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Opinion

Long-lived cellular molecules in the brain

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In long-lived mammals, including humans, brain cell homeostasis is critical for maintaining brain function throughout life. Most neurons are generated during development and must maintain their cellular identity and plasticity to preserve brain function. Although extensive studies indicate the importance of recycling and regenerating cellular molecules to maintain cellular homeostasis, recent evidence has shown that some proteins and RNAs do not turn over for months and even years. We propose that these long-lived cellular molecules may be the basis for maintaining brain function in the long term, but also a potential convergent target of brain aging. We highlight key discoveries and challenges, and propose potential directions to unravel the mystery of brain cell longevity.

Highlights

Long-lived proteins (LLPs) and long-lived RNAs (LLRs) might be required for maintaining functional integrity and the longevity of long-lived cells in the brain, such as neurons and adult neural stem cells.

Hypotheses have been raised regarding the functions of long-lived molecules, but current proposals are largely speculative and remain to be experimentally tested. Based on the localization of LLPs/LLRs, they seem likely to be involved in synaptic and epigenetic regulation, which could be critical for long-term memory and the maintenance of cellular identity.

To dissect the physiological and pathological roles of LLPs/LLRs, novel methods are needed to measure them at subcellular resolution and manipulate them selectively.

Unraveling the mechanisms underlying LLP/LLR maintenance will also improve current understanding of lifespan regulation and the emergence of age-related diseases.

Long-lived proteins (LLPs) and long-lived RNAs (LLRs) in the mammalian brain

Neurons, unlike most other cell types, do not divide and are not replaced over an organism's lifetime [1]. This lack of turnover necessitates robust mechanisms to maintain cellular integrity and function across prolonged periods of time, up to many decades. One key aspect of neuronal longevity is protein homeostasis, or proteostasis, which involves the balance between protein synthesis, folding, and degradation [2]. Advances in metabolic labeling techniques have provided unexpected insights into protein turnover rates in neurons, and identified LLPs in the brain (reviewed in [3,4]). In addition to proteins, recent studies assessing the longevity of RNA have led to the identification of LLRs in the brain [5], challenging the prevailing consensus that RNA molecules are unstable.

Investigating the mechanisms underlying the long-term maintenance of these long-lived molecules – and the consequences of their dysfunction during brain aging or in the pathogenesis of age-related disease – is crucial to understanding their pathophysiological roles. However, due to limitations in measurement sensitivity and the lack of tools to selectively manipulate long-lived molecules, current proposals regarding their roles in brain aging remain largely speculative. In this opinion article, we discuss recent developments in characterizing LLPs and LLRs, as well as advances in emerging technologies to detect long-lived molecules in the brain. We also examine the mechanisms underlying the maintenance of long-lived molecules and these molecules' potential physiological roles. We finally delineate future directions to improve current understanding of the biological roles of long-lived molecules in brain aging and longevity.

Dissecting LLPs in different subcellular regions and their potential functions

Some of the fundamental goals of aging research are to determine the impact of aging on cellular homeostasis at the molecular level and to identify the causal mechanisms that link aging to age-related diseases. As dysregulation of proteostasis is strongly associated with several neurodegenerative diseases, there has been significant interest in proteostasis mechanisms in post-mitotic neurons. Initially, metabolic labeling methods in other cell types have revealed that certain proteins, such as crystallins in the eye lenses and cohesins in oocytes, can persist for extended periods. More recent studies have employed sophisticated techniques to identify and characterize LLPs in the rodent brain, which appear to have important regulatory functions [6,7]. Stable

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isotope labeling by amino acids in cell culture (SILAC) has advanced our understanding of protein turnover in the brain, identifying numerous LLPs and determining their half-lives across various brain regions. A comprehensive analysis employing dynamic SILAC measured the half-lives of over 5100 proteins in rat primary hippocampal cultures and revealed a wide range of protein half-lives and key differences in proteostasis between neurons and glial cells [8].

