Structure and Functions of RNA G-quadruplexes

Prakash Kharel and Pavel Ivanov

Contents

Abstract G-quadruplexes (G4s) are four-stranded nucleic acid secondary structures that are formed by the stacking of square planar guanine arrangements and stabilized by favorable cations. Potential G4-forming sequences are distributed in the regulatory regions of the genome and transcriptome. G4s are proposed to modulate various physiological and pathophysiological cellular processes. As such RNA G4s (rG4s) have been implicated in several key processes of gene regulation such as RNA maturation, mRNA translation, and RNA transport. rG4s often impact cellular biology by associating different RNA binding proteins, both of which could act as crucial therapeutic targets in the fight for developing novel therapeutics for the diseases associated with rG4-containing transcripts.

P. Kharel · P. Ivanov (✉)
Division of Rheumatology, Inflammation, and Immunity, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA

P. Kharel
e-mail: pkharel@bwh.harvard.edu

J. Barciszewski (ed.), *RNA Structure and Function*, RNA Technologies 14,
https://doi.org/10.1007/978-3-031-36390-0_9

Keywords G-quadruplex · RNA metabolism · Translation · G-quadruplex targeting

1 Introduction

Guanine (G)-rich nucleic acid sequences can fold into four-stranded secondary structures called G-quadruplexes (G4s) via the stacking of two or more square planar G arrangements known as G-quartets (Sen and Gilbert 1988). It was first noted in 1910 that high concentrations of impure guanylic acid formed a gel in an aqueous solution (Bang 1910). 50 years later, Khorana and co-workers found similar highly ordered aggregation with the first synthesized deoxyguanosine oligonucleotides. These earlier observations were structurally rationalized using X-ray diffraction studies that could be explained by a hydrogen-bonding arrangements of four G bases, thus proposing the G-quartet assembly (Gellert et al. 1962). More than a century after the initial discovery of Bang, the G4 field has moved quite a remarkable distance.

G-quartets (or G-tetrads), structural units of G4s, are formed when guanines are organized into square planar arrangements where each G base is connected to two other bases. The G-quartet involves two edges of each of the four G bases with Watson–Crick and Hoogsteen base pairings (Fig. 1a) (Gellert et al. 1962). Hydrogen bonds (H-bond) between each G pair involve four donor/acceptor atoms, the N1, N7, N2, and O6 atoms, such that a G-quartet has eight total hydrogen bonds (four N2−H…N7 and four N1−H…O6 bonds). Importantly, four carbonyl oxygen (O6) atoms form a negatively charged core in the center of the G-quartet (Fig. 1b). Under the favorable condition, two or more G-quartets stack onto one another to form a right-handed helical G4 structure (Fig. 1c). The central anionic core of a G-quartet or the central space between two quartets provides a perfect space for the binding of a cation, which in turn provides key stability to the quartets and G4s (Bhattacharyya et al. 2016). Because of the defined geometry and size of the central channel, only cations with an adequate charge, size, and dehydration energy can coordinate a G4. Of particular biological importance, Na^+, K^+, and NH_4^+ cations are most physiologically relevant and G4-stabilizing (Fig. 1c). Cations like K^+ and NH_4^+ are too large to fit into the plane of G-quartet, but readily accommodate into the space between two G-quartets and coordinate with eight O atoms. On the other hand, smaller Na^+ is embedded into the middle of a single G-quartet and coordinates only four O atoms, thus contributing less to G4 stability. In contrast, cations with very small ionic radii such as Li^+ do not favor G4 formation.

Generally speaking, potential intramolecular G4-assembling sequences can be formed by repetition of a G-tract sequence motif within a single run of sequence. In such repetitive motif $G_mX_nG_mX_oG_mX_pG_m$, m is the number of G bases in every short G-track, which are connected by intervening X_n, X_o, X_p sequences with any combination of bases including Gs (Puig Lombardi and Londoño-Vallejo 2020). Despite having a lot of similarities in their basic building units, in fact, G4s are a diverse family of nucleic acid structures that can fold into various topologies (Lightfoot et al. 2019;

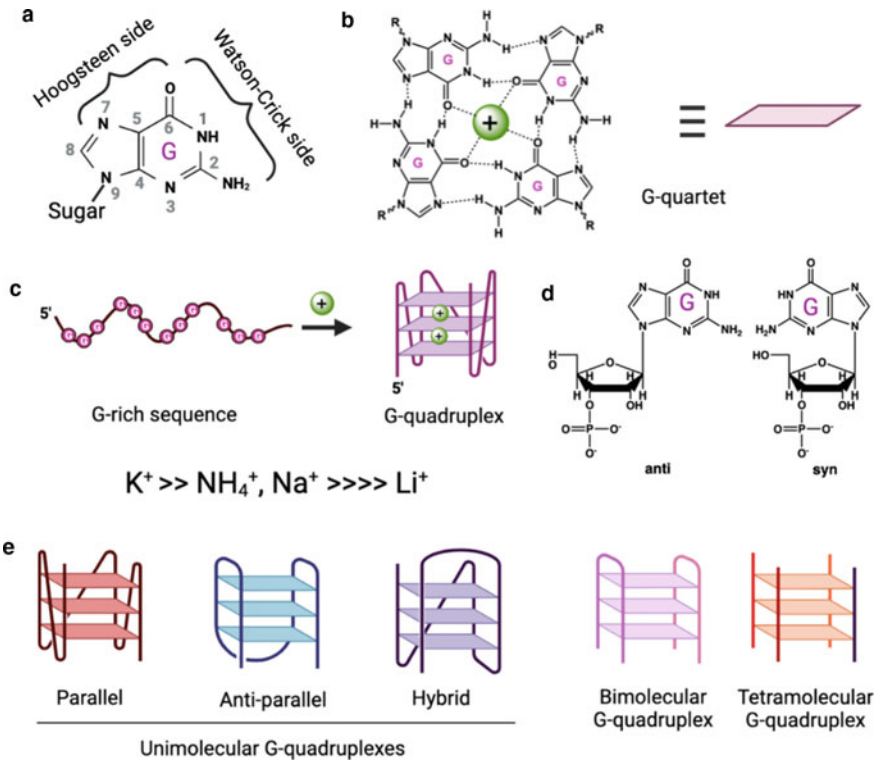

Fig. 1 Watson–Crick and Hoogsteen base pairing interaction sites in a guanine base. **b**. A square planar G-quartet arrangement stabilized by a centrally located cation. H-bonding between each pair of guanines involves four donor/acceptor atoms (the N1, N7, N2, and O6 atoms) resulting in 8 H-bonds per quartet. Four carbonyl oxygen (O6) atoms form a negatively charged core in the center of the G-quartet that favors the binding of monovalent cations (⊕). **c**. A G-rich sequence with at least 4 G-stretches with at least 2 Gs each can fold into G4 under favorable conditions (top); and Preferential binding of mostly used monovalent cations to G4s (bottom). **d**. syn and anti-conformation of glycosidic bond in Gs. In RNA, this remains almost exclusively in anti-conformation resulting in all parallel rG4 topology. **e**. G4s with different topologies and molecularities

