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SURVEY AND SUMMARY

G-Quadruplexes as pathogenic drivers in neurodegenerative disorders

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ABSTRACT

G-quadruplexes (G4s), higher-order DNA and RNA secondary structures featuring guanine-rich nucleic acid sequences with various conformations, are widely distributed in the human genome. These structural motifs are known to participate in basic cellular processes, including transcription, splicing, and translation, and their functions related to health and disease are becoming increasingly recognized. In this review, we summarize the landscape of G4s involved in major neurodegenerative disorders, describing the genes that contain G4-forming sequences and proteins that have high affinity for G4-containing elements. The functions of G4s are diverse, with potentially protective or deleterious effects in the pathogenic cascades of various neurological diseases. While the studies of the functions of G4s *in vivo,* including those involved in pathophysiology, are still in their early stages, we will nevertheless discuss the evidence pointing to their biological relevance. A better understanding of this unique structural element in the biological context is important for unveiling its potential roles in the pathogenesis of diseases such as neurodegeneration and for designing new diagnostic and therapeutic strategies.

INTRODUCTION

Nucleic acids, including DNA and RNA, represent the basic molecular code of life. The functions of nucleic acids are determined not only by their primary sequences but also their secondary or higher-order structures. The most abundant and thus the most studied secondary nucleic acid structures are formed by base-pairing through conventional Watson–Crick hydrogen bonding. However, alternative stable structures can also arise, such as G-quadruplexes (G4s), in which guanine bases are connected with Hoogsteen hydrogen bonds (1,2). Originally identified in *in vitro* experimental settings, G4s are four-stranded secondary structures formed in guanine-rich DNA or RNA sequences. The resulting structures exhibit high thermodynamic stability under near-physiological conditions as well as resistance to nuclease activity (3,4). G4s are increasingly recognized for playing many different roles in cellular environments associated with both normal physiology and pathology. There have been several reviews on the disease relevance of G4s (5–7), with oncological studies in particular drawing attention to these structural elements as candidates for therapeutic intervention (8,9). Interestingly, G4s have increasingly also been associated with a diverse set of genes and pathways implicated in neurological disorders. While the involvement of G4s in the regulation of selected neurological diseases and non-coding RNAs has been described previously (10), what has been lacking is a comprehensive discussion of the role of G4s within many neurological disorders, with an emphasis on the predicted outcomes and established mechanisms underlying the G4-induced pathological cascade. Here, we summarize and discuss the roles of G4s associated with various genetic players in neurodegeneration, such as Alzheimer's disease (AD), fragile X syndrome (FXS), amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Parkinson's disease (PD), and prion disease (Table 1).

Formation of G-quadruplex structures

The unique structural formation of G4s governs their role in the cell. Four guanine bases bonded together, often referred to as a G-quartet or G-tetrad, form a square planar

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Table 1. Summary of the roles and predicted effects of G-quadruplexes in neurodegenerative diseases

Disease	*Gene*/protein	Role of G4s
Alzheimer's disease (AD)	*APP*	Overproduction is suppressed by a 3′ UTR G4 motif (101).
	ADAM10	Translation is inhibited by a parallel 5′ UTR G4 (80).
	BACE1	Full-length (501) transcript production is activated by a G4 motif in 3rd exon during alternative splicing (106).
Fragile X Syndrome (FXS)	*FMR1*/FMRP	Inhibits translational machinery of SMNDC1 (97), Shank1 (95), PSD-95(98) via interactions with G4 motifs to prevent pathogenic upregulation. Alternative splicing is negatively regulated by G4s found in the coding region of the FMR1 mRNA (105).
Amyotrophic Lateral Sclerosis/Frontal-temporal Dementia (ALS/FTD)	*C9orf72*	Transcription is negatively regulated by DNA G4s formed at C9orf72 HRE (37). C9orf72 RNA G4s are implicated in regulation of many cellular processes.
	TDP-43	Transport of mRNA to neurites for translation is dependent on the presence of 3′UTR G4s in mRNAs (124).
	ANG	Cleavage product-induced G4 formation is critical for stress granule formation and translational inhibition (131).
Parkinson's Disease (PD)	*SNCA*	5′ UTR G4 motifs suppress cap-dependent translation (79) and influence cap-independent translation.
Prion Disease	PrP	Deleterious conversion to PrP^{Sc} is triggered by PrP binding to G4 motifs (136).
Progressive Myoclonus Epilepsy Type I (PME1)	*CSTB*	Dodecamer repeat forms parallel G4s at physiological pH (138).

arrangement in which each of the four bases acts as a donor and acceptor of two hydrogen bonds (Figure 1A) (11). G4s are the result of two or more G-quartets stacked sequentially. Depending on the topology and sources of the folding strands, G4s can adopt parallel, antiparallel, or hybrid complexes intra- or inter-molecularly (Figure 1B). G4s can be formed by DNA, RNA or a hybrid of both. Several distinctive features and structures contribute to the stability of G4s. First, G4s are stabilized by Hoogsteen hydrogen bonds between the G-rich strands of the nucleotides (2) and by π-orbital interactions among the stacked quartets (12). One of the most important factors involved in stabilizing G4s is the coordination of a monovalent metal ion, usually K^+ (13). Metal ion coordination occurs when metal ions enter an interior channel, formed by Hoogsteen binding patterns and stacking interactions, and interact with the oxygen at position 6 (O6) of each guanine atom throughout the length of the structure, counteracting the negative electrostatic effects of carbonyl groups (14). Several factors dictate which physiologically relevant cation stabilizes the G4 structures, including the ionic radius, hydration energy, and binding strength toward O6. K^+ is typically the favored cation because of its smaller effective ionic radius and lesser dehydration free energy. Accordingly, physiologically relevant concentrations of K^+ have been shown to stabilize G4s *in vitro* (15).

Multifaceted cellular functions of G-quadruplexes

There are hundreds of thousands of sites in the human genome containing G-rich sequences that are predicted by computational methods to form G4s (16,17). The chemical properties of G4s have been extensively characterized *in vitro*, although the nature and prevalence of these structures *in vivo* remain a subject of debate (18). However, with the recent development of G4 structure-specific antibodies, small molecule ligands, and chemical probing, DNA and RNA G4s have been increasingly detected in cells under physiological conditions (19–22). Studies of the biological implications of G4s can be classified into three categories of research that provide different levels of evidence: (i) bioinformatic and computational predictions of G4 motifs, (ii) *in vitro* studies of G4s and (iii) *in vivo* studies of G4s. Although the study of G4 function in a cellular context is ideally elucidated through *in vivo* evidence, the purpose of our review is to highlight and offer timely discussion concerning the interesting roles and functions of G4s that are revealed through all three types of research, in order to promote further study of the implications of G4s in neurological disorders. To provide context for our later discussion, we will first present an overview of the impact of G4s on transcription, translation, splicing and other cellular functions.

G4s are thought to play an important role in regulating transcription (Figure 2A). G4 motifs are enriched in promoter regions of the human genome, with their regulation of oncogenic promoters especially well characterized (17,23). G4-specific antibodies have been utilized in chromatin immunoprecipitation (ChIP) and immunohistochemical analyses to detect the *in vivo* formation of G4s in the cell. For example, a G4-specific antibody hf2 or BG4 has been used to demonstrate the influence of G4s on transcription (24,25). By analyzing gene expression patterns associated with DNA G4s using ChIP analysis, G4s have been shown to be capable of enhancing or inhibiting transcription (25,26). The extent of studies elucidating mechanisms by which G4s affect transcription is still limited, but we will discuss the possible mechanistic scenarios in the following sections on DNA G4s and transcription.