Another study identified several LLPs in synapses [9], suggesting a potential role for these proteins in synaptic plasticity, learning, and memory (Figure 1). These findings indicate that while many synaptic proteins undergo rapid turnover, a subset exhibits extended stability, which may be crucial for maintaining long-term synaptic functions and ultimately memory. Furthermore, several studies utilizing *in vivo* isotopic labeling combined with mass spectrometry and mathematical modeling have accurately measured the lifetimes of thousands of brain proteins [10], as well as the heterogeneity in protein turnover and associated phosphorylation levels across different tissue types [11]. The findings underscore the complexity of proteostasis in the brain and the

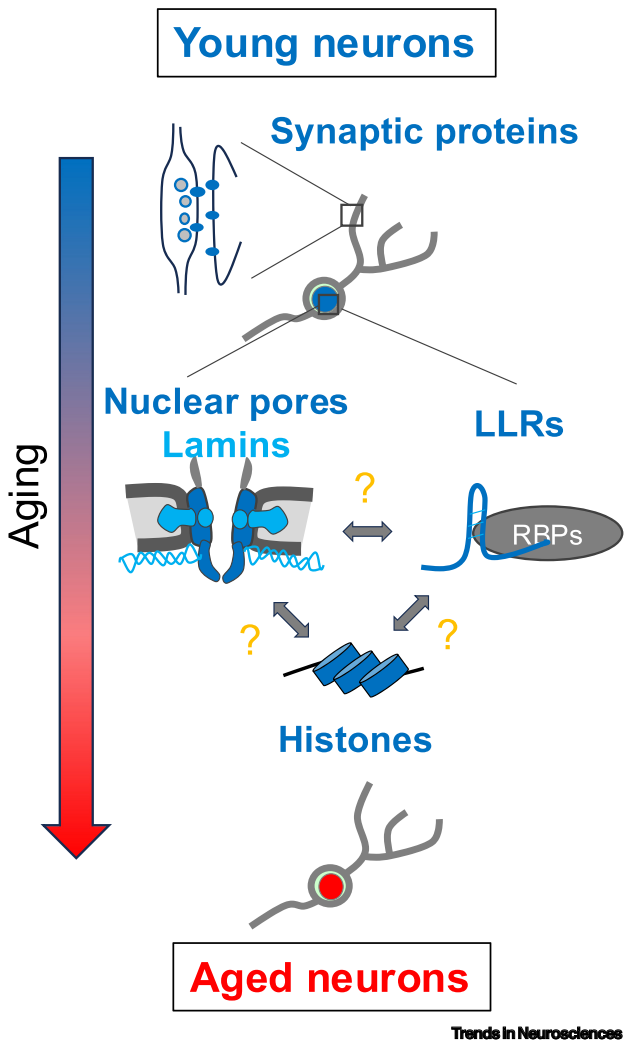

Figure 1. Schematic illustration of the major classes of long-lived molecules in the brain and their hypothesized roles in brain maintenance. Synaptic proteins and nuclear structural proteins as well as long-lived RNAs (LLRs) have been identified as long-lived molecules in the brain. Synaptic proteins have different lifetimes depending on synapse/cell types [11,23]. Since they are involved in synaptic plasticity, it has been hypothesized that long-lived synaptic proteins play a key role in memory formation and memory maintenance. It should be experimentally tested whether the impairment of long-lived synaptic proteins deteriorates cognitive function. Similarly, nuclear pore proteins, lamins, and histones [7], as well as LLRs [5], are long-lived molecules in cellular nuclei. They are tightly associated with chromatin and may contribute to robust cell-type-specific epigenetic regulation and/or chromatin integrity. Further investigation is needed to assess whether they are ultimately important in maintaining brain function and longevity. Abbreviation: RBP, RNA-binding protein.

necessity for precise measurement techniques to fully understand protein dynamics at a subcellular resolution.

New technologies to detect LLPs with cellular function and their limitations

Recent studies employing isotope labeling approaches to infer the function of LLPs have provided valuable insights into protein turnover rates, along with associated cellular processes and spatial information *in situ*. A notable example is the development of the turnover and replication analysis by isotope labeling (TRAIL) methodology [12]. This approach integrates ^{15}N isotope labeling with mass spectrometry to simultaneously measure protein degradation and cell division rates in multiple tissues. The TRAIL method has revealed that protein lifespans vary significantly, ranging from minutes to years, depending on the tissue context.

High-resolution mass spectrometry (HRMS) has been instrumental in analyzing the proteome of various brain regions, leading to the identification of LLPs [10,13]. By integrating isotopic labeling, the turnover rates of numerous proteins across different brain tissues (cortex, cerebellum) and subcellular fractions and potential links with neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis, were assessed. The findings revealed significant variability in protein lifespans across brain tissue types, with certain proteins exhibiting extended stability, underscoring the complexity of proteostasis in the brain.