Winnerdy and Phan 2020). In bona fide G4s, the G4 topologies are dictated by the pattern of strand polarities and the orientation of interconnecting loops. The G4s can have parallel (all backbones running in the same direction), anti-parallel (adjacent backbones run in the opposite direction), or mixed topologies (Fig. 1e). While the different topologies bring structural diversity, their influence on G4 formation and contributions to cellular functions is largely unknown. Another aspect of G4 structural diversity arises from the difference in the number of G-quartet stacks and the number of molecules involved. Based on the number of G-quartets, G4s can be 2-tier, 3-tier, 4-tier, and so on. Depending upon the number of nucleic acid molecules involved, besides unimolecular (intramolecular), G4s can also be bimolecular, or

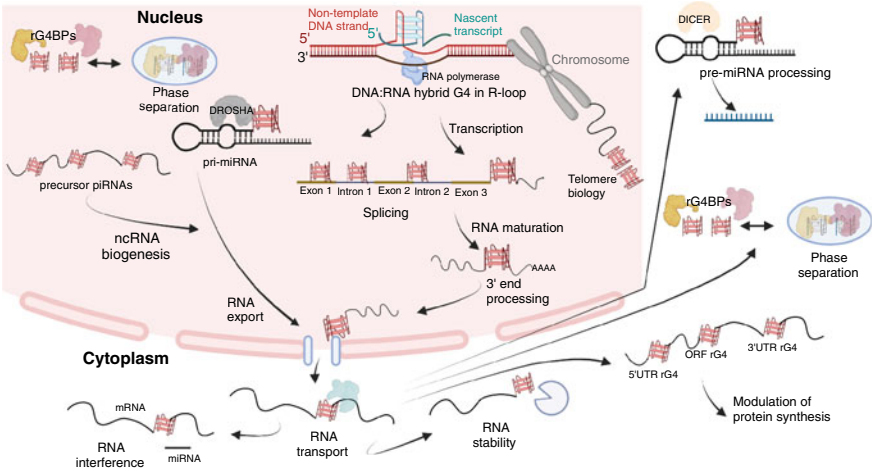

Fig. 2 Role of RNA G-Quadruplexes (rG4s) in the nucleus and the cytoplasm. rG4s have implications in almost every step of RNA life that ranges from the regulation of transcription, splicing, and 3′ end maturation in the nucleus to RNA transport, and the regulation of mRNA translation, ncRNA maturation, and RNA interference in the cytoplasm. Additionally, rG4s contribute to phase separation and/or aggregate formation in both the nucleus and cytoplasm

tetramolecular (Fig. 1e). Moreover, the nature of the flanking sequence can have a direct impact on the function of an rG4 (Zheng et al. 2022).

2 DNA G4s (dG4s) Versus RNA G4s (rG4s)

Both dG4s and rG4s look very similar at first glance. However, an assumption that rG4s are DNA counterparts is oversimplified. One of the key differences between dG4 and rG4 comes from the presence of a 2′-hydroxyl group (2′−OH) in the ribose sugar (Zaccaria and Fonseca Guerra 2018; Zhang et al. 2010). Not only, 2′−OH allows more intramolecular interactions within RNA G4s but also, they are favored to bring water molecules, making rG4s often more stable compared to their DNA counterparts. Additionally, the steric constraints posed by 2′-OH strongly favor the *anti*-conformation (via restrains on the glycosidic torsion angle), and imposition of additional constraints on sugar puckering (the ribose having a preference for C3′-*endo* puckering) (Fig. 1d). Consequently, the rG4 topology is almost always parallel where all four strands are oriented in the same direction (Fig. 1e). In contrast, dG4s are polymorphic and can adapt parallel, antiparallel or mixed conformations (Fig. 1e). rG4s also differ from dG4s in their cation interaction specificity. In a study based on a pair of G4 oligos, it was shown that while K^+ dramatically stabilizes both dG4s and rG4s, Na^+ only had a strong effect on dG4s. For divalent cations, only Sr^{2+} increases the stability of the rG4s. On the other hand, biologically relevant divalent cation Mg^{2+}

actually can destabilize rG4s (Balaratnam and Basu 2015). These unique features make rG4s more compact, less hydrated, and often more thermodynamically stable than dG4s. Furthermore, their presence in the cellular context makes the folding possibility of rG4s very different than that of dG4s; while the cellular DNA is almost always in a double-stranded form, cellular RNA is mostly in a single-stranded form.

3 Functions of rG4s in the Nucleus

3.1 Transcriptional Regulation

Putative G4s are commonly found in the genomic DNA, thus making it possible that corresponding rG4s are also formed upon transcription. In turn, the nascent RNA can base pair with the complimentary template DNA strand to form an RNA:DNA hybrid, which together with the displaced DNA strand, forms R-loop (Belotserkovskii et al. 2018). Bioinformatic analysis identified that such hybrid putative G4s (pG4s) are enriched downstream of the transcription start sites and are found in >97% of human genes, with an average of 73 hybrid pG4s per gene (Xiao et al. 2013). Indeed, the formation of R-loop G4s was confirmed using T7 RNA polymerase in vitro transcription. Such assay suggested that R-loop G4s inhibit transcription in vitro and represent *cis*-elements that are built into a gene and can be activated co-transcriptionally. The nascent RNA and non-template DNA strand of mitochondrial *CSBII* can co-transcriptionally form a stable DNA–RNA hybrid G4, which was suggested to promote transcription termination (Zheng et al. 2014). Furthermore, hybrid G4s formed by nascent transcript with DNA are shown to be dominating in number and thermodynamically more stable, which can help populate G4s in expense of duplex DNA (Shrestha et al. 2014). Furthermore, post-transcriptionally formed switch from rG4 to R-loop have been suggested to promote the class switch recombination (CSR) in the mouse immunoglobulin heavy chain (IHC) locus (Almeida et al. 2018). In mouse IHC, RNA helicase DDX1 directly binds to rG4s present in the intronic switch region, dissolving the structure thereby leading to a structural switch from rG4 to an R-loop form. R-loop formation results in a non-template single-strand DNA that could be a substrate for activation-induced cytidine deaminase (AID), the enzyme that initiates CSR by converting cytidines to uracils. Additionally, DNA:RNA hybrid G4s could contribute to transcription termination as potential G4s are proposed to act as terminator sequences that can stall RNA Polymerase II transcription. For example, R-loops formed behind elongating polymerase II are prevalent over G-rich sites located downstream of poly(A) signals, and are capable of G4 formation (Skourti-Stathaki et al. 2011). The DNA damage response protein Senataxin (SETX) is a DNA/RNA helicase which plays a key role in the resolving of R-loops thereby allowing $5' \rightarrow 3'$ exonuclease Xrn2 access to the $3'$

