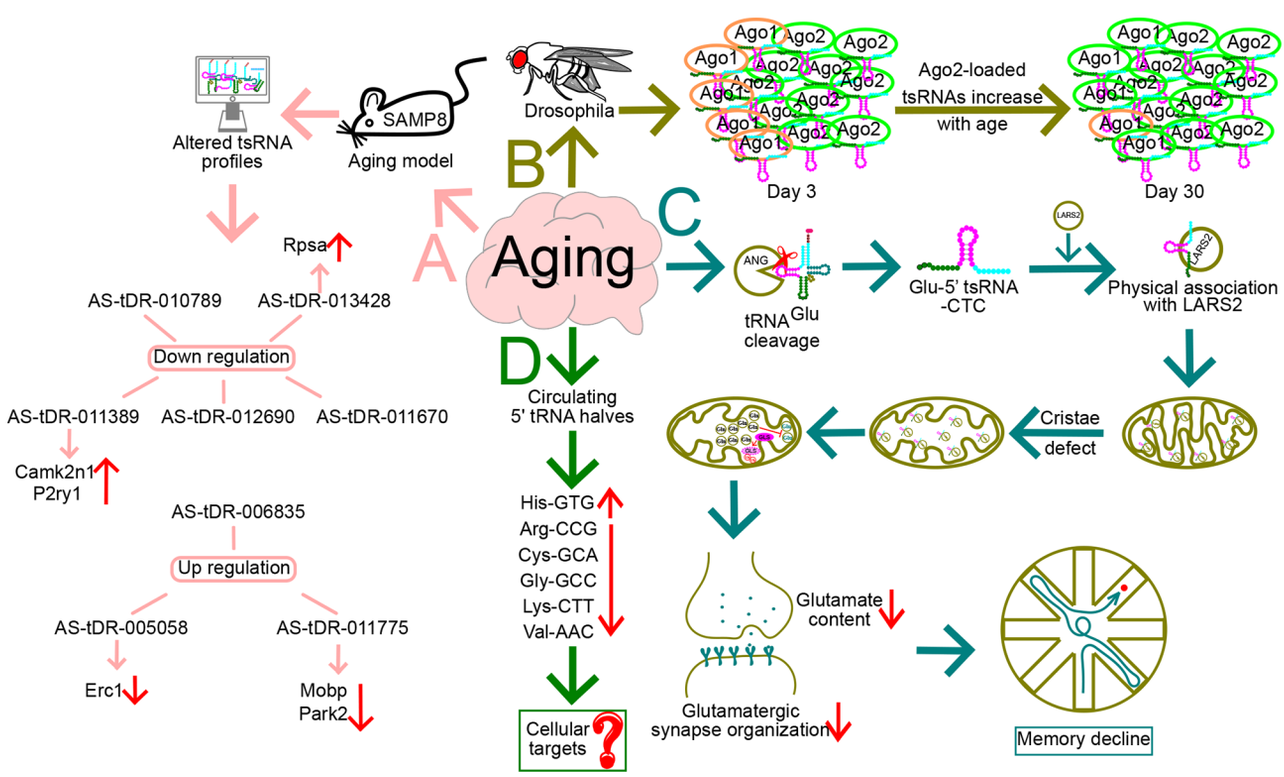

Figure 4 Schematic of tsRNA roles in aging-associated biological processes

The expression profiles of tsRNAs change dynamically with age: A: In a senescence-accelerated mouse model, differentially expression of tsRNAs may impact brain function; B: In drosophila, Ago2-loaded tsRNAs increase with age, suggesting that a potential shift from Ago1 to Ago2 may influence age-related pathways; C: The serum of aged mice shows significant changes in the expression patterns of many 5' tsRNAs. D: In the medial prefrontal cortex (mPFC) of aged mice, cytoplasmic-to-mitochondrial translocation of Glu-5' tsRNA-CTC fragments compromises mitochondrial cristae and glutamate production, ultimately leading to synaptic dysfunction and memory decline. Abbreviations: SAMP8, senescence-accelerated mouse prone 8; LARS2, leucyl-tRNA synthetase 2.

tsRNAs in brain aging

The aging brain undergoes significant changes in RNA expression, particularly of noncoding RNAs, linking these changes to cognitive decline. The tsRNAs derived from the 3' end of tRNAs show a consistent increase in expression with age in rat brains, whereas the tsRNAs derived from the 5' end exhibit a less uniform pattern of change (Karaiskos & Grigoriev, 2016). In the senescence-accelerated mouse prone 8 (SAMP8) model, multiple tsRNAs were differentially expressed when compared to the senescence-accelerated mouse resistant 1 (SAMR1) control. These tsRNAs are predicted to represent target genes involved in various brain functions, including synapse formation and the synaptic vesicle cycle pathway (Zhang et al., 2019) (Figure 4A).