G4s can also influence translation (Figure 2B). G4s that are enriched in the regulatory regions of mRNAs, such as the 5′ untranslated region (5′-UTR) and the 3′-UTR, can exert control over the translation process (27,28). Studies focused on the most regulated stage of translation, translation initiation, have identified G4s as possible inhibitors of initiation that could act to prevent cap-dependent translation from occurring. In various mRNAs, G4s have also been identified in the internal ribosome entry site (IRES), an RNA element allowing for the initia-

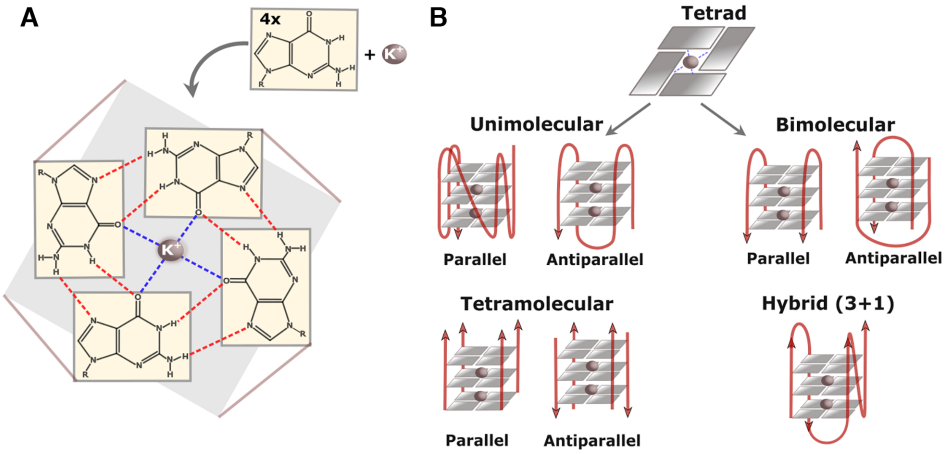

Figure 1. Experimentally observed G-quadruplex structures, orientations, and conformations. (**A**) G4s are nucleic acid secondary structures consisting of four guanine bases organized in a square planar arrangement and connected through Hoogsteen hydrogen bonds. Within the central channel, dipole interactions between guanine O6 and a cation, usually K^+, lend additional stability to the G4 structure. (**B**) There is great structural heterogeneity in the G4s, with these motifs capable of adopting parallel, antiparallel, or hybrid conformations in a unimolecular, bimolecular or tetramolecular manner.

tion of cap-independent translation. Regulation of IRES-mediated, cap-independent translation by G4 motifs is well documented although, as we discuss later, the exact effect of the G4 motifs remains controversial. The well-established binding between fragile X mental retardation 1 protein (FMRP) and G4s has also hinted at a potential regulatory loop in which G4s found in FMRP-binding mRNAs help suppress the translation of these mRNAs, which as discussed below has been linked to the pathogenesis of FXS or other neurological diseases.

RNA G4s are also involved in the regulation of splicing (Figure 2C). These secondary structures have been implicated as regulators of pre-mRNA processing, including adenylation and alternative splicing (29). Although studies elucidating the exact mechanisms through which G4-involved splicing occur are limited, as we discuss in our later section on G4's regulation of splicing in both cis- and trans-acting mechanisms, G4 binding to splicing factors is a common theme shared by the examples we provide.

G4s have also been identified as regulatory players in other cellular processes. Given the multifaceted influences of G4s and their high prevalence within the cell, they are likely involved in a myriad of cellular functions. As selected examples, we will discuss the influence of G4s in RNA-protein interactions, including those involving membraneless RNP granules, nucleocytoplasmic transport, the tRNA stress response, and more, when we take a deeper look at the mechanisms of G4-dependent regulations relevant to several neurological diseases in the sections below.

DNA G-QUADRUPLEXES AND TRANSCRIPTION

Several studies have pointed to a role for DNA G4s in the regulation of transcription. For the transcription-linked disease genes that we discuss in this section, the most thoroughly studied role of G4s in transcription belongs to their effect on *C9orf72*, which has been implicated in ALS and FTD (Figure 3). ALS is a neurological disorder marked by progressive degeneration of motor neurons within the brain and spinal cord. Closely related to ALS is FTD, a disease characterized by continuous neuronal loss in the frontal and temporal cortices. Given their shared genetic causes and other neuropathological similarities, ALS and FTD are thought to exist within the same spectrum of disease (30,31). The most common cause of ALS-FTD is a hexanucleotide repeat expansion (HRE), (GGGGCC)n, in a non-coding region of the chromosome 9 open reading frame 72 (*C9orf72*) gene (32,33). ALS-FTD patients typically harbor thousands of HRE repeats, in contrast to healthy controls that normally possess fewer than 25 repeats (32). The proposed mechanisms of pathogenesis in ALS-FTD include loss of *C9orf72* function, toxicity of the HRE RNA, and generation of aberrant poly-dipeptides through repeat-associated non-ATG-dependent translation (34). It has been established that the *C9orf72* HRE adopts stable G4 structures, potentially implicating these motifs in the pathology of ALS and FTD (35–37).

DNA G-quadruplex-mediated negative regulation of transcription

It has been demonstrated through *in vitro* transcriptional assays that DNA G4s formed at the *C9orf72* HRE block transcription by impairing RNA polymerase processivity, leading to decreased expression of the *C9orf72* gene (Figure 3) (37). In addition to *C9orf72*, DNA G4s have also been shown to inhibit transcription in *BRCA1*, a gene encoding a critical DNA repair factor that has also been linked to AD because of its reduced levels in AD mouse models and patient tissues (38,39), and in the prion protein gene (*PRNP*). In the case of *BRCA1*, Pyridostatin, a G4-binding small molecule, stabilizes G4s in the *BRCA1* promoter and represses *BRCA1* transcription in rat cortical neurons, compromising double-stranded break repair activity and inducing neurotoxicity resulting from cumulative insults to genomic integrity (40). A recent study investigating interactions between G4s and the prion protein has reported the presence of two G4 motifs in the promoter region of *PRNP* that can form hybrid G4 structures (41). One of the G4 motifs has been shown to inhibit transcription, and prion pro-