cleavage poly(A) sites causing nascent RNA release, 3′ cleavage product degradation and RNA polymerase II termination (Skourti-Stathaki et al. 2011). The depletion of SETX causes such pause-mediated transcription termination. RNA:DNA hybrid G4s can also contribute to transcription termination via coupling with 3′-end polyadenylation in association with heterogeneous nuclear ribonucleoprotein factors (HNRNP H/F) (Decorsière et al. 2011). For example, in tumor protein 53 (*TP53)* mRNA, rG4s interact with the splicing/polyadenylation factor HNRNP H/F to regulate polyadenylation. Under normal circumstances, mRNAs lacking rG4s at poly(A) signals are efficiently processed, whereas efficient 3′-end processing of *TP53* mRNA is inhibited presumably because of the rG4, resulting in the reduced gene expression. However, under genotoxic stress, there is global repression of mRNA 3′-end processing resulting in decreased mRNA maturation. In contrast, 3′-end processing of *TP53* mRNA is up-regulated to increase the expression of TP53. This anomalous mechanism is possible due to the recognition of rG4 in the *TP53* pre-mRNA by HNRNP H/F causing efficient recruitment 3'-end processing factors, which ultimately leads to an increased p53 expression. Another study showed that a G4 helicase DHX36 binds to TP53 rG4 under genotoxic condition and resolves the rG4 once the stress is removed thereby making TP53 mRNA available for immediate expression (Newman et al. 2017).

3.2 mRNA Maturation

Furthermore, rG4s also contribute to pre-mRNA splicing. Genome-wide analysis of alternatively spliced transcripts found over 3 million rG4 capable sites mapped to approximately 30,000 mammalian genes (Kikin et al. 2008). Alternative splicing is regulated by the synergic action of many RBPs with RNA elements that impact spliceosome assembly at neighboring splice sites (Wang et al. 2015), therefore rG4s assembled in the vicinity of splice sites may directly impact the binding of regulatory RBPs. For example, two rG4s are found within the FMRP-binding site (FBS) on its pre-mRNA (*FMR1*), which give rise to different FMRP isoforms (Didiot et al. 2008). As observed in a minigene system, the *FMR1* FBS can be a potent exonic splicing enhancer and acts as a control element that regulates alternative splicing in response to intracellular levels of FMRP isoforms. The binding of the longer FMRP isoform to FBS results in decreased synthesis of the longer FMRP isoforms (carrying a complete exon 15) concomitant with an increase of shorter isoforms. rG4 abrogating mutations in the FBS resulted in decreased FMRP binding, ablate exonic splicing enhancer activity and change the splicing pattern of *FMR1* pre-mRNA (Didiot et al. 2008). On the other hand, rG4s in intron 6 of the human telomerase reverse transcriptase (hTERT), the rate-limiting component of telomerase, can serve as an intronic splicing silencer as observed by G4-specific ligand-mediated impairment of hTERT splicing (Gomez et al. 2004). Additionally, an rG4 located in intron 3 of *TP53* pre-mRNA acts as an intronic splicing enhancer as it stimulates the splicing of intron 2 leading to a differential expression of transcripts encoding distinct p53 isoforms (Marcel

et al. 2011). Furthermore, using a reporter system that consists of rG4 WT 3'UTR of *FXR1* mRNA, it has been shown that the presence of rG4 results in a more prominent shorter mRNA isoform while a G4 mutated version produced a prominent longer mRNA isoform, suggesting the role of 3′UTR mRNA rG4s in increasing alternative polyadenylation efficiency (Beaudoin and Perreault 2013).

3.3 Non-coding RNA Maturation in the Nucleus

In addition to mRNA maturation, rG4 structures can modulate the nuclear biology of noncoding RNAs, including both long non-coding RNA (lncRNAs) and short non-coding RNAs. There are relatively fewer studies in the role of rG4s in lncRNA. In the nucleus, nascent NEAT1 lncRNA binds to the non-POU domain-containing octamer-binding protein (NONO) through rG4 motifs (Simko et al. 2020). NONO plays an essential role in the initial paraspeckle formation stabilizing nascent NEAT1 transcript and providing the foundation necessary for the recruitment of the additional protein components needed for the subsequent steps of NEAT1 assembly and maturation (Clemson et al. 2009).

As such rG4s are also implicated in pre-miRNA maturation. Using computational analyses, two different groups proposed that 13–16% of pre-miRNAs harbor at least one putative rG4 motif in their sequence (Mirihana Arachchilage et al. 2015; Pandey et al. 2015). Based on in vitro data, rG4s in some pre-miRNAs exist in equilibrium with the canonical stem-loop structure such that their folding unwinds the stem-loop, thus hindering Dicer-mediated cleavage of the pre-miRNA and consequently affecting the pre-miRNA maturation process. First, it has been demonstrated that the maturation of the clinically relevant human *pre-miR92b* can be regulated by rG4 formation (Mirihana Arachchilage et al. 2015). Since the Dicer enzyme is stem-loop structure specific, disruption of the stem-loop because of the ion-dependent rG4 formation was found to inhibit Dicer-mediated maturation of *pre-miR-92b*, leading to reduction of mature *miR-92b* and de-repression of its targets. Similarly, it was found that rG4s in pre-miRNAs govern the biogenesis of mature miRNAs through a 'structural interference' mechanism (Pandey et al., 2015). A two-tier rG4 within *pre-let7e* interferes with Dicer-mediated processing, thus leading to a reduction of mature *miR-let7e* levels (Pandey et al. 2015). Furthermore, it has been proved that the formation of an rG4 structure in *pre-miR149* inhibits Dicer processing in vitro and this can be stabilized by the C8 acridine orange derivative and is used as a supramolecular carrier for the cancer-selective delivery of the ligand, considering the ability of such rG4 to bind to nucleolin (NCL) protein overexpressed on the surface of prostate cancer cells (Kwok et al. 2016). Interestingly, several pre-miRNA rG4s, such as *pre-miR-1229*, and *miR-1229-3p*, have been implicated in Alzheimer's disease, and *pre-miR-26a-1* rG4 has been linked to obesity regulation (Imperatore et al. 2020). Similarly, rG4s are implicated in Moloney leukemia virus 1 like (MOV10L1) mediated piRNA biogenesis (Zhang et al. 2019a).