Another emerging technology employing multi-isotope imaging mass spectrometry (MIMS) in combination with ^{15}N metabolic labeling has provided significant insights into the identification and visualization of long-lived cells and subcellular structures [14–16]. MIMS has been used to monitor the replacement of long-lived components of nuclear pore complexes in post-mitotic cells [17]. Certain nucleoporins, particularly those in scaffold components like the Nup107/160 and Nup205 subcomplexes, have extremely long lifespans in neuronal nuclei, persisting for more than 1 year [6,7] (Figure 1). Core histones H3 and H4 exhibit long lifespans, indicative of minimal replacement over several months. These long-lived histones are predominantly enriched at silent gene loci, suggesting their involvement in epigenetic memory and transcriptional repression. Stable isotope labeling and tandem mass spectrometry has identified long-lived mitochondrial proteins in mouse heart and brain tissues [18]. Consistent with other methods, a subset of mitochondrial crista proteins persisted for months [19].

The aforementioned methods leverage stable isotope labeling to quantitatively study protein turnover with high spatial and temporal resolution. However, they come with critical technical challenges. One major limitation is the difficulty in distinguishing between retained and resynthesized molecules, especially in long-lived or post-mitotic cells. Additionally, detection sensitivity can be a bottleneck, particularly for low-abundance targets or in heterogeneous tissues. Biochemical fractionation of tissue material can mitigate some of these limitations. The process of labeling itself can also introduce biological perturbations, as excessive or prolonged isotope exposure may impact cellular physiology. Finally, the complexity of data analysis – especially in spatially resolved methods like MIMS – requires careful interpretation to avoid confounding artifacts.

To mitigate these limitations, several approaches have been proposed. One such approach is data-independent acquisition proteomics, which can be used to detect protein modifications such as isomerization. This method allows us to estimate protein lifetime based on the degree of modifications, without the use of isotopes [20–22]. Another approach is a targeted imaging method that visualizes the turnover of specific proteins such as PSD-95 and GluA2, in combination with HaloTag and fluorescent ligands [23,24]. Although these methods are low throughput, they allow higher-resolution spatiotemporal data to be obtained, to align with behaviorally relevant

events. Multiplexing this imaging approach could resolve the heterogeneous nature of protein turnover from single-cell to single-synapse resolution. In the future, it will be essential to combine these various methods to not only determine the lifetime of endogenous proteins across human tissues, but to measure protein lifetime in relation to cellular events.

Now that several studies have identified numerous LLPs in the brain, the question arises: what are the possible implications of LLPs for neuronal function? The exceptional stability of LLPs, particularly nuclear and structural proteins, could have significant implications for neuronal longevity, cellular aging, and the pathogenesis of neurodegenerative diseases. Neurons are among the longest-lived cells in the body and rarely divide, placing extraordinary demands on the integrity of their proteome over a lifetime. In this context, LLPs such as nuclear pore proteins, lamins, and histones must maintain their structural and functional integrity for up to decades without replacement. This biological constraint makes them uniquely susceptible to cumulative damage from oxidative stress, misfolding, or post-translational modifications, potentially leading to progressive functional decline. Age-dependent deterioration of nuclear pore complexes has been shown to impair nucleocytoplasmic transport, disrupting the nuclear permeability barrier [25,26] and leading to aberrant exchange of proteins and RNAs between the nucleus and cytoplasm. Such defects can have broad downstream effects on gene expression, RNA processing, and protein localization – key processes essential for neuronal identity and function. Furthermore, beyond their canonical transport roles, recent studies have revealed that nuclear pore complexes also serve as scaffolds for gene regulatory complexes and play a role in the spatial organization of the genome [27–30]. Although these studies utilized proliferating progenitors, this structural function appears to be cell-type-specific, suggesting that deterioration of nuclear pore complex components may result in selective transcriptional dysregulation in vulnerable neuronal populations [19–22]. Therefore, to fully understand their contribution in brain aging, it is critical to address the role of long-lived nuclear proteins in epigenetic regulation and chromatin organization in long-lived neurons.