A recent study demonstrated that, during brain aging, the Glu-5' tsRNA-CTC fragment selectively accumulates in the mitochondria of glutamatergic neurons in the medial prefrontal cortex (mPFC). No accumulation of Glu-5' tsRNA-CTC was detected in other brain cell types. This accumulated tsRNA interferes with the aminoacylation of mitochondrial tRNALeu, thereby disrupting mitochondrial protein synthesis in neurons. The resulting impairment compromises mitochondrial crista structure, reduces glutaminase levels, and lowers glutamate production. This cascade ultimately leads to synaptic dysfunction and memory decline. Importantly, reducing Glu-5' tsRNA-CTC levels restored glutamate metabolism and improved memory performance in aged mice (Li et al., 2024) (Figure 4C).

tsRNAs in neurodegenerative disorders

A growing body of research indicates the potential for inactivating mutations in tRNA-processing enzymes and disrupted tRNA metabolism to contribute to the development of various pathological conditions, including neurological disorders (Hanada et al., 2013; Karaca et al., 2014). Both healthy and diseased nervous systems produce tsRNAs, with the most abundant expression observed in the hippocampal region, where over 80% originate from the 5' end of tRNAs. Among these tsRNAs, Gly-5' tsRNA-GCC and Glu-5' tsRNA-CTC are highly enriched across multiple brain regions, including the hippocampus, prefrontal cortex, and cerebellum (Jehn et al., 2020). Notably, both the hippocampus and prefrontal cortex are highly sensitive to neurological diseases. Emerging evidence increasingly supports the involvement of tsRNAs in the pathogenesis of neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS) (Baindoor et al., 2024; Gusella et al., 2021; Pandey et al., 2021; Wu et al., 2021; Zhao et al., 2025).

Alzheimer's disease: AD, the most common form of dementia, is characterized by progressive memory loss, aphasia, apraxia, and agnosia (da Silva Filho et al., 2017). In familial AD (FAD), the neurodegenerative phenotype is most often associated with mutations in genes encoding amyloid precursor protein (APP) and presenilins 1 and 2 (PSEN1, PSEN2) (Li et al., 2020; Delabio et al., 2014). These mutations promote the accumulation of amyloid-β (Aβ) plaques and the formation of neurofibrillary tangles (NFTs), leading to neuronal damage and synaptic loss (Hampel et al., 2021). However, FAD accounts for only 5%–10% of all AD cases, and the genetic basis of FAD remains incompletely understood (Van Cauwenberghe et al., 2016). FAD has a relatively young age of onset and is therefore referred to as early-onset AD (EOAD). In contrast, the vast majority of AD cases are sporadic, with onset usually after the age of 65, and they are classified as late-onset AD (LOAD), which accounts for over 90% of all patients with AD (Lv et al., 2023).

In recent years, increasing attention has been directed toward the role of tsRNAs in AD progression. Both ANG expression and tsRNA production were significantly elevated in the hippocampal neurons of patients with either EOAD or LOAD (Wu et al., 2021) (Figure 5A). Notably, the total abundance of tsRNAs in AD brains surpasses that of conventional small RNAs, such as microRNAs (miRNAs) and PIWI-interacting RNAs (piRNAs), indicating the importance of tsRNAs in AD pathology. Intriguingly, the top ten tRFs are all derived from the 5' ends of tRNAs (Wu et al., 2021). Several 5' tsRNAs, including Pro-5' tsRNA-AGG, Gly-5' tsRNA-GCC, Cys-5' tsRNA-GCA, and Glu-5' tsRNA-CTC, were markedly upregulated in the hippocampus of patients with AD compared to age-matched controls (Figure 5A). Furthermore, the degree of tsRNA upregulation appears to be age-dependent and disease-stage-dependent. For example, the expression of Pro-5' tsRNA-AGG positively correlates with Braak staging, highlighting a strong association between tsRNA dysregulation and AD progression (Braak et al., 1991; Wu et al., 2021).