4 RNA Transport

Subcellular RNA transport is a crucial post-transcriptional process that is key to spatiotemporal control of gene expression. RNA export from the nucleus to the cytoplasm is a ubiquitous phenomenon that is essential in the transport of a wider class of RNAs including mRNA, rRNA, tRNA, lncRNA, and miRNA. rG4s can play a crucial role in regulating the transport of G4-containing transcripts from the nucleus to the cytoplasm. In addition to nucleo-cytoplasmic export, the cytoplasmic mRNA transport mechanism is especially important in asymmetric cells such as neurons where transcribed mRNAs travel large distances to their sites of translation (Loya et al. 2010). It has been shown that 3′UTRs rG4s of PDS-95 (post-synaptic density protein 95; contains three G4s) and CaMKIIa (Ca2 + /calmodulin-dependent protein kinase II; contains one G4) mRNAs can regulate their dendritic localization (Subramanian et al. 2011). Furthermore, mRNA 3'UTR rG4s were shown to contribute to dendritic mRNA localization in an FMRP dependent manner (Goering et al. 2020).

5 Functions of rG4s in the Cytoplasm

5.1 Translation Regulation

Translation of mRNA to protein codes is one of the most important steps in RNA metabolism, and its regulation is tightly controlled. Secondary structures such as internal ribosome entry site (IRES)-like elements and rG4s in 5′UTR (untranslated regions) can significantly impact the translation efficiency (Georgakopoulos-Soares et al. 2022). Putative rG4s are overrepresented in the 5′UTRs of mRNAs implying important regulatory functions. When present, rG4s in mRNA 5′UTRs mostly inhibit translation (Kumari et al. 2007). However, 5′-UTR rG4s in the context of IRES-like elements, are known to augment the translation (Morris et al. 2010). mRNA 3'UTR rG4s also contribute to translation both negatively and positively (Arora and Suess 2011; Beaudoin and Perreault 2013; Thandapani et al. 2015).

Several cell-based reporter assays showed that rG4s in the mRNA 5'UTRs cause reduction in the efficiency of their translation (Kumari et al. 2007; Morris and Basu 2009). It has been shown that the rG4 density and position relative to the 5′ caps along with their stability contribute to their respective influence in translation (Kumari et al. 2008). Depletion or pharmacological inhibition of eukaryotic initiation factor 4A (eIF4A), a helicase that unwinds RNA secondary structures and facilitates the recruitment of the 43S preinitiation complex, generally reduces the translation efficiency of mRNAs. However, rG4-bearing transcripts are more sensitive to eIF4A depletion indicating that rG4s directly influence recruitment or scanning of preinitiation complexes/ribosome (Bordeleau et al. 2006; Wolfe et al. 2014). Unwinded rG4s in 5′UTRs can promote the formation of 80S ribosomes on alternative, upstream start codons, thus inhibiting the translation of the main open reading frame. rG4s in *FGF2*

(Bonnal et al. 2003), *α-Syn* (Koukouraki and Doxakis 2016), and *VEGF* (Morris et al. 2010) mRNAs are proposed to stimulate translation as a part of an internal ribosome entry site (IRES) or IRES-like elements, potentially by helping recruit the 40S ribosomal subunit (Bhattacharyya et al. 2015). Of note, rG4s in the mRNA open reading frame (ORF) have a much lower abundance than in the UTRs, and when present may act as translational repressors/ roadblocks for the elongating ribosomes (Mirihana Arachchilage et al. 2019). For example, rG4 within the ORF of *APP* mRNA inhibits its translation via association with FMRP, a known translational silencer (Westmark and Malter 2007). However, some rG4s, such as in *MLL1/4* mRNA ORF, can potentially enhance their translation. *MLL1/4* rG4 is recognized by the RGG-containing factor AVEN in a complex with rG4 helicase DHX36 (Thandapani et al. 2015). The binding of DHX36 stimulates *MLL1/4* mRNA translation presumably via its rG4-resolving activity, thus removing structure mediated blockade for elongating ribosomes. rG4s in the 3′ UTR of mRNA are shown to inhibit translation (e.g., *PIM1, APP*) (Arora and Suess 2011; Crenshaw et al. 2015)., however the molecular mechanism of such effects is unclear.

5.2 mRNA Stability

The stability of a given mRNA transcript is determined by the presence of sequence motifs (Koh et al. 2019; Siegel et al. 2021) and structures (Fischer et al. 2020), which can be bound by *trans*-acting RNA-binding proteins to inhibit or enhance mRNA decay. As such rG4s present in mRNA 3'UTRs can contribute to mRNA stability. Although the ubiquitous presence of rG4s in mRNA is clear and their role in the translation regulation has been a matter of several studies, their role in mRNA stability has only being recently being explored. We (Kharel et al. 2023) and others (Yang et al. 2022) have demonstrated that mRNA G4s are stress responsive elements such that rG4 folding is enhanced under different cellular stresses and 3'UTR mRNA rG4 folding contributes to mRNA stability. Additionally, 3'UTR rG4 folding has been shown to interfere with miRNA-mediated gene regulation (Rouleau et al. 2017).

5.3 ncRNA Biology

When present within transcripts, rG4s can directly influence RNA biogenesis and their downstream function in the cytoplasm as well. It has been independently reported by two laboratories that an rG4 present in pre-miRNAs can modulate their DICER-mediated maturation (Mirihana Arachchilage et al. 2015; Pandey et al. 2015). The formation of stable rG4s was reported in the ribosomal RNA as well (Mestre-Fos et al. 2019), although their functional roles in ribosome functions are still unclear. It has been reported that the formation of an rG4 in piRNA-48164 hinders PIWI protein

binding thereby inhibiting the target reporter gene silencing in the cells (Balaratnam et al. 2019).

Furthermore, in response to various stresses cytosolic transfer RNAs are cleaved by ribonucleases in the anticodon loops (Akiyama et al. 2022; Yamasaki et al. 2009). Such stress-induced cleavage of tRNAs in the cytoplasm yields a novel class of small RNAs called tRNA-derived stress-induced tRNA fragments (tiRNAs), which represent tRNA 5′- and 3′-halves (Yamasaki et al. 2009). We have shown that 5′tiRNAs derived from $tRNA^{Ala}$ and $tRNA^{Cys}$ contain 5′G-rich motifs, which can adopt tetramolecular G4 structures that are functionally active and inhibit translation by directly interacting with eIF4G1 under stress (Ivanov et al. 2014; Lyons et al. 2017, 2020). As a consequence of such inhibition, cells undergo translational reprogramming, which aims on stress adaptation and cell survival. Partially, adaptation to stress can be explained by the abilities of G4-assembling tiRNAs to promote formation of stress granules (Emara et al. 2010), RNA granules with pro-survival roles in RNA metabolism (Ivanov et al. 2019).