Long-lived chromatin-associated proteins, including core histones and lamins, may also influence gene expression patterns over the course of aging and pathology progression. Although direct evidence remains limited [31,32], it is plausible that the persistence of modified or damaged histones/lamins contributes to the loss of youthful, plastic gene expression programs observed in aged neurons. If not properly maintained or remodeled, these LLPs could gradually encode maladaptive epigenetic states, thus reinforcing transcriptional profiles associated with cellular aging or disease [33].

Understanding the molecular mechanisms that preserve the function of LLPs – whether through selective autophagy, chaperone-mediated repair, or damage-sensing degradation pathways – will be critical in deciphering how proteome stability contributes to neuronal health span. Conversely, identifying how and why these mechanisms fail with age could shed light on early molecular events driving neurodegenerative disease, particularly in sporadic cases where no clear genetic cause is known.

To fully explore the functional relevance of LLPs, there is a pressing need to develop novel tools for their manipulation *in vivo*. This may involve the engineering of conditional degradation systems, targeted manipulation of age-related modifications of LLPs, developing methods to pulse label and track old versus new protein pools, or the application of spatially resolved proteomic techniques to identify where and when LLP dysfunction arises. Following these developments, the contribution of LLPs in synaptic plasticity or epigenetic regulation could be addressed. Ultimately, studying LLPs not only offers a window into the fundamental biology of long-lived cells

but may also unveil new therapeutic avenues to promote healthy brain aging and mitigate neurodegenerative processes (see Outstanding questions).

RNA stability in mammalian cells

In addition to LLPs, what other long-lived cellular molecules might support the long-term maintenance of brain function? Classically, RNA has been considered an unstable, short-lived molecule compared with DNA. The half-life of mRNA has been estimated at around 3–10 h [34]. More recently, by combining metabolic labeling of RNA [with 4-thiouridine (4sU) or 5-ethyl-uridine (5-EU)] and next-generation sequencing [35–39], the half-life of transcripts has been quantitatively measured in mouse embryonic stem cells (mESCs) and immune cells. The half-life of RNA transcripts varies depending on transcript type, and the RNA half-life of housekeeping genes tends to be longer (~30 h) than that of regulatory RNAs (~30 min). In addition, rRNA has a half-life of 50–72 h, whereas tRNA has a half-life of 36–60 h, with RNA half-life being, to some extent, dependent on cellular state [34] (Table 1). Similar observations have been made in rodent primary culture neurons [40]. Overall, the range of RNA stability is in the order of hours to a few days, and it has been considered that rapid RNA turnover allows cells to adapt their transcriptome in response to changes in their cellular state (e.g., cell cycle) or environment.

However, some RNAs can be very stable in specific cases. One example is the long-term maintenance of mRNA in metazoan oocytes [41,42]. During oogenesis, transcription terminates at the onset of meiosis and maternally derived mRNA must be maintained to initiate the processes necessary for embryonic development at a later stage. These maternally derived mRNAs possess distinct characteristics, such as shorter polyA tails, and can be maintained for weeks without engaging in translation [43]. Recent evidence suggests that maternal mRNAs are differentially modified or protected compared with mRNAs in somatic cells, and these modifications are likely to promote escape from degradation in several species [44–46]. Similarly, mRNAs in cotton

Table 1. Detection of RNA half-lives among various cell types

Type of RNA	Cell type	Cellular state	Method	Half-life/*Longest detection*	Refs
mRNA	Mouse fibroblast (3T3 and 3T3)	Proliferative	^{3}H uridine	9 h	[34]
rRNA	Mouse fibroblast (3T3 and 3T3)	Proliferative	^{3}H uridine	50–72 h	[34]
tRNA	Mouse fibroblast (3T3 and 3T3)	Proliferative	^{3}H uridine	36–60 h	[34]
mRNA	*Xenopus laevis* oocytes	Dormant	^{3}H uridine and ^{3}H guanosine	2–3 years	[41]
Nonspecific	Mouse cortical neural nuclei	Post-mitotic	^{3}H uridine	*12 days*	[50]
lncRNA	Dendritic cells	Proliferative	4sU	10 min	[35]
Coding RNAs	Dendritic cells	Proliferative	4sU	2.3 h	[35]
mRNA	Mouse ESCs	Proliferative	4sU/SLAM-seq	4.3 h/*24 h*	[36]
Nonspecific	Snail brain cells	Post-mitotic?	5-EU	*2 months*	[70]
Nonspecific	Mouse cerebellum	Post-mitotic	5-EU	*2 weeks*	[71]
Nonspecific	Mouse neurons/adult neural stem cells in the hippocampus and cerebellum	Post-mitotic/quiescent	5-EU	*~2 years*	[5]

seeds have been shown to be remarkably stable [47], supporting the idea that dormant or quiescent cells have the ability retain certain RNAs for very long periods of time.