ANG usually cleaves tRNAs around the anticodon arms, resulting in the production of tsRNAs 30 or 40 nt in length, whereas Dicer-dependent cleavage often produces tsRNAs with a length of around 20 nt (Cole et al., 2009; Fu et al., 2009; Haussecker et al., 2010). In human hippocampus tissues, the majority of AD-affected tsRNAs are 30–40 nt long. Notably, patients with AD exhibit enhanced expression of ANG, but Dicer expression is similar to that in healthy individuals, suggesting a dominant role for ANG in tRNA cleavage during AD pathogenesis (Wu et al., 2021).

Parkinson's disease: PD is the second most prevalent neurodegenerative disorder, affecting over 6 million people worldwide—a number projected to double by the middle of the 21st century (Schapira et al., 2017). Such neurodegenerative disorder is primarily characterized by the progressive degeneration and loss of dopaminergic neurons.

Compared to healthy controls, patients with PD display a differential abundance of tsRNAs in various biological materials, including the prefrontal cortex, cerebrospinal fluid (CSF), and serum (Magee et al., 2019). Although many of these tsRNAs are commonly found in all three of these biological sources, patients with PD show distinct expression patterns from those of healthy controls (Magee et al., 2019; Pliatsika et al., 2016, 2018). A subset of these tsRNAs is sufficient to distinguish patients with PD from controls with relatively high sensitivity and specificity (Magee et al., 2019), suggesting their potential utility as diagnostic biomarkers (Figure 5B).

The SAMP8 model, which exhibits PD-like symptoms at around 7 months of age, shows distinct expression profiles of tsRNAs when compared to the SAMR1 controls. Notably, the tRF-1 fragment AS-tDR-011775 is upregulated and suppresses the expression of the PARK2 gene, resulting in reduced levels of the parkin protein. Parkin plays a critical role in mitochondrial autophagy and neuronal homeostasis; therefore, its downregulation likely contributes to neuronal damage and loss, thereby accelerating PD progression (Zhang et al., 2019) (Figure 5B). These findings suggest that, in addition to aggregated α-synuclein, tsRNAs may serve as prognostic biomarkers and therapeutic targets in PD.