6 rG4 Binding Proteins

While the dynamics of rG4 vs non-rG4 equilibrium is largely controlled by their ionic environment in vitro, within the cells, proteins potentially could solely (individually or as a part of protein–protein or RNP complexes) dictate or contribute to the cation-assisted G4 folding-unfolding dynamics (reviewed in (Kharel et al. 2020b)). A G4 binding protein can recognize and bind to a G4 in a multistep process involving main binding domains recognizing the G4 structure with the assistance of interactions from neighboring disordered regions (reviewed in (McRae et al. 2017)). In some cases, previously unstructured (intrinsically disordered) regions of rG4BPs become ordered upon canonical RNA binding to stabilize G4-interacting conformations. Several pull-down and cross-linking coupled with immunoprecipitation experiments show that many proteins can specifically bind to G4s in the cells. The analysis of reported rG4 interacting proteins reveals the presence of certain specific domains and motifs, or unstructured regions in the established or predicted binding regions of the rG4BPs (Kharel et al. 2020b). By virtue of their chemical nature and structural features, RRM (RNA-recognition motif) and RGG (Arginine-Glycine-Glycine) motifs within the RNA- binding proteins are mostly reported to be involved in the interaction with rG4s and hence are the most studied. In addition, some RBPs like Heterogeneous nuclear ribonucleoprotein H (HNRNPH1) and CCHC-type zinc finger nucleic acid binding protein (CNBP) bind to the G-rich motifs of RNA and actually prevent rG4 formation (Benhalevy et al. 2017a; Russo et al. 2010). Both CNBP and HNRNPH1 can also recognize, bind and destabilize the folded rG4s (Benhalevy et al. 2017a; Vo et al. 2022). On the other hand, helicase RBPs like DEAH-Box Helicase 36 (DHX36) bind to rG4s and actively resolve the structure (Booy et al. 2012). There are numerous other rG4 binding proteins that are recruited

by rG4s to perform other cellular functions, such as splicing and translation regulation. Some rG4s might act as rG4BP sequestering elements thus preventing these factors from their other cellular functions (Conlon et al. 2016). Additionally, rG4-rG4BP interaction also contributes to RNA transport (discussed before) and RNA–protein condensate formation (discussed later). Some of the major rG4BPs and their known/ proposed functions are summarized in the Table 1 (also reviewed at (Fay et al. 2017b)).

Table 1 Representative list of rG4BPs and their functions

rG4BPs	Function
AFF3, AFF4	Modulate splicing by recognition of the exonic splicing enhancer Melko et al. (2011)
CNBP	Promotes the translation of G-rich mRNAs by preventing rG4 formation Benhalevy et al. (2017)
DHX36	Acts as dG4 and rG4 helicase Chen et al. (2018a); Tippana et al. (2019)
eIF4A	Alters translation efficiency of mRNAs with rG4 and other structural elements in the 5′ UTR (Schmidt et al. 2022; Wolfe et al. 2014)
eIF4G1	Under cellular stress, 5′tiRNA^{Ala} rG4 binds to HEAT1 domain of eIF4G1 thereby inhibiting translation Lyons et al. (2020)
FMR2	Modulates splicing by recognition of the exonic splicing enhancer Bensaid et al. (2009)
FMRP	Binds to rG4s and modulates the activity of microRNA (miRNA)-mediated silencing in the 3′ UTR of a subset of mRNAs through its interaction with RNA helicase Moloney leukemia virus 10 (MOV10) Kenny et al. (2019). Promotes mRNA localization in the neuronal cells Goering et al. (2020)
FUS/TLS	Binds rG4s and results in liquid–liquid phase separation and cellular condensate formation. Notably, ALS-linked mutations result in the dysregulation of liquid–liquid phase separation Ishiguro et al. (2021)
GRSF1	Melts mitochondrial rG4s and enhances degradosome-mediated degradation of G4 RNAs Pietras et al. (2018)
hnRNP A1	Regulates *MST1R* mRNA splicing and translation Cammas et al. (2016)
hnRNP A2	Promotes the translation of FMR1 by preventing G4 from forming; unfolds LTR promoter G4s Khateb et al. (2007)
hnRNP A3	Binds to G4C2 repeats and is a constituent of inclusions in the hippocampus of patients with C9orf72 mutations Mori et al. (2013)
hnRNP H1	Destabilizes rG4s and modulates splicing Vo et al., (2022). Sequestration of hnRNP H1 to G4C2 foci causes alterations in splicing Conlon et al. (2016)
Lin28	Remodels rG4s in its target mRNA and miRNA and affects mRNA stability and miRNA metabolism O'Day et al. (2015)
Nucleolin	Preferentially binds long-looped rG4s Lago et al. (2017). Binds to HCV viral core RNA G4 and suppresses its replication
TRF2	Binds TERRA rG4 and contributes to telomeric integrityMei et al. (2021)
YB1	Binds 5′tiRNA^{Ala} rG4s and contributes to stress granule formation and translation inhibition Lyons et al. (2016)

7 RG4s and Membrane-Less Biomolecular Condensates

Liquid–liquid phase separation (LLPS) is a biophysical phenomenon that contributes to the formation of membrane-less RNA–protein assemblies (or biocondensates) in the cells, such as cytoplasmic nuclear paraspeckles, cytosolic P bodies, and stress granules (Ivanov et al. 2019). Interestingly, some transcripts containing G-rich RNA repeat sequences can seed RNA only foci in vitro or RNA-containing protein complexes in lysates and live cells (Fay et al. 2017a; Ivanov et al. 2014; Yamasaki et al. 2009). rG4s have been heavily linked in the formation of such ribonucleoprotein (RNP) granules or RNA granules which are involved in various cellular processes and linked to several diseases including neurodegeneration and cancers (Wolozin and Ivanov 2019). Biophysical and structural gel-like features of poly(G) RNA assembly at higher concentrations could be the key contributing factor to LLPS and follows condensate formation. Furthermore, rG4s and their sequestered protein partners could assemble or aggregate to form RNP condensates. Several rG4 features qualify them as candidate contributors to LLPS (Asamitsu and Shioda 2021). First, at high concentrations, poly-guanosine can form gel-like structures in aqueous solutions. These largely static gel-like condensates might stimulate LLPS by increasing the local concentration of liquid phases. Second, RG4s formed *in cis* or *in trans* may promote the recruitment of multivalent protein factors leading to promotion of protein condensates that further contribute into LLPS-induced formation of RNA granules. Additionally, the arginine-/glycine-rich domains (RGG) of several rG4 binding proteins are structurally intrinsically disordered which brings conformational flexibility and degenerates specificity in RNA binding. Degeneracy in RNA binding could result in RBP oligomerization or multivalent interactions with other proteins or multiple RNAs/mRNPs at the same time, which is important to build RNP assemblies further promoting LLPS. Importantly, RGG domains mediate protein–protein interactions and can induce liquid–liquid phase separation even in the absence of RNA both in vitro and in live cells (Schuster et al. 2018).