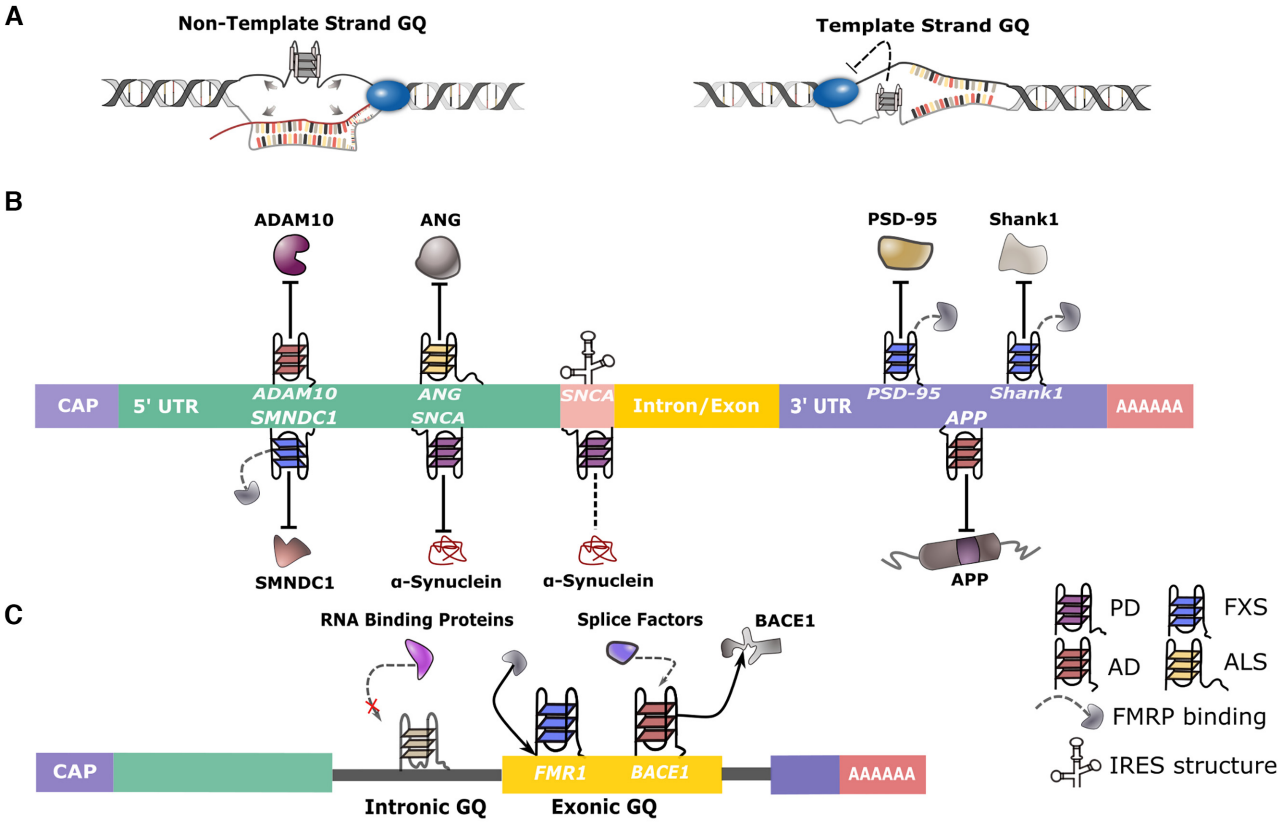

Figure 2. Proposed G-quadruplex regulation of biological processes. G4s regulate transcription, translation, and splicing. (**A**) Non-template strand G4s can regulate transcription by keeping the template strand single-stranded or by promoting formation of R-loops. Template strand G4s could regulate transcription by blocking RNA polymerase progression. (**B**) G4s found in mRNAs, particularly in regulatory regions such as the 5′-UTR, IRES and 3′-UTR, can potentially regulate the translation of proteins associated with AD, FXS, ALS and PD. G4s may influence both cap-dependent and cap-independent translation, and G4-interacting proteins such as FMRP are also involved. Arrows indicate inhibition of translation. (**C**) G4 motifs are found upstream and downstream of splice junctions. G4 structures may prevent access to RNA protein binding sites, mediate the binding of regulatory proteins such as FMRP, or recruit splice factors to influence alternative splicing, such as the selective generation of *BACE1* isoforms.

tein (PrP) apparently has the capacity to interact with and induce the unfolding of that same G4, suggesting that PrP can bind to G4 motifs in its own promoter to auto-regulate transcription (41). Given the enrichment of G4 motifs at the promoters in the human genome (17,24), it is likely that many genes whose transcription is regulated by these secondary structures are relevant to the neurological diseases discussed here.

The functions of G4s as transcriptional switches likely depend on their conformation and stability, which can be influenced by their repeat and flanking sequences, the lengths of the repeats, and environmental factors. For example, the repeat length of the *C9orf72* HRE DNA, $d(G4C2)_n$, influences whether the *C9orf72* HRE adopts a parallel or antiparallel topology (37,42). *In vitro*, the dominant and most stable conformation formed from the *C9orf72* HRE DNA, an antiparallel G4, arises from $d(G4C2)_4$ and leads to decreased transcription (37). The antiparallel G4 formed from the $d(G4C2)_4$ repeat has a monomeric chair-type conformation with a characteristic antiparallel G-tetra core and three edgewise loops, but with distinct 4-layer stacking (43). Other repeat lengths, such as $d(G4C2)_2$, $d(G4C2)_3$ and $d(G4C2)_5$, have been shown to form heterogeneous G4 mixtures with parallel and antiparallel topologies (37,43,44). A recent study using homogenous $d(G4C2)_2$ samples isolated through anion exchange chromatography has demonstrated that the $d(G4C2)_2$ repeat can form intermolecular G4s with a parallel conformation that fold as symmetric tetramers or with an antiparallel conformation that fold as asymmetric dimers (44). While the topologies of G4 structures formed in the *PRNP* gene have not been elucidated to the same extent as those in *C9orf72*, there is evidence to indicate that the G4 motif found at the *PRNP* promoter region likely adopts a hybrid (3+1) topology. Circular dichroism (CD) spectroscopy has revealed a CD profile with positive peaks around 260 and 290 nm and a negative peak around 240 nm (41), indicating a hybrid structure with three parallel strands and one antiparallel strand (45). To date, most studies on G4 and related structures are limited to *in vitro* analyses; probing these structures under native conditions in the cells awaits new technologies and has yet to be achieved.

While the mechanisms explaining the regulation of transcription by G4s have not been completely resolved yet, there are several possible scenarios by which G4s may regulate transcription that are suggested by past studies. After transcription is initiated, the transcription bubble generates positive or negative supercoiling regions that can propagate and induce stress, eventually forming single-stranded seg-