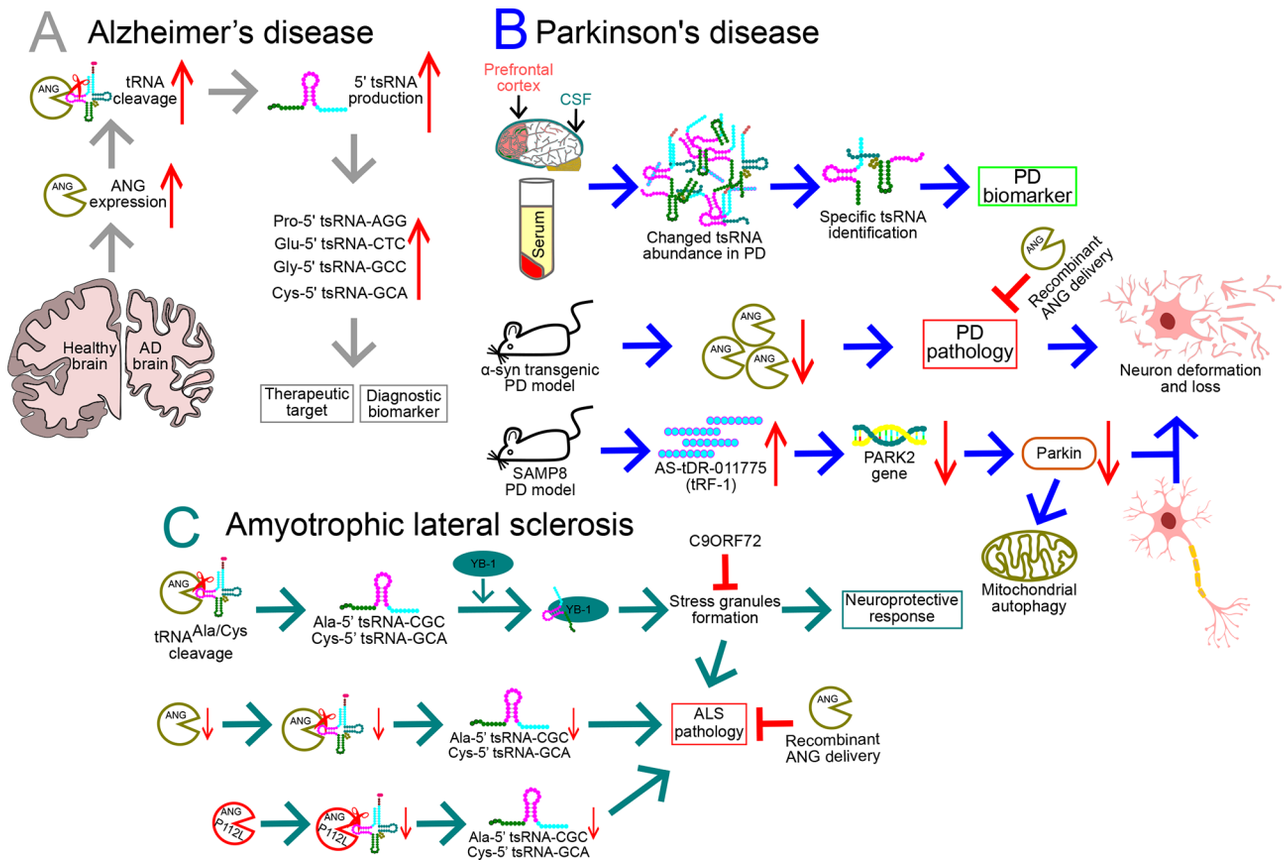

Figure 5 Schematic of tsRNA roles in neurodegenerative disorders

A: In Alzheimer's disease (AD), increased expression of ANG is accompanied by upregulation of several 5' tsRNAs in the hippocampus. These upregulated 5' tsRNAs may represent promising diagnostic biomarkers and therapeutic targets. B: In Parkinson's disease (PD), tsRNAs exhibit differential abundance in the prefrontal cortex, CSF, and serum of patients compared to healthy controls, suggesting a potential for tsRNAs as diagnostic biomarkers. Similar patterns are observed in the SAMP8 mouse model. In α-synuclein transgenic mice with reduced ANG expression, ANG supplementation confers protective effects on damaged motor neurons. C: In amyotrophic lateral sclerosis (ALS), 5' tiRNAs promote the formation of stress granules (SGs) and contribute to neuroprotective responses. Impaired SG formation due to C9ORF72 mutations, reduced ANG levels, or ANG variants with deficient ribonuclease activity may contribute to ALS pathology. Recombinant ANG treatment has been shown to protect injured neurons. Abbreviation: CSF, cerebrospinal fluid.

ANG—the endonuclease responsible for cleaving tRNAs at the anticodon arm to produce tiRNAs—is highly expressed and enriched in motor neurons. ANG appears to exert a neuroprotective effect on injured motor neurons; however, multiple ANG variants, including K40I, R31K, K17I, Q12L, I46V, and C39W, can reduce this neuroprotective activity, possibly by affecting the nuclear import, localization, or ribonucleolytic activity of ANG (Kieran et al., 2008; Sebastià et al., 2009) (Figure 5B). α-Synuclein transgenic mouse models that replicate key pathological features of PD show reduced ANG expression (Steidinger et al., 2011, 2013), but the relationship between changes in ANG expression and alterations in tsRNA production remains largely unknown.

Amyotrophic Lateral Sclerosis: ALS is a fatal and currently incurable neurodegenerative disorder characterized by the progressive degeneration of motor neurons (Baindoor et al., 2024). Patients with ALS possess mutations in the ANG gene that promote the generation of 5' tiRNAs. Specific tsRNAs, such as Ala-5' tsRNA-CGC and Cys-5' tsRNA-GCA, bind directly to the translational repressor YB-1 and displace the eIF4F complex from the 5' cap of mRNAs, thereby inhibiting translation initiation (Ivanov et al., 2014) (Figure 2C). These tsRNAs can also assemble into G-quadruplex structures, which facilitate their entry into motor neurons and promote the formation of SGs—the membraneless organelles associated with neuroprotective responses (Pandey et al., 2021). The C9ORF72 gene, which contains expanded GGGGCC hexanucleotide repeats, is the most common genetic cause of ALS. These repeats interfere with SG assembly induced by Ala-5' tsRNA-CGC, thereby disrupting tsRNA-mediated protective mechanisms and contributing to ALS pathogenesis (Ivanov et al., 2014; Wu et al., 2007) (Figure 5C).