We and others showed the role of rG4s in the formation of RNA granules in the transcripts generated by the GGGGCC hexanucleotide repeats (rG4C2) in the C9orf72 gene which is the most common genetic mutation associated with amyotrophic lateral sclerosis and frontotemporal dementia (C9-ALS/FTD) (Fay et al. 2017a). rG4C2 RG4s can also influence ALS/FTD-linked LLPS by modulating repeat-associated non-AUG (RAN) translation occurring at C9orf72 repeats, which generates toxic arginine-rich dipeptides that in turn can also promote LLPS. Similarly, short root mRNA rG4 was shown to induce a phase separation-like phenomenon in the plant cells (Zhang et al. 2019b). It has also been suggested that 3-tier rG4s are specially more efficient at inducing phase separation than their 2-tier counterparts (Zhang et al. 2019b). Contributions of rG4s into formation of biomolecular condensates is an area of active research currently and a matter of studies by several laboratories.

8 rG4s as Therapeutic Targets

Increasing evidence supporting the idea that rG4s can contribute or even regulate a variety of physiological and pathological processes has encouraged the design and development of rG4-interacting ligands that may act as therapeutic agents. Small molecule ligands have been the most investigated therapeutics and have been primarily used to stabilize the rG4s, enhancing their inherent repressive role in mRNA translation by obstructing ribosomal activity or interfering with translation machinery. G4 ligands often share common structural features such as an aromatic core, which permits stacking interactions with planar G-quartet, and one or more positive moieties that may interact with nucleic acids backbone phosphate groups in grooves and loops. Currently, several labs are working to develop ligands that are rG4-specific. There are numerous generic G4 targeting small molecule ligands that nonspecifically target and mostly stabilize both dG4s and rG4s (e.g., listed at http://www.g4ldb.com database). Nevertheless, a few of them show a low or high specificity toward rG4 (Select rG4 ligands in Fig. 3a) (reviewed in Kharel et al. 2020a; Santos et al. 2021).

It has been shown that the interaction of bisquinolinium ligands such as PhenDC3 with *TRF2* mRNA rG4 results in the suppression of its expression (Halder et al. 2011). The driving mechanism for the binding of bisquinolium ligands towards rG4s was proposed to be π–π stacking with square-planar G-quartets. Similarly, Miglietta et al. identified anthrafurandione derivatives as potential therapeutics that target 5'UTR *KRAS* mRNA rG4s to repress the mRNA translation in pancreatic cancer cells (Miglietta et al. 2017). The binding mechanism seems to involve the π–π stacking interactions of anthrafurandione core with G-quartets, whereas the cationic side chains bind to grooves and loops via electrostatic interactions. Despite of not being reported as a therapeutic, parallel G4 interacting porphyrin molecule, N-methyl mesoporphyrin-IX, brings selectivity towards rG4s and parallel dG4s (Sabharwal et al. 2014). This selectivity allows the use of NMM-IX as the rG4 trapping ligand under appropriate environmental conditions (Kharel et al. 2023). A polyaromatic molecule, RGB-1 has been shown to interact with TERRA and *NRAS* mRNA G4s where RGB-1 is proposed to selectively recognize rG4s due to the presence of H-bonding acceptors that interact with the 2′-OH group of the rG4s (Katsuda et al. 2016). However, a deeper structural analysis of the interacting RGB-1:rG4 complex is still lacking. Similarly, carboxy-pyridostatin has been used to selectively stabilize cytosolic G4s in the cells. c-PDS has been shown to establish π–π stacking interactions with TERRA G-quartets, and several hydrogen bonds with guanine residues (Rocca et al. 2017). Importantly, cPDS showed a stabilizing effect on TERRA rG4 ($\Delta T_m = 20.7$ °C), which was not affected by the addition of up to 100 equivalents of a dG4 competitor.

A small molecule library was used to screen ligands that could discerningly bind to the $(G_4C_2)_4$ rG4 formed by the mutagenic G_4C_2 repeats found in the first intron of the C9orf72, the most common genetic cause of C9-ALS/FTD (Simone et al. 2018). This repeat-associated intron sequence can be translated through the non-canonical

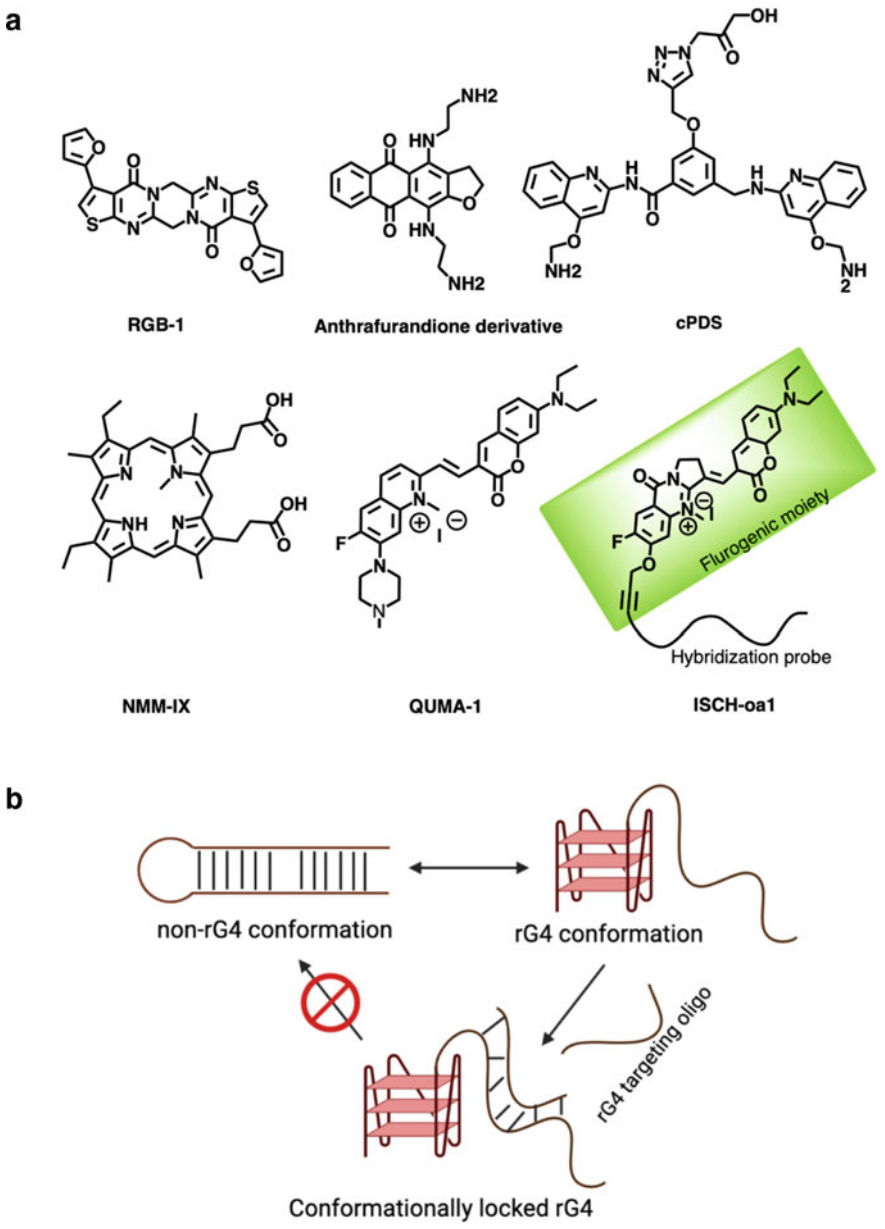

Fig. 3 rG4s targeting. **a**. Some of the representative small molecule ligands with a higher selectivity towards rG4s, and **b**. schematic of one of the strategies to target rG4 using engineered oligonucleotide therapeutic