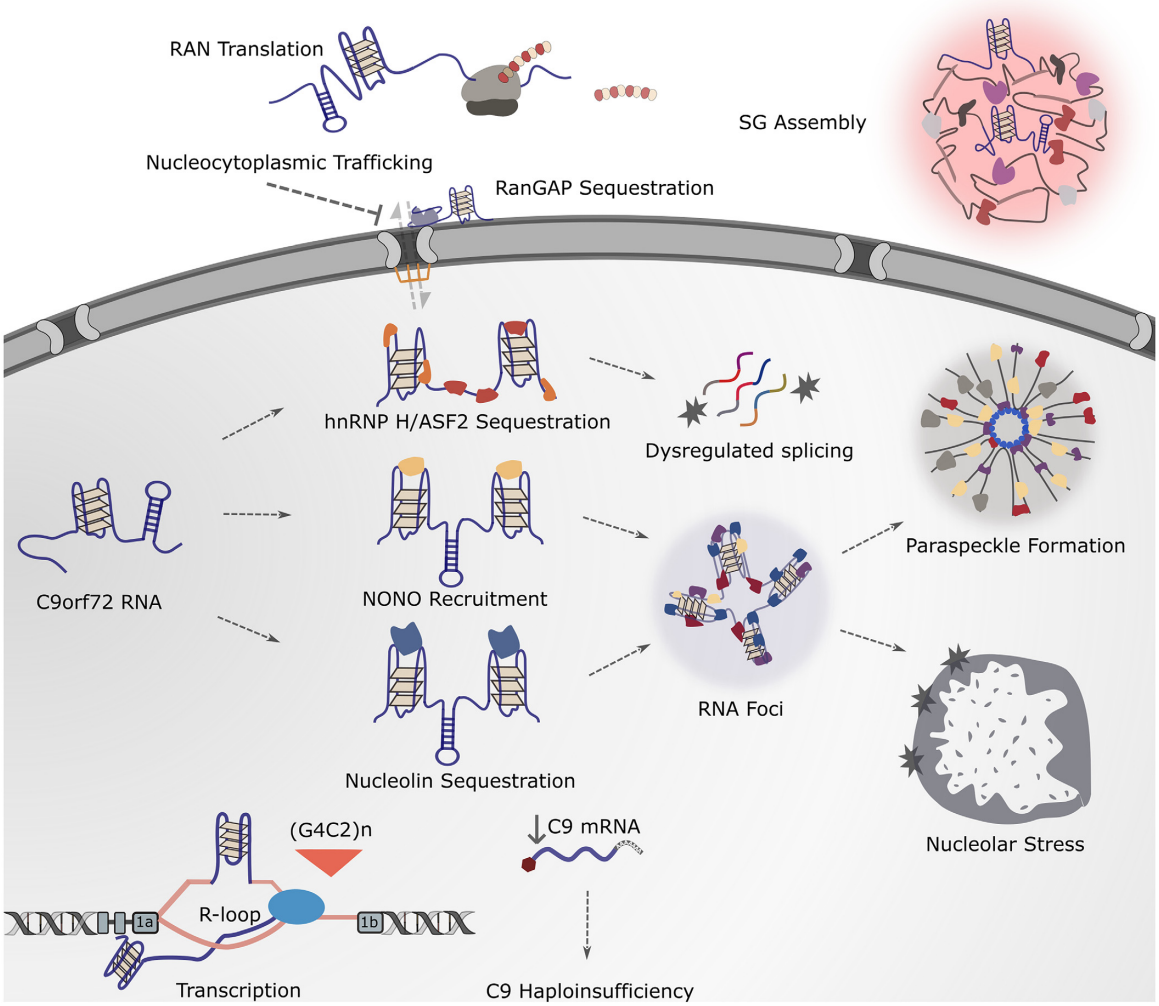

Figure 3. The role of G-quadruplexes in pathogenic cascades associated with the hexanucleotide repeat expansion in C9orf72. The C9orf72 DNA and RNA HRE are involved in a range of molecular cascades underlying ALS-FTD pathology. DNA G4s stabilize R-loop formation, which together results in the suppression of transcription. RNA G4s recruit several protein factors, including the splicing factors hnRNP H and ASF2, the paraspeckle protein NONO, and the nucleolar component nucleolin. As a result, a variety of functional consequences arise, including splicing dysregulation, RNA foci formation, paraspeckle dysregulation, and nucleolar stress. C9orf72 RNA G4s are also involved in nucleocytoplasmic trafficking, stress granule assembly, and possibly non-canonical repeat-associated translation.

ments that can fold into G4s (46,47). It is possible that G4s that form upstream of the transcription start site (TSS) can impede transcription by acting as an obstacle to block RNA polymerase in the transcribed region (48–51), by recruiting G4-binding proteins that inhibit transcription (47,52), or by failing to maintain the open DNA conformation that facilitates transcription re-initiation (46,52–54). G4s that form downstream of the TSS can cause transcription reinitiation if located on the coding or sense strand by keeping the template strand single-stranded, or they can block RNA polymerase progression if located on the template or antisense strand by serving as a physical obstacle (46,47).

The G-quadruplex and R-loop feedback loop

In the context of double-stranded DNAs, one of the consequences of G4s formed on one DNA strand, such as the non-template strand in the case of the *C9orf72* HRE, is the formation of an R-loop, a three-stranded structure composed of the displaced single-stranded DNA together with a DNA:RNA hybrid formed through Watson-Crick base pairing that involves the other DNA strand (55). The *C9orf72* HRE has been shown to readily form R-loops *in vitro* (Figure 3) (37,56). The G4C2 repeats can adopt stable secondary DNA structures, including G4s and hairpins, which can stabilize the displaced strand and therefore the R-loops. Recent studies of non-*C9orf72* model G4 motifs have pointed to the interplay between G4s and R-loop formation as a potential positive feedback loop. For example, results from single-molecule fluorescence assays that have modeled the G4s and R-loops *in vitro* suggest that R-loop formation can occur prior to G4 formation and subsequently stabilize the R-loops, via positive feedback, during further rounds of transcription (57,58).

R-loops have been extensively linked to the regulation of transcription, with these secondary structures thought

to regulate transcription activation, elongation, and termination (59). Studies have shown that the formation of R-loops leads to transcriptional stalling, and therefore decreased gene expression *in vitro* (60,61). R-loops formed from the *C9orf72* HRE have also been shown to stall RNA polymerase II and increase abortive transcription *in vitro* (37). The influence of G4 and R-loop formation on *C9orf72* gene expression can presumably initiate a number of different pathological cascades, including those related to bidirectional transcription and epigenetic modification, in which R-loops have been reported to participate (61,62).

DNA methylation affects G-quadruplex stability

The stability of G4s can be affected by DNA methylation. For example, in the case of the *C9orf72* HRE, methylation of its DNA is present in most patients harboring more than 90 repeats (63). Both 5-methylcytosine (5mC) and its oxidized form, 5-hydroxymethylcytosine (5hmC), have been suggested as the forms in which *C9orf72* HRE DNA methylation occurs. It has been reported that both the G-rich strand and the C-rich antisense strand of the *C9orf72* $(G4C2)_n$ DNA repeat can form four-stranded quadruplex structures, and that the 5mC and 5hmC modifications have differential effects on the stability and protein binding of the DNA structures (64). In another case, the 5mC modification of a DNA G4 found in the promoter region of *bcl-2* has been shown to increase the stability of the G4 (65). Furthermore, DNA methylation of G4 motifs in *VEGF* has been reported to decrease the initial elongation efficiency of PCR, which could indicate G4 stabilization (66). Another study investigating the effect of CpG methylation on the binding affinity of G4s to associated proteins has found that the influence of CpG methylation on the binding affinity also simultaneously affects G4 structure and topology (67). In addition, R-loop structures, which have been found at CpG islands, have been proposed to suppress DNA methylation. R-loop formation has been suggested to maintain the unmethylated state of CpG islands, and R-loop formation also provides protection from DNMT3B1-mediated DNA methylation (68).

DNA secondary structures, including G4s as well as their companion R-loop structures, may have a profound effect on transcription activity and epigenetic modifications at the HRE or gene locus, consistent with multiple reports showing that DNA methylation plays a role in reduced *C9orf72* gene expression (69–71). Consequently, the dysregulation of gene expression that occurs as a result of modifications at the transcriptional level may contribute to the development of neurodegenerative diseases that involve the loss of *C9orf72* expression and production of aberrant repeat-containing RNAs and polypeptides. However, analyses of the correlation between CpG hypermethylation of the *C9orf72* promoter and disease progression have not yielded consistent results (72,73). Given the complicated roles that DNA methylation is likely to play in disease development, further molecular studies and analysis of larger clinical datasets are clearly needed in the future.

Other functions of DNA G-quadruplexes

G4s are also linked to the disruption of DNA replication as well as the induction of DNA damage and genome instability (74,75). These higher-order structures may contribute to repeat instability, a common feature of most repeat expansion diseases, in which expansion or contraction of the repeat can occur. The human *C9orf72* allele contains 2–25 units of the G4C2 repeat, whereas ALS- and FTD-linked alleles have been found to contain up to thousands of the repeats. Varying lengths of the *C9orf72* repeats have been observed across generations or in cells from different tissues of individual patients; thus, the repeat instability could arise during meiosis, somatic cell division, or post-mitotic stages. The G4C2 repeat has been shown to affect DNA replication in a length- and orientation-dependent manner in human cells (76). The formation of R-loops at the *C9orf72* G4C2 repeat has also been linked to repeat instability. In addition, the *C9orf72* G4C2 repeat has been shown to be capable of forming bidirectionally transcribed double R-loops *in vitro*, with these double R-loops more prone to repeat instability than are single R-loops (56). Understanding the roles of G4s and R-loops may help elucidate the mechanisms of repeat instability in the neurodegenerative diseases.

RNA G-QUADRUPLEXES AND TRANSLATION

RNA structures play critical roles in biological processes that include translation. The bioinformatic observation that G4s are enriched in the non-coding regions but rarely found in coding sequences suggests that exons may have evolved to avoid the stable higher-order structures that likely impede ribosome scanning (77). At the same time, RNA G4s located in the 5′-UTR and 3′-UTR regions have been shown to play important regulatory roles in enhancing or inhibiting translation of various proteins associated with a range of neurological diseases, including AD, PD and FXS (Figure 2B).