Multiple ANG variants identified in patients with ALS affect either ANG ribonuclease activity or the shuttling of ANG between the nucleus and cytoplasm (Rayaprolu et al., 2012; Van Es et al., 2011, Prehn & Jirström, 2020). The ANG gene in a subset of patients with ALS contains a P112L missense mutation that suppresses the ribonuclease activity of ANG, thereby impeding the biogenesis of neuroprotective 5' tsRNAs and impairing their downstream protective functions (Ivanov et al., 2014; Wu et al., 2007) (Figure 5C). ANG delivery can improve motor function in SOD1^{G93A} mice, an established ALS mouse model (Kieran et al., 2008), suggesting that ANG-directed therapeutics may benefit patients with ALS.

ANIMAL MODELS FOR tsRNA RESEARCH

In the life sciences, the use of appropriate animal models is

essential for mimicking in vivo physiological and pathological conditions. These models enable researchers to obtain direct and reliable experimental data that can offer critical insights into potential therapeutic strategies for human diseases. The study of tsRNAs commonly employs two primary strategies: manipulating upstream enzymes that regulate tsRNA biogenesis or directly targeting specific tsRNAs using short hairpin RNAs (shRNAs) or antisense oligonucleotides (ASOs).

Manipulation of upstream enzymes

CRISPR-based knockout or knock-in of specific genes to create genetically modified models: Conditional genetic manipulation is often preferable when an enzyme is also essential in nontarget tissues, as global knockout or overexpression could lead to unintended or exaggerated phenotypes that complicate interpretation. A widely used solution is to flank the target gene with loxP sites and cross the mice with transgenic lines expressing Cre recombinase under tissue-specific or time-specific promoters. Alternatively, adeno-associated viruses (AAVs) encoding promoter-specific Cre can be microinjected to achieve spatial and temporal gene deletion. For example, we previously generated ANG knock-in mice that abundantly produce Glu-5' tsRNA-CTC fragments in the brain (Li et al., 2024).

Direct inhibition of tsRNAs: An alternative approach is the specific inhibition of individual tsRNAs using shRNAs or ASOs. This strategy allows researchers to dissect the precise role of each tsRNA. However, since pathological conditions are often driven by the collective effect of multiple tsRNAs, targeting only one or a few tsRNAs may not fully reverse disease phenotypes. Therefore, a combined approach involving both upstream enzyme manipulation and specific tsRNA inhibition may provide a more comprehensive understanding.

SUMMARY

In this review, we explored the biogenesis and classification of tsRNAs, their subcellular localization and biological functions, and their roles in aging and in the most prevalent neurodegenerative diseases. Enzyme-mediated production of tsRNAs has been observed across various biological systems and pathological conditions, suggesting that tRNA cleavage is a conserved process throughout evolution. A key feature of tsRNAs and their associated enzymes is the dynamic shift in their localization and expression patterns under stress conditions, highlighting their significance in stress responses. Recent studies have offered valuable insights into how these small RNAs contribute to diverse pathophysiological processes and have pointed to their potential as diagnostic and therapeutic tools for distinguishing between disease types.

Among the tsRNA subtypes, 5' tiRNAs are the most abundantly expressed in the brain (Haack et al., 2019). These are predominantly generated by ANG, particularly under stress conditions such as aging and neurodegenerative disease (Li et al., 2024; Pandey et al., 2021; Prehn & Jirström, 2020; Wu et al., 2021). In ALS, ANG-induced 5' tiRNAs derived from tRNAAla and tRNACys have demonstrated neuroprotective effects (Ivanov et al., 2014). However, certain ANG variants (e.g., ANG K40I) result in losses of ribonuclease activity and are associated with more severe disease phenotypes (Kieran et al., 2008; Prehn & Jirström, 2020). Notably, the administration of recombinant ANG in ALS mouse models can delay neurodegeneration, improve motor function, and slow disease progression (Kieran et al., 2008; Prehn & Jirström, 2020).