LLRs in the rodent brain

These observations suggest that RNA is not necessarily unstable, but depending on cell type or cellular state, cells employ machineries to maintain certain RNAs in the long term. Previous studies have mostly measured the half-life of RNAs in proliferating cells. However, the majority of cells in the brain, including neurons and adult neural stem cells, are post-mitotic or quiescent cells. Is it possible that post-mitotic or quiescent cells have different RNA turnover dynamics? In addition, is it possible that different RNA dynamics contribute to the regulation of long-lived cells?

Several groups, including ours, have recently explored this possibility by labeling RNA with 5-EU or 5-bromouridine (BrU) in postnatal mouse brains and assessing the lifetime of RNAs *in vivo* through combination with click chemistry [5,48,49]. To our surprise, some brain cells retained 5-EU-positive signals up to 2 years after 5-EU injection – almost the lifetime of rodents. 5-EU signals are exclusively retained in the nuclei, in agreement with a previous report [50]. Intriguingly, only a subset of cell types in specific brain regions, such as the hippocampus and the cerebellum, retained 5-EU signals. Other cell types in the brain such as cortical neurons did not retain 5-EU signals, although they are capable of metabolizing 5-EU and can use it for transcription [5]. Consistent observations were made following metabolic labeling with BrU and BrUTP [5]. How are these LLRs retained in a cell-type-specific manner? One possibility is through developmental timing. The hippocampus and cerebellum develop in mice during the first 2 weeks of the postnatal period, meaning that the majority of the cells are generated during the period of 5-EU injection. By contrast, cortical neurons are generated during embryonic development. The generation of LLRs may be coupled with cell cycle exit, as observed in the hippocampus [5]. To investigate this possibility, 5-EU was injected during embryogenesis and the retention of LLRs in the cortex was examined [5]. LLRs were not detected in the cortex, suggesting three, non-mutually exclusive possibilities. First, LLRs could be transcribed only in specific cell types. Second, only specific cell types retain machineries to preserve LLRs. Third, the critical period for LLR generation/maintenance may differ depending on cell type. These possibilities should be explored in future investigations.

In parallel, the aforementioned study verified that long-retained 5-EU signals are RNA derived using RNase, DNase, and RNA polymerase inhibitors, as well as via RNA-seq. However, it would be important to systematically assess LLRs by other methods as well. While inhibition of *de novo* transcription should not affect the maintenance of LLRs, specifically inhibiting the transcription of LLRs with CRISPR interference (CRISPRi) should provide insight into LLR function. Currently, one must rely on uridine analogs to measure RNA lifetime. Of note, however, incorporation of uridine analogs into LLRs may alter their stability and structure. Therefore, similar to LLPs, it is critical to develop independent methods to assess endogenous LLRs. Importantly, treatment with RNA polymerase inhibitors after 5-EU incorporation did not change the levels of 5-EU-labeled transcript levels, suggesting that 5-EU is not recycled [5]. However, RNA polymerase inhibitors are cytotoxic and can therefore be used for only a limited time. Furthermore, RNA-seq and qRT-PCR data suggested that transcripts derived from the same gene or genomic region can contribute to both LLRs and short-lived RNAs [5]. Nevertheless, it is difficult to estimate their respective proportions. Since LLRs are low in abundance, to be sequenced EU-labeled transcripts must currently be enriched using click chemistry or similar methods. The proportion of LLRs compared with other, short-lived transcripts from the same locus must be taken into account when assessing the stability of LLRs *in vivo*. Even following enrichment, the number of LLRs detected in *in vivo* hippocampal samples is much lower than in the *in vitro* quiescent neural

precursor cell model, in which all cells are labeled with 5-EU [5]. To this end, more accurate and sensitive methods are needed to detect LLRs alongside all other transcripts.