RNA G-quadruplexes inhibit translation initiation

Within the 5′-UTR of mRNAs, G4s have been identified as structural elements responsible for regulating the initiation stage of the translation process. The presence of Lewy bodies, cytoplasmic inclusions consisting of the protein α-synuclein (encoded by *SNCA*), within dopaminergic neurons is a hallmark of PD, the second most common neurodegenerative disorder after AD. Because the overaccumulation and aggregation of the protein α-synuclein is thought to initiate a cascade that imparts neurotoxicity to neurons and other cells, understanding the regulation of α-synuclein translation could shed light on the pathogenic process in PD (78).

Bioinformatic analysis has revealed the presence of three non-overlapping G4 motifs at the proximal 5′-UTR of *SNCA* that operate together to repress *SNCA* translation (79). These G4 motifs are only effective at suppressing translation when the inhibitory effect is measured cumulatively. Analysis of the mutated G4 motifs found within the 5′-UTR *SNCA* has revealed that two of the mutated G4 motifs exhibit enhanced translation, along with higher mRNA

levels, when compared to the wild-type control (79). Hence, mutating these two G4 motifs should promote *SNCA* translation at least in part by either enhancing transcription or stabilizing the *SNCA* 5′-UTR reporter mRNA. However, because the third mutated G4 motif did not exhibit altered mRNA levels when translation was enhanced, it is likely that translation initiation is directly affected.

The involvement of G4s in the regulation of translation initiation may not be limited to *SNCA*, since it has been proposed that G4s found in the 5′-UTR of the metalloprotease *ADAM10* can potentially affect translation initiation (Figure 4). Effective elimination of neurotoxic Aβ plaques, which are characteristic of AD, relies on amyloid precursor protein (APP) processing by ADAM10, an α-secretase that cleaves APP within its Aβ domain and releases the neuroprotective N-terminal portion of APP, sAPP-α (80,81). A unimolecular, parallel G4 formed in the 5′-UTR of *ADAM10* mRNA has been shown to inhibit *ADAM10* translation *in vitro*, and mutations of the 5′-UTR G4 exhibited enhanced *ADAM10* translation, secretion of sAPP-α, and anti-amyloidogenic processing of APP (80). A subsequent study found that a methylquinolinium derivative known as compound 24 binds to the G4-forming sequence of *ADAM10* via a high-affinity interaction, with compound 24 exposure promoting *ADAM10* translation and decreasing Aβ production in cells (81). These studies demonstrate a role for the 5′-UTR G4 in the translation of disease-relevant enzymes such as *ADAM10* and suggest that their RNA secondary structure could be a potential therapeutic target.

The roles that G4s play in the regulation of translation initiation are particularly important to elucidate because it is believed that most regulation of translation occurs at the initiation stage in eukaryotes (82). Although the evidence thus far points to an inhibitory role for G4s in translation, the relevant mechanisms are not limited to the impediment of ribosome scanning at 5′-UTRs of mRNAs, as exemplified by the G4-containing tRNA fragments discussed in the section below regarding stress granules. Future studies that further elucidate the mechanisms by which G4s affect translation initiation may clarify the role of G4s in neurodegenerative diseases with pathological features associated with altered cap-dependent translation.

G-quadruplexes in non-canonical translation

Unlike most eukaryotic translation initiation, which requires an m7G cap at the 5′ end of the mRNA to initiate ribosome scanning in order to locate the start codon, there are alternative non-canonical mechanisms that initiate translation without these standard features. IRES is an RNA element that allows for internal ribosome entry to initiate translation in a non-cap-dependent manner. G4 motifs have been identified within IRES sequences and have been clearly shown to exert an influence, albeit with varied effects, on IRES activity. Several studies have shown that intramolecular G4 motifs found within the IRES promote IRES-mediated translation (83,84) and that removal of the G4 motif prevents IRES initiation (85), whereas another study has shown that G4 motif stabilization inhibits IRES-mediated translation (86). Of relevance to neurodegeneration is the fact that an IRES element located in the 5′-UTR of *SNCA* has been shown to enhance *SNCA* translation and synthesis in response to cellular stress (79). However, mutations in the three G4 motifs that we mentioned in our discussion of G4s and translation initiation did not affect the IRES activity and thus their role in the translation remain unclear (79). Thus, although the role of G4 elements in negatively regulating cap-dependent translation is established (87,88), the precise interplay of these elements in stress-induced cap-independent translation remains in need of future elucidation.

C9orf72 repeat-associated non-ATG (RAN) translation refers to the observation that the *C9orf72* HRE repeats in a non-coding region can still enable translation in the absence of an ATG start codon when the repeat length exceeds certain limits (89,90). *C9orf72* HRE-containing sense and antisense transcripts undergo RAN translation and produce aggregation-prone dipeptide repeat proteins. The dipeptide repeat proteins are toxic and induce neurodegeneration in experimental models. G4s are formed on the *C9orf72* HRE RNAs, but a direct role for the quadruplex structures in the regulation of the RAN translation remains to be established. Nonetheless, the potential involvement of G4s in pathologically relevant RAN translation may provide a target for therapeutic intervention.

G-quadruplex-dependent translational regulation in FXS

Fragile X syndrome (FXS) is the most common inherited intellectual disability and is induced by the silencing of the RNA binding protein known as fragile X mental retardation 1 protein (FMRP) (91). The expansion and hypermethylation of trinucleotide (CGG)n repeats within the 5′-UTR of *FMR1* mRNA, along with aberrant CpG island hypermethylation preceding the open reading frame of *FMR1*, underlie FMRP repression in FXS. FMRP is an mRNA-binding protein that is deemed essential for normal neurological function because of its suggested role in dendritic mRNA transport and postsynaptic translation (92). Phosphorylated FMRP generally operates as a *cis*-acting translational repressor of a subset of dendritically localized RNAs, inhibiting their premature translation during neuronal trafficking (93). In FXS pathology, the absence of FMRP-mediated translational blockade can result in translational overactivation, leading to aberrant and constitutive production of synaptic proteins, internalization of receptors, and impaired synaptic plasticity (94).

The binding affinity of FMRP for its mRNA targets depends on the presence of specific secondary structures, including G4s. It has been shown that the RGG box domain of FMRP binds with high affinity to G4s of its target mRNAs and regulates their expression (95,96). FMRP binds to a subset of its mRNA targets through G4 motifs located in the various regions of the transcripts.