mechanism of translation (Repeat associated non-AUG translation), synthesizing toxic dipeptides. High quantities of dipeptides (poly-GA, GR, GP, PA, PR) can aggregate and induce pathogenic RNA granule formation, leading to cellular cytotoxicity. Targeting of such rG4s by small molecules in the iPSC-derived motor neurons and C9orf72 mutated Drosophila led to the reduction of dipeptide products, decreasing disease-related cytotoxicity. The small molecule ligands potentially destabilized the G4s formed by $(G_4C_2)_n$ sequence within the mutated intron and inhibit RNA foci formation, resulting in the inhibition of RAN translation of the dipeptide repeats.

Small molecules like QUMA-1 and ISCH-based fluorogenic probes provide a powerful platform to study rG4 folding dynamics inside the cells. A red-emitting fluorescent probe, QUMA-1, has been successfully developed for the selective, continuous, and real-time visualization of rG4s in both live and fixed cells (Chen et al. 2018b). Furthermore, G-quadruplex-triggered fluorogenic hybridization (GTFH) probes have been successfully engineered and synthesized, which are capable of specifically tracking a particular specific rG4 based on the covalently linked complimentary probe attached to the dye (ISCH) (Chen et al. 2016).

Engineered oligonucleotides that directly or indirectly target rG4 regions to stabilize the structure or to bring a molecular lock to a particular structure (Fig. 3b) further provide with a more specific rG4 targeting alternative over the ligand-based approaches (reviewed in Cadoni et al. 2021; Kharel et al. 2020a). However, nuclease-mediated cleavage of the phosphodiester bonds, unfavorable pharmacokinetics, and sub-optimal complex stability limit the direct clinical application of chemically unmodified nucleic-acids-based therapeutics. Chemical modifications, including the phosphate backbone (e.g., phosphorthioates), the sugar moiety (e.g., locked nucleic acids or 2′-O-*alkyl* ribonucleic acids), and the nucleobases (e.g., modified bases) have been designed to improve the stability and bioavailability of such therapeutics.

A strategy to lock miRNA-92b in the rG4 form using a complementary locked nucleic acid-based approach was used to minimize the canonical stem-loop structure resulting in blocked miRNA processing (Fig. 3b) (Mirihana Arachchilage et al. 2018). A rationally designed locked nucleic acid sequence that specifically binds to a region near the 3′-end of *pre-miR92b*, regulates the equilibrium between rG4 and stem-loop. Upon treatment, such equilibrium was shifted toward the G4 conformation. This, in turn, reduced the amount of mature miRNA-92, thus resulting in a therapeutic effect on its mRNA targets as demonstrated by the rescue of PTEN tumor suppressor gene expression in human non-small-cell lung cancer cells. Additionally, a strategy using complementary γ-peptide nucleic acid oligomers to invade an rG4 resulting in translation repression of a reporter gene was used (Oyaghire et al. 2016).

While nucleic acid-based therapeutic strategies for targeting rG4s possess incredible promise in terms of specificity and therapeutic output, challenges remain based on the poor pharmacodynamics of these larger therapeutic molecules. Cellular uptake of the larger, negatively charged molecules has poor efficiency in crossing the cell membrane or maintaining bio-stability, making use of co-delivery materials almost necessary. However, given the prevalence of rG4s in transcriptome and the lack of specificity of the currently used set of small molecules, finding ligands that will precisely bind to a particular rG4 remains immensely challenging. Thus, systematic

efforts to identify and characterize unique rG4 features with clever drug engineering would be needed to develop effective structure–function-based rG4 drugs.

9 Concluding Remarks

Unfortunately, only few 3-D structures of rG4s have been determined to date. Recent advances in the G4 field clearly indicate the formation of rG4s in vivo and their broader role in RNA biogenesis, transport, stability, subcellular localization, and mRNA translation. Thus, understanding of structural features of rG4s in the context of endogenous transcripts is particularly important. Whenever present, rG4s and rG4BPs interact dynamically to guide RNA biology and cell biology. The dissecting of molecular details of such rG4-rG4BP interactions is particularly challenging. Nonetheless, recent advances in RNA biology fostered by cutting edge technologies at both proteome and transcriptome scales have already pushed rG4-rG4BP interaction studies to the nucleotide-amino acid resolutions. Furthermore, such approaches will allow detailed compositional characterization of dynamic rG4-RNP complexes in subcellular compartmentalization- and stimuli-dependent manners (e.g., under stress conditions). Expectedly, rG4s have the potential to serve as therapeutic targets. This partially stems from the fact that rG4-bearing RNA targets have only limited lifetime once transcribed, when compared to G4 DNA targets that embedded in the genomic context. We expect that future rG4 research will continue focusing on the atomic details of the molecular partnership between rG4s, rG4BPs, and rG4 ligands.

References

Akiyama Y, Lyons SM, Fay MM et al (2022) Selective cleavage at CCA ends and anticodon loops of tRNAs by stress-induced RNases. Front Mol Biosci 9:791094

Arora A, Suess B (2011) An RNA G-quadruplex in the 3' UTR of the proto-oncogene PIM1 represses translation. RNA Biol 8(5):802–805

Asamitsu S, Shioda N (2021) Potential roles of G-quadruplex structures in RNA granules for physiological and pathological phase separation. J Biochem 169(5):527–533

Balaratnam S, Basu S (2015) Divalent cation-aided identification of physico-chemical properties of metal ions that stabilize RNA g-quadruplexes. Biopolymers 103(7):376–386

Balaratnam S, Hettiarachchilage M, West N et al (2019) A secondary structure within a human piRNA modulates its functionality. Biochimie 157:72–80

Bang I (1910) Untersuchugen uber die Guanylsaure. Z Physiol Chem 31:407

Beaudoin JD, Perreault JP (2013) Exploring mRNA 3'-UTR G-quadruplexes: evidence of roles in both alternative polyadenylation and mRNA shortening. Nucleic Acids Res 41(11):5898–5911