Mechanisms underlying the maintenance of LLRs and their physiological roles

The findings discussed in the previous section have raised additional questions about RNA stability and function. First, how can LLRs be preserved for 2 years? LLRs were primarily observed in the nuclei in a cell-type-specific manner. Therefore, it is likely that specific mechanisms are required to maintain LLRs in the nucleus and protect them from the nuclear RNA exosome. RNAs are degraded by 5′ or 3′ exonucleases, with RNA-binding proteins (RBPs) protecting the 5′ or 3′ untranslated regions (UTRs) of RNAs to allow escape from exonuclease activity. Since RBPs often recognize the secondary/tertiary structure of RNAs [51–53], it is essential to investigate whether LLRs share common sequences or structures, and to identify which RBPs can bind to LLRs, given that there are more than 500 RBPs in the mammalian genome [54,55] (see Outstanding questions). As LLRs mainly comprised pre-mRNA/mRNAs, long noncoding RNAs (lncRNAs), and several repeat-derived RNAs with various structures [5], it is plausible that more than one mechanism contributes to the retention of these distinct RNA classes. LLRs themselves may also have specific structures to promote stability, such as the stable triple helix structure in the 3′ ends of the metastasis-associated lung adenocarcinoma transcript 1 (*MALAT-1*) and the nuclear retention element (ENE) of Kaposi's sarcoma-associated herpesvirus polyadenylated nuclear RNAs [56–58]. In addition, RNA modifications also alter RNA stability [59]. In line with this idea, it would be intriguing to determine the structure of LLRs, using long-read RNA-seq to recover the full-length sequence, splicing variants, UTR usage, intron retention, and RNA modifications. Various potential RNA structures or motifs essential for LLR function could then be investigated, such as stem-loop or R-loop structures. These analyses could also help to explain why LLRs are retained only in certain cell types, possibly through interactions with specific nuclear RBPs.

Second, what are the physiological functions of LLRs? Are they important for neuronal longevity? The aforementioned study addressed the role of major satellite RNAs as LLRs, since they were enriched in both *in vitro* quiescent neural precursor cells and *in vivo* in the hippocampus [5], and identified roles for major satellite RNAs in heterochromatin stability and somatic stem cell maintenance. However, more than 1000 LLRs have been identified from quiescent neural precursor cells. Some of the LLRs identified in neural precursor cells are known to be associated with heterochromatin, whereas others are known to be associated with euchromatin. Interestingly, another study has shown that euchromatin-associated CoT-1 scaffold RNAs are enriched for repeat-derived RNAs, pre-mRNAs, and lncRNAs [59]. Since LLRs are associated with euchromatin as well as heterochromatin, and many of the contributing RNA species are shared between CoT-1 RNAs and LLRs, it is very likely that CoT-1 RNAs represent a component of LLRs. CoT-1 scaffold RNAs are tightly associated with euchromatin and therefore may support maintenance of open chromatin and ultimately drive cell-type-specific genetic programs. Conversely, heterochromatin-associated LLRs could facilitate heterochromatin stabilization and contribute to 3D nuclear architecture over long periods of time. Since LLPs, including lamins and nuclear pore proteins, interact with heterochromatin, and LLPs and LLRs are spatially well colocalized at the nuclear periphery, it would be interesting to investigate whether LLPs and LLRs cooperate to support their own maintenance and synergistically increase the stability of chromatin and gene programs. To this end, it would be important to investigate whether they interact with specific chromatin regions in concert, and whether they recruit other chromatin regulators such as histone and DNA modifiers. If this is the case, one of the roles of LLRs (with LLPs) could be to serve as a structural platform to stabilize cell-type-specific chromatin structure and function. Epigenetic regulation plays a central role in cellular identity and transcriptional memory, with loss of heterochromatin an established hallmark of cellular aging [60–63]. Therefore, it is vital to determine whether

the deterioration of LLPs/LLRs precedes heterochromatin loss and to functionally assess the contribution of LLRs to epigenetic regulation.

Another possibility is that the pre-mRNA stored in the nucleus acts as a reservoir of mRNA during differentiation. When quiescent neural precursor cells are activated, they need to activate genetic programs for proliferation and differentiation. If pre-mRNAs required for stem cell activation are stored and ready to be transported and translated, the cells could be activated smoothly to generate their progeny. LLRs from quiescent neural precursor cells contain various transcripts related to cell cycle regulation and neural development [5], and the onset of adult neural stem cell differentiation is known to be post-transcriptionally controlled [64]. Therefore, it would be interesting to test whether LLRs can be translated on stem cell activation. However, in the case of neurons, this scenario is less likely, given that LLRs are maintained for years with minimal reduction [5].