FMRP has also been shown to bind to G4s located in the 5′-UTR and 3′-UTR of its other target mRNAs. For example, the FMRP RGG box has been observed to bind to an intramolecular, parallel G4 formed in the 5′-UTR of survival motor neuron domain containing 1 (*SMNDC1*) *in vitro* (97). G4 motifs formed in the 5′-UTR of mRNAs typically operate as inhibitory translational elements, and

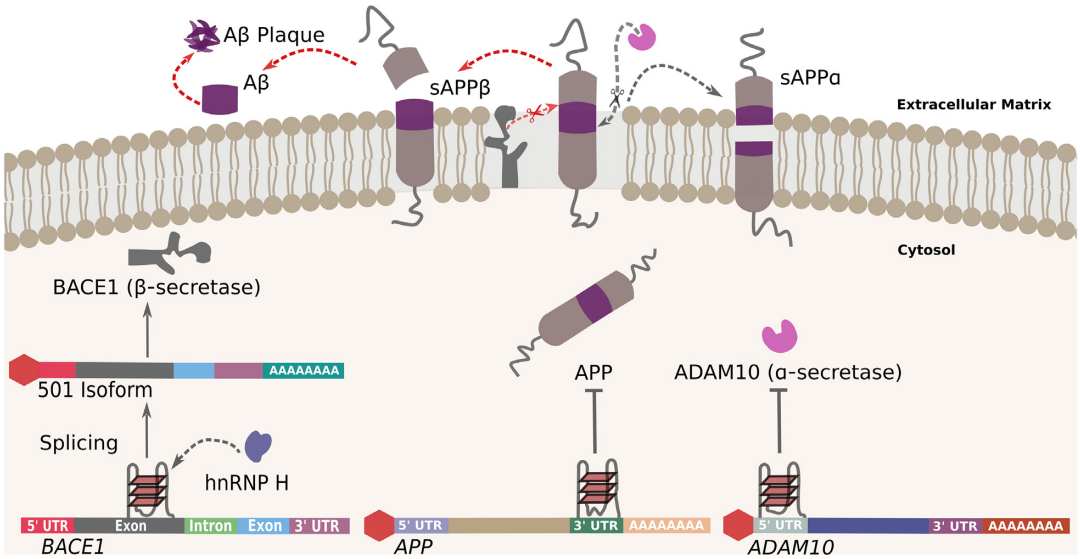

Figure 4. Proposed involvement of G-quadruplexes in Alzheimer's disease. Through the differential cleavage of APP, G4s participate in the regulation of Aβ biogenesis, either by affecting either the expression of APP itself or that of APP-processing enzymes. A G4 motif in the 3′-UTR of *APP* mRNA has been found to negatively regulate APP translation. A G4 motif within the coding region of *BACE1* mRNA may act as a recruitment site for splice regulator hnRNP H, resulting in favored production of the full-length *BACE1* 501 transcript isoform and leading to enhanced APP proteolysis and Aβ production. Also, a G4 motif within the 5′-UTR of *ADAM10* mRNA negatively regulates the production of ADAM10 α-secretase activity, thereby suppressing cleavage of APP and contributing to Aβ production.

FMRP binding may repress translation by impeding the accessibility of the translation initiation sites. At 3′-UTRs, FMRP binding to G4s can also promote translational repression through less clear mechanisms that may be mediated by microRNAs in some cases. It has been shown that FMRP binds to two sequential parallel 3′-UTR G4 structures in *Shank1* mRNA and presumably contributes to its translational repression (95). FMRP has been implicated in G4-microRNA interplay through an observation that FMRP can negatively regulate the translation of postsynaptic density protein 95 (*PSD-95*) via a microRNA-dependent mechanism involving the 3′ UTR G4 motifs found in *PSD-95*. The guanine-rich region of the 3′UTR *PSD-95* mRNA is capable of folding into two alternate parallel G4 conformations that exist in equilibrium with each other, with the dominant G4 conformation exposing the complementary nucleotide seed sequence of miR-125a. The two G4 structures, connected by a linker region, bind to miR-125a and form a stable complex within the 3′UTR *PSD-95* mRNA (98). Subsequently, phosphorylated FMRP and miR-125a operate in conjunction to repress *PSD-95* translation (99).

Interestingly, fused in sarcoma (FUS), a protein linked to ALS/FTD that plays a role in synaptic function regulation and local translation, has been shown to bind with high specificity to parallel G4s formed in the mRNA of *PSD-95* or *Shank1* (100). The nanomolar dissociation constants for complexes containing FUS and *PSD-95* or *Shank1* G4s are comparable to those of complexes containing FMRP, suggesting that FUS can potentially compete with FMRP binding to the G4s in these mRNAs and thereby influence their translation.

G4s have been shown to regulate the translation of APP, the key protein in AD; a G4 motif in the 3′-UTR of APP mRNA has been found to negatively regulate APP translation (Figure 4) (101). FMRP has also been linked to the regulation of APP translation, with FMRP shown to repress *APP* translation by recruiting *APP* mRNA to processing bodies, where non-translating mRNAs are stored or degraded (102). FMRP was also observed to bind to the coding region of *APP* mRNA at a guanine, G-quartet-like sequence and regulate the translation of APP in a manner dependent on the activation of a subtype of glutamate receptor (103). Heterogeneous nuclear ribonucleoprotein C (hnRNP C) has been found to compete with FMRP for binding at the coding region, with hnRNP C binding promoting *APP* translation by displacing FMRP and alleviating its translational blockade (102). Therefore, FMRP and hnRNP C appear to work in concert to regulate *APP* translation through a mechanism that may suggest a role for G4s in the process.

RNA G-QUADRUPLEXES AND SPLICING

RNA G-quadruplexes regulates alternative splicing as cis elements

G4s found in the exonic regions of *FMR1* and *BACE1* mRNAs (which are implicated in FXS and AD, respectively) have been shown to regulate alternative splicing by acting as a molecular switch, controlling the distribution of the various isoforms produced.

Interestingly, it has been demonstrated that a selective mRNA target of FMRP is *FMR1* itself. FMRP has been shown to bind to a specific and high-affinity site located in

the nucleotide sequence of the 3′ terminal coding region of *FMR1* mRNA that is responsible for encoding the RGG domain of FMRP. It was initially thought that through binding to the coding region site, FMRP could repress its own translation through a negative-feedback loop (104). However, a follow-up study has shown that the coding region site within *FMR1* mRNA, which contains two alternative G4 structures, is a potent exonic splicing enhancer (105). The exonic splicing enhancer activity of the coding region site was nullified when G4 formation was inhibited, indicating that the splicing activity of the coding region site is dependent on G4 formation. FMRP binding to the coding region site has been shown to control the relative amounts of short and long FMRP isoforms produced by alternative splicing of exon 15. Importantly, G4 structures are thought to mediate the binding between FMRP and the coding region site, since removal of both G4s reduces the FMRP binding to nonspecific levels (105). It has been suggested that increased expression of the full-length FMRP isoform alters *FMR1* splicing events around the coding region site to favor short-isoform production. Hence, the presence of G4s at the coding region site can potentially allow for an additional layer of FMRP self-regulation through splicing, mediating its expression through a negative autoregulatory loop.

BACE1 encodes a transmembrane protease that is responsible for the production of the first cleavage product in the conversion of APP to Aβ. Alternative splicing of *BACE1* yields six shorter isoforms, but only the full-length transcript, known as isoform 501, is involved in APP proteolysis. It has been shown that *BACE1* mRNA harbors a G-rich sequence on its third exon that has G4-forming potential (106). This G-rich sequence has been reported to recruit splicing regulator heterogeneous nuclear ribonucleoprotein H (hnRNP H) to *BACE1* mRNA, thereby facilitating the alternative splicing of *BACE1* mRNA isoforms. The G-rich sequence-dependent hnRNP H recruitment selectively activates the production of isoform 501, while simultaneously inhibiting generation of the shorter isoforms (Figure 4). Accordingly, deletion of the G-rich sequence has been found to silence the production of isoform 501, whereas knockdown of hnRNP H results in reduced isoform 501 translation, repressed APP proteolysis, and a consequent decrease in Aβ (106).

RNA G-quadruplexes sequester splicing factors

C9orf72 HRE G4s have been shown to sequester proteins involved in splicing regulation, leading to dysregulated splicing and RNA toxicity via a trans-acting mechanism (Figure 3). *In vitro* cellular studies have revealed that *C9orf72* RNA G4s bind to the splicing regulator ASF/SF2, inducing RNA toxicity through protein-binding interactions (36). Similarly, G4 formation is responsible for high-affinity associations between *C9orf72* HRE RNA and the essential splicing factor hnRNP H *in vitro* (107). Dysregulated splicing of several hnRNP H target transcripts, as a result of the sequestration of the protein by G4s formed from the *C9orf72* HRE, contributes to neurodegeneration in *C9orf72* HRE-carrying patients' brains (107). Other splicing factors, such as hnRNP A3, have been found to associate with RNAs containing hexanucleotide G4C2 repeats (108), potentially contributing to the splicing dysregulations in the patients.