The tsRNAs are found in the prefrontal cortex, cerebrospinal fluid (CSF), and serum of patients with PD and healthy controls. However, significant differences in their expression profiles exist between these groups. Certain differentially expressed tsRNAs can distinguish patients with PD from controls with high sensitivity (89%–100%) and specificity (79%–98%), making them promising diagnostic biomarkers (Magee et al., 2019). As in ALS, ANG appears to play a protective role in PD. Several ANG mutations compromise its ribonuclease activity, nuclear-cytoplasmic shuttling capability, or protein stability—impairments that hinder its ability to generate cytoplasmic tsRNAs (Wu et al., 2007). Overexpression of recombinant ANG in α-synuclein transgenic mouse models of PD can protect dopaminergic neurons and mitigate neurodegeneration (Steidinger et al., 2011).

Collectively, the available literature suggests that the neuroprotective effects of ANG-mediated tsRNA generation in ALS and PD arise through the support of motor and dopaminergic neuron survival. In contrast, in AD, aberrantly induced tsRNAs appear to exacerbate neuronal degeneration, particularly in the hippocampus and cortex. Patients with AD exhibit significantly elevated ANG expression, implicating ANG in tsRNA dysregulation during disease progression. ANG-induced fragments, such as Gly-5' tsRNA-GCC and Glu-5' tsRNA-CTC, are markedly increased in both early- and late-onset AD, suggesting their potential contributions to disease progression and their potential value as therapeutic targets. Overall, the suppression of ANG overexpression may have therapeutic benefits in AD.

Notably, tsRNAs derived from tRNAPro are also upregulated in both EOAD and LOAD, and the observation of stage-specific increases across Braak stages 3 to 6 suggests the potential utility of tsRNAs as biomarkers for AD staging (Wu et al., 2021). A recent study identified Glu-5' tsRNA-CTC as an age-accumulated fragment in the glutamatergic neurons of the mPFC, where its accumulation correlated with cognitive decline. Interestingly, the Glu-5' tsRNA-CTC level was also significantly higher in AD brains than in brains without AD (Li et al., 2024). Furthermore, the delivery of ASOs targeting Glu-5' tsRNA-CTC into the bilateral ventricles of aged mice significantly alleviates aging-associated phenotypes. These findings highlight the therapeutic potential of ASO-based interventions for age-related cognitive impairment and neurodegenerative disorders involving tsRNA dysregulation.

CONCLUDING REMARKS

This review summarizes the current scientific findings on the biological functions of tsRNAs and their involvement in nervous system disorders. Emerging evidence underscores the crucial roles of abnormally expressed tRNA-derived small RNAs—and their upstream processing enzymes—in regulating the function and survival of motor and cortical neurons in various neurological diseases. Notably, some tsRNAs exhibit spatiotemporal specificity, highlighting their potential as biomarkers for accurately distinguishing between disorders with overlapping clinical symptoms, as well as for therapeutic targeting.

However, as a relatively new and evolving field within noncoding RNA (ncRNA) research, tsRNA studies face several key challenges:

Limited detection scope: Due to current technical limitations in sequencing technologies, the diversity of the identified tsRNA species remains narrow. The high sequence similarity between tsRNAs, their parental tRNAs, and other small RNA subtypes further complicates their precise classification and annotation.

Incomplete coverage of tsRNA subtypes: Most of the existing research focuses on tiRNAs, 5’ tRFs, and 3’ tRFs, while other subtypes, such as itRFs, tRF-1s, and tRF-2s, remain underexplored.

Lack of robust functional validation: Although the potential diagnostic and therapeutic benefits of tsRNAs in neurological disorders have been proposed, conclusive experimental evidence supporting their utility as reliable biomarkers or therapeutic targets is still insufficient.

To address these challenges, advancements in high-resolution sequencing technologies are urgently needed to expand the known repertoire of tsRNAs and improve the accuracy of their detection. Additionally, more comprehensive and mechanistic studies are essential to provide a deeper understanding of tsRNA biology and to evaluate their true potential in the diagnosis and treatment of neurological diseases.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS’ CONTRIBUTIONS

All authors contributed to the conceptualization and overall framing of the work, participated in drafting and revising the manuscript, and approved the final version.

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