One open question is why LLRs are observed in only certain cell types in the brain if they are fundamentally important for the long-term maintenance of neural function (see Outstanding questions). What would happen if LLRs were introduced into neural cell types that they were not endogenous to? Cellular robustness could be maintained at the expense of cellular plasticity. Understanding how LLRs are involved in regulating cellular robustness and plasticity, both ontogenetically and evolutionarily, is important. Of course, it is possible that LLRs have not been detected in other neural cell types due to technical issues, such as measurement sensitivity, or due to the different critical periods of LLR generation among different neural cell types. Addressing LLRs from several perspectives using various methods would be necessary.

Concluding remarks and future perspectives

Recent studies, facilitated by methodological advances, have documented the presence of long-lived molecules in brain cells, including post-mitotic neurons and quiescent somatic stem cells. To unravel the relationship between long-term maintenance of brain function and brain aging, it is necessary to understand the mechanistic link between physiological aging and pathological development. LLPs and LLRs are potential links to bridge this knowledge gap. However, because most long-lived cellular molecules are not abundant, it is critical to develop methods to detect and manipulate them at single-cell resolution *in vivo*, allowing researchers to identify them and investigate their biological roles.

In addition to brain cells, it is important to identify long-lived cellular molecules in different organs and cell types and to investigate their biological functions. Besides neural cells, there are many other cell types that need to maintain their cellular function and identity for long periods of time, such as cardiac myocytes, oocytes, plasma cells in the immune system, and somatic stem cells specific to each organ. It is likely that oocytes and neural cells use different mechanisms to maintain LLRs, based on their differing subcellular localization. Thus, the basic principles for maintaining long-lived cellular molecules may differ depending on the cell type and its function. Assessing the diversity and identifying the cell-type-specific functions of long-lived cellular molecules would be critical to understanding how long-lived organisms have evolved to maintain cellular function over extremely long periods of time.

Finally, future studies should explore both the mechanisms that confer molecular longevity and methods to replace long-lived molecules. Recent evidence has shown that these long-lived molecules accumulate damage during aging and in the pathological development of neurodegenerative diseases [25,26,31,32,34,65–69]. Due to the nature of long-lived molecules, replacing them is not mechanistically straightforward. However, if these long-lived molecules are common and

Outstanding questions

Are LLPs important for long-term neuronal function in the brain, including synaptic and epigenetic regulation? Are LLPs in the brain essential for long-term memory (e.g., via their involvement as synaptic proteins or perineuronal net components)?

How do long-lived nuclear proteins contribute to maintaining the cell-type-specific epigenetic landscape? Do they stabilize cell-type-specific epigenetic modifications or organize 3D chromatin architecture to maintain cellular identity? Does damage to LLPs during aging initiate age-related synaptic dysfunction and age-related epigenetic aberration?

What is the role of LLRs in biological function beyond maintaining chromatin integrity? Do they work as a scaffold to recruit other epigenetic enzymes or nuclear structural proteins, including LLPs, to cooperatively regulate robust epigenetic modifications, chromatin openness, or 3D chromatin architecture?

Do distinct LLRs play a role in the regulation of euchromatin and heterochromatin, and if so, do the regulatory mechanisms differ? Can LLRs be translated during the differentiation of adult neural stem cells to smoothly activate gene expression?

What mechanisms underlie the long-term maintenance of LLRs? How are (pre-)mRNAs, lncRNAs, and repeat-derived RNAs retained in the nucleus in the long term?

Do LLRs have common motifs/structures or contain modifications? Are they bound by specific RBPs? How do they escape from RNA degradation machineries? Is it possible to replace damaged LLPs/LLR? Can cells be rejuvenated by replacing LLPs or LLRs?

Why are LLRs specific to certain subpopulations of brain cells? Are brain cells with LLRs more robust or do they need LLRs because they are more vulnerable? Do other organs or cell types have LLRs, and what are their biological functions?

convergent targets of age-related diseases, a fundamental objective would be to develop therapeutic strategies to target them.

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Declaration of interests

The authors declare no competing interests.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used DeepL in order to improve the language. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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