G-QUADRUPLEX-DEPENDENT RNA-PROTEIN INTERACTIONS

As mentioned in the discussion of splicing dysregulation above, RNA G4s have been shown to recruit and sequester cellular proteins from their normal functions, leading to pathological consequences that can include neurodegeneration. In fact, RNA G4s formed from the *C9orf72* HRE repeat have been shown to interact with a wide range of different types of proteins and influence multiple cellular processes, including nucleolar stress, paraspeckle formation, phase separation, and stress granule formation. Here we highlight several cellular events that are pathologically relevant, with the understanding that the pathways mediated by G4 RNA-protein interactions in neurological disease are likely much more diverse than what we discuss here.

RNA G-quadruplexes mediate the formation of membraneless RNP structures

The RNA- and protein-containing membraneless organelles referred to as RNP granules are maintained through protein-protein, protein-RNA, and RNA-RNA interactions (109). These membraneless organelles, which include both nuclear and cytoplasmic bodies such as stress granules, nucleoli, Cajal bodies, P-bodies, and paraspeckles (110), are formed by phase separation of components, allowing for the condensation of proteins into subcellular membraneless compartments (111). Phase separation, which has been reviewed extensively (112,113), has been used to conceptualize the formation of membraneless organelles that behave as liquid droplets (111).

The *C9orf72* HRE RNA forms foci in patients' brains that are potentially pathogenic (32). The neurotoxic consequences of the sequestration of proteins in HRE RNA foci are seen in the mislocalization of nucleolin, an essential nuclear protein that is critical for the function of the nucleolus. Nucleolin is known to preferentially bind to *C9orf72* HRE RNA in a G4-dependent manner (37). The *C9orf72* HRE RNA colocalizes with nucleolin in the nucleoli of patients' brains to impair nucleolar function and induce nucleolar stress, linking abnormal *C9orf72* HRE nucleic acid structures to ALS pathology induced by nucleolar stress (Figure 3) (37). *C9orf72* RNA G4s may have promise as a therapeutic target given that small molecules stabilizing C9orf72 RNA G4s have been shown to reduce levels of RNA foci and dipeptide repeat proteins in *Drosophila* and neuronal models (114).

Paraspeckles, which are nuclear ribonuclear bodies assembled on the long non-coding RNA (lncRNA) NEAT1, have been linked to ALS when paraspeckle formation is observed in the early stages of ALS pathology, and the ALS-linked proteins TDP-43 and FUS, among others, are found to be enriched in paraspeckles (115,116). A potential role for paraspeckles in *C9orf72* pathology was recognized when RNA foci from the *C9orf72* HRE were shown to possess paraspeckle-like characteristics. Like paraspeckles, *C9orf72*

RNA foci co-localize with the paraspeckle proteins SFPQ, NONO, RBM14, hnRNP H and FUS (117). Interestingly, it has been observed that *C9orf72* RNA foci can also form paraspeckle-like bodies in a NEAT1-independent manner, suggesting that the *C9orf72* RNA HRE can act as a scaffold for paraspeckle-like structures (117). Notably, the assembly of endogenous paraspeckles may be driven by the G4 structures on its scaffold, lncRNA NEAT1. An abundance of G4 motifs has been observed on NEAT1, and paraspeckle proteins such as NONO have been found to bind to NEAT1 *in vitro* and *in vivo* through these G4 motifs (118). Furthermore, the enrichment of G4 motifs is conserved among NEAT1 homologs despite their low sequence homology, highlighting the potential role for G4s as a structural element that recruits NONO and contributes to the seeding of paraspeckle assembly. *C9orf72* HRE RNAs compete with NEAT1 for the binding of paraspeckle proteins such as NONO in a G4-dependent manner (118), suggesting that the HRE RNAs can potentially cause disruption of paraspeckle function as part of the pathogenic cascades in the disease (Figure 3).

Stress granules are cytoplasmic foci composed of proteins and RNA, including translationally stalled mRNAs, that are formed under stressful conditions and have been proposed to lead to pathological protein aggregates in neurodegenerative diseases that include AD, ALS, and FTD (119). The *C9orf72* HRE RNA has been shown to affect stress granule dynamics. A study determining the effect of the HRE RNA on phase separation, a process considered important for RNA granule formation because it enables the compartmentalization of proteins, has found that the *C9orf72* HRE RNA can enhance the formation of both stress granules and nuclear foci, as well as promote the condensation of RNA granule proteins and phase separations *in vitro* (120). *C9orf72* RNA HRE-mediated condensation is reported to respond to ionic conditions, which can directly contribute to G4 stabilization. Indeed, G4s formed from the *C9orf72* HRE RNA have been shown to promote phase transitions *in vitro* and in cells. It has therefore been proposed that *C9orf72* G4s can phase-separate *in vitro* through the condensation of RNA granule components, leading to stress granule formation (Figure 3) (120).

RNA G-quadruplexes and nucleocytoplasmic transport

The trafficking of proteins and RNAs across the nuclear envelope through the nuclear pores is a major transport system in the cell, and pathologies of nucleocytoplasmic transport have been increasingly observed in neurodegenerative diseases. *C9orf72* HRE RNA G4s have been linked to disrupted nucleocytoplasmic transport through the observation that RanGAP, a regulator of nucleocytoplasmic transport, influences HRE-mediated neurodegeneration in experimental models (Figure 3) (121). RanGAP exhibits a higher binding affinity for HRE sense-strand G4s than for hairpin structures *in vitro*, suggesting that RanGAP may operate by preferentially binding the sense RNA G4 formed from the *C9orf72* HRE. It has been reported that RanGAP function is impaired in *C9orf72* ALS iPS neurons and, correspondingly, that *C9orf72* HREs decrease nuclear import (121). Treatment of TMPyP4, a compound that destabilizes RNA G4s, decreases the affinity of RanGAP for the G4s and rescues the nuclear import defects, suggesting that unfolding the G4s can suppress nuclear transport deficits caused by the *C9orf72* HRE.

Recognition of G-quadruplexes by TDP-43

TAR DNA-binding protein 43 (TDP-43) is a DNA/RNA-binding protein that is responsible for long-distance mRNA transport and the regulation of local protein synthesis related to conserving neural cell polarity and synaptic plasticity (122). The TDP-43 proteinopathy, the deposition of TDP-43 protein in ubiquitinated and hyperphosphorylated aggregates, is present in the majority of ALS cases and nearly 50% of all FTD cases (123). Both the loss of TDP-43's RNA processing functions and the gain-of-toxicity from the TDP-43 proteinopathy have been proposed to underlie the pathogenesis of the relevant diseases.

When RNA motifs for TDP-43 binding have been explored through *in vitro* systematic evolution of ligands by exponential enrichment (SELEX) screening, all the top RNA targets of TDP-43 have been found to harbor G4 motifs (124). TDP-43 has been confirmed to bind to natural DNAs and RNAs in a parallel G4-specific manner, and its targets include PSD-95 and CaMKIIα mRNAs, which are transported on the basis of the recognition of their 3′UTR G4 motifs (96,124), suggesting that TDP-43 binds to and transports these G4-containing mRNAs into neurites for local translation. TDP-43 has also been found to bind to the *C9orf72* HRE RNA *in vitro*, and pre-incubation of TDP-43 with G4C2 repeat RNAs decreases TDP-43's binding affinity for G4-containing PSD-95 and CamKIIa mRNAs, suggesting that sequestration of TDP-43 by the *C9orf72* HRE RNAs may impair TDP-43's RNA transport function (124).

Interestingly, mutant ALS-associated TDP-43^{M337V} exhibits a lower binding affinity for G4-containing mRNAs, indicating that Met337 within the C-terminal Gly-rich domain of TDP-43 is critical for G4 binding (124). A follow-up study from the same group has expanded upon these findings by showing that the Gly-rich region of TDP-43 is the region that is responsible for recognition and binding of specific G4-containing mRNAs (125). The implications of these observations for ALS-FTD pathology are potentially significant, given that as much as 30% of neuronal mRNAs harbor 3′-UTR G4 motifs (126).

G-quadruplexes are integral to the tRNA-mediated stress response

G4 structures on tRNAs may also play a regulatory role in stress granule formation and translation. A subset of ALS in patients is linked to missense mutations in angiogenin, a ribonuclease with the ability to cleave both rRNAs and tRNAs. In response to stress stimuli, angiogenin cleaves mature tRNAs into 5′ and 3′ tRNA-derived stress-induced RNAs (tiRNAs) in order to suppress unwanted translation (127,128). ALS-linked mutant angiogenin has been found to exhibit limited catalytic activity and to fail to induce tRNA cleavage (129). Under conditions of stress, certain tiRNA fragments, i.e. 5′-tiRNA^{Ala} and 5′-tiRNA^{Cys},

inhibit protein synthesis by displacing cap-binding eIF4F complexes from capped mRNA, leading to the formation of stress granules and inhibition of translation (127). The ability of these tiRNA fragments to perform these functions depends on a 5′ terminal oligoguanine (5′TOG) motif, a stretch of guanine residues located at the 5′ end, and a secondary structure characterized by the presence of a stem–loop sandwiched between the 5′ and 3′ regions (127,130). The 5′TOG motif found in 5′-tiRNA^{Ala} has been shown to be capable of forming a highly symmetric, parallel tetramolecular RNA G4 with five tetrad layers (131). The formation of G4s within the 5′TOG motif is correlated with the association of 5′-tiRNA^{Ala} and 5′-tiRNA^{Cys} with the translational repressor Y-box binding protein 1 (128); furthermore, the removal of G4 from the 5′TOG motif prevents stress granule formation (131), with both findings indicating a requisite role for G4s in the tRNA-mediated stress response that results in stress granule formation and translational inhibition.

Concerning the mechanism governing the tiRNA G4-triggered stress granule formation, it has been shown that eIF2α phosphorylation, the canonical trigger of stress granule formation, is not involved in the tiRNA-induced stress response. Rather, G4s formed from the 5′TOG motif of 5′-tiRNA^{Ala} directly interact with eIF4G, inhibiting the assembly of the eIF4F translation initiation complex and in turn stimulating stress granule formation (132). A larger pool of tiRNAs of varying fragment sizes with the propensity to form G4s has been shown to contain the 5′TOG motif, indicating that the number of potential tiRNAs capable of inhibiting translation is larger than initially suspected (133).

A potential role for G-quadruplexes in oxidative stress

G4s formed by G4C2 DNAs and RNAs from the *C9orf72* repeat region have been reported to bind to heme, an iron-containing compound of the porphyrin class, and form tight complexes under physiologically relevant conditions (134). This heme-quadruplex complex containing G4C2 repeat nucleotides exhibits both peroxidase and oxidase activity in a quadruplex-dependent manner (37,134). These *in vitro* observations have raised the possibility that the *C9orf72* HRE-induced heme-quadruplex complexes may catalyze aberrant oxidative reactions or interfere with iron homeostasis or mitochondrial function, in which heme plays an important role, leading to cell damages responsible for neurodegeneration. Recently, physiological evidence for the binding of G4 and heme has been gleaned outside of a *C9orf72*-specific context, in experiments that indirectly detected the release of heme from G4s by analyzing how treatment with a G4 ligand (predicted to displace sequestered heme) affected the expression of genes involved in heme catabolism and iron homeostasis. These experiments showed that PhenDC3, a G4 ligand, displaces G4-bound heme *in vitro* and causes heme oxidase 1 induction in human cells, supporting the concept that G4s sequester heme in the cell (135). Such heme-quadruplex complexes may have physiological functions in living cells that need to be further explored.

G-QUADRUPLEXES IN OTHER NEUROLOGICAL DISEASES

Prion diseases

Prion proteins are thought to be responsible for transmissible spongiform encephalopathies (TSEs), or prion diseases, which are characterized by the deleterious transition from the normal, soluble, alpha-helix-rich cellular form of prion protein (PrP^{C}) to the insoluble, beta-sheet-rich prion proteins susceptible to aggregation (PrP^{Sc}). In addition to the abovementioned G4 motifs at the promoter of the PrP gene, which may mediate autoregulation at the transcriptional level (41), there are putative G4 motifs in the PrP mRNA as well. In the human PrP protein, the octa-repeat domain, which is found in the N-terminal domain of PrP and is crucial for aggregation, consists of five repeats of an octapeptide sequence. The underlying mRNA sequence harbors five putative G4 motifs. The secondary structure of this RNA segment is dynamic and assumes various conformations, including hairpins and G4s. It was initially determined, when short sequences (24 nt) of the PrP mRNA segment were tested, that G4s could be formed, and that these structures may interfere with translation (136). PrP^{c} binding to the G4s in PrP mRNA has been suggested to regulate the co-translational folding of the PrP protein and thus potentially lead to spontaneous conversion of PrP^{Sc} (136). However, a later study has demonstrated that the full sequence containing the five G4 motifs in the PrP RNA segment exhibits preferential double-stranded A-helical hairpin structures instead of G4s, suggesting that the sequence context may influence the confirmation or stability of the secondary structures (137). Therefore, future studies are needed to ascertain the pathological consequences of the structures and functions of PrP mRNA *in vivo*.

Progressive myoclonus epilepsy

The most common cause of progressive myoclonus epilepsy type I, also known as Unverricht-Lundborg disease, is a d(C4GC4GCG)-d(CGCG4CG4) dodecamer repeat expansion at the cystatin B (CSTB) promoter on chromosome 21q22.3 (138). The dodecamer repeat mutation leads to significantly lower CSTB mRNA levels in patients, which in turn leads to a loss-of-function of CSTB as a cysteine protease inhibitor. Emerging evidence points to a possible role for G4 secondary structures in relation to the functions of the dodecamer repeat. The G-rich bottom strand of the promoter region has been shown to form stable secondary structures featuring parallel G4s under physiologically relevant conditions *in vitro* (138). Although a clear link has been established between secondary structures and the CSTB promoter, further study is needed to elucidate the direct effects and regulatory mechanism of G4s on the functions of the dodecamer repeat in the disease.

CONCLUSION

Research on G4s as they relate to biology and neurodegeneration remains one of the most fascinating fields as well as one of the most complex, given the concurrent prevalence of G4 involvement in neurological diseases and the

existing uncertainties regarding *in vivo* functions and biological mechanisms. G4s can act as a regulatory structural element in neurological diseases, initiating neuroprotective and neurotoxic cascades by influencing a plethora of cellular processes. Given their unique structural properties, G4s can serve as effective targets for therapeutic interventions employing aptamers, antisense oligonucleotides, or small molecules. For these approaches to be therapeutically viable, however, they must achieve a great degree of specificity in recognizing unique genomic G4s that possess high variability in their sequence, orientation, and stability. Future studies are needed to uncover the dynamics of G4s and the genetic players involved in neurodegeneration. These approaches may reveal previously unknown culprits in the intricate etiology of neurodegenerative disorders, while supporting the development of novel therapeutic strategies.

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