Belotserkovskii BP, Tornaletti S, D'Souza AD et al (2018) R-loop generation during transcription: Formation, processing and cellular outcomes. DNA Repair 71:69–81

Benhalevy D, Gupta SK, Danan CH (2017) The human CCHC-type zinc finger nucleic acid-binding protein binds G-Rich elements in target mRNA coding sequences and promotes translation. Cell Rep 18(12):2979–2990

Bensaid M, Melko M, Bechara EG et al (2009) FRAXE-associated mental retardation protein (FMR2) is an RNA-binding protein with high affinity for G-quartet RNA forming structure. Nucleic Acids Res 37(4):1269–1279

Bhattacharyya D, Diamond P, Basu S (2015) An independently folding RNA G-quadruplex domain directly recruits the 40S ribosomal subunit. Biochemistry 54(10):1879–1885

Bhattacharyya D, Mirihana Arachchilage G, Basu S (2016) Metal cations in G-quadruplex folding and stability. Front Chem, 4(38)

Bonnal S, Schaeffer C, Créancier L et al (2003) A single internal ribosome entry site containing a G quartet RNA structure drives fibroblast growth factor 2 gene expression at four alternative translation initiation codons. J Biol Chem 278(41):39330–39336

Booy EP, Meier M, Okun N et al (2012) The RNA helicase RHAU (DHX36) unwinds a G4-quadruplex in human telomerase RNA and promotes the formation of the P1 helix template boundary. Nucleic Acids Res 40(9):4110–4124

Bordeleau ME, Cencic R, Lindqvist L et al (2006) RNA-mediated sequestration of the RNA helicase eIF4A by Pateamine A inhibits translation initiation. Chem Biol 13(12):1287–1295

Cadoni E, De Paepe L, Manicardi A et al (2021) Beyond small molecules: targeting G-quadruplex structures with oligonucleotides and their analogues. Nucleic Acids Res 49(12):6638–6659

Cammas A, Lacroix-Triki M, Pierredon S et al (2016) hnRNP A1-mediated translational regulation of the G quadruplex-containing RON receptor tyrosine kinase mRNA linked to tumor progression. Oncotarget 7(13):16793–16805

Chen SB, Hu MH, Liu GC et al (2016) Visualization of NRAS RNA G-quadruplex structures in cells with an engineered fluorogenic hybridization probe. J Am Chem Soc 138(33):10382–10385

Chen MC, Tippana R, Demeshkina NA et al (2018a) Structural basis of G-quadruplex unfolding by the DEAH/RHA helicase DHX36. Nature 558(7710):465–469

Chen XC, Chen SB, Dai J et al (2018b) Tracking the dynamic folding and unfolding of RNA G-quadruplexes in live cells. Ang Chem Int Ed 57(17):4702–4706

Clemson CM, Hutchinson JN, Sara SA et al (2009) An architectural role for a nuclear noncoding RNA: NEAT1 RNA is essential for the structure of paraspeckles. Mol Cell 33(6):717–726

Conlon EG, Lu L, Sharma A et al (2016) The C9ORF72 GGGGCC expansion forms RNA G-quadruplex inclusions and sequesters hnRNP H to disrupt splicing in ALS brains. eLife 5.

Crenshaw E, Leung BP, Kwok CK et al (2015) Amyloid precursor protein translation is regulated by a 3'UTR guanine quadruplex. PLoS ONE 10(11):e0143160

Decorsière A, Cayrel A, Vagner S et al (2011) Essential role for the interaction between hnRNP H/F and a G quadruplex in maintaining p53 pre-mRNA 3'-end processing and function during DNA damage. Genes Dev 25(3):220–225

Didiot MC, Tian Z, Schaeffer C et al (2008) The G-quartet containing FMRP binding site in FMR1 mRNA is a potent exonic splicing enhancer. Nucleic Acids Res 36(15):4902–4912

Emara MM, Ivanov P, Hickman T et al (2010) Angiogenin-induced tRNA-derived stress-induced RNAs promote stress-induced stress granule assembly. J Biol Chem 285(14):10959–10968

Fay MM, Anderson PJ, Ivanov P (2017a) ALS/FTD-Associated C9ORF72 repeat rna promotes phase transitions in vitro and in cells. Cell Rep 21(12):3573–3584

Fay MM, Lyons SM, Ivanov P (2017b) RNA G-quadruplexes in biology: principles and molecular Mmchanisms. J Mol Biol 429(14):2127–2147

Fischer JW, Busa VF, Shao Y et al (2020) Structure-mediated RNA decay by UPF1 and G3BP1. Mol Cell 78(1):70-84.e76

Gellert M, Lipsett MN, Davies DR (1962) Helix formation by guanylic acid. Proc Natl Acad Sci U S A 48(12):2013–2018

Georgakopoulos-Soares I, Parada GE, Hemberg M (2022) Secondary structures in RNA synthesis, splicing and translation. Comp Str Biotech J 20:2871–2884

Goering R, Hudish LI, Guzman BB et al (2020) FMRP promotes RNA localization to neuronal projections through interactions between its RGG domain and G-quadruplex RNA sequences. eLife 9, e52621

Gomez D, Lemarteleur T, Lacroix L et al (2004) Telomerase downregulation induced by the G-quadruplex ligand 12459 in A549 cells is mediated by hTERT RNA alternative splicing. Nucleic Acids Res 32(1):371–379

Halder K, Largy E, Benzler M et al (2011) Efficient suppression of gene expression by targeting 5′-UTR-based RNA quadruplexes with bisquinolinium compounds. ChemBioChem 12(11):1663–1668

Imperatore JA, Then ML, McDougal KB et al (2020) Characterization of a G-quadruplexsStructure in pre-miRNA-1229 and in its Alzheimer's Disease-associated variant rs2291418: implications for miRNA-1229 maturation. Int J Mol Sci 21(3)

Ishiguro A, Lu J, Ozawa D et al (2021) ALS-linked FUS mutations dysregulate G-quadruplex-dependent liquid-to-liquid phase separation and liquid-to-solid transition. J Biol Chem 297(5)

Ivanov P, O'Day E, Emara MM, Wagner, et al (2014) G-quadruplex structures contribute to the neuroprotective effects of angiogenin-induced tRNA fragments. Proc Natl Acad Sci U S A 111(51):18201–18206

Ivanov P, Kedersha N Anderson P (2019) Stress granules and processing bodies in translational control. Cold Spring Harb Perspect Biol 11(5).

Katsuda Y, Sato S, Asano L et al (2016) A small molecule that represses translation of G-quadruplex-containing mRNA. J Am Chem Soc 138(29):9037–9040

Kenny PJ, Kim M, Skariah G et al (2019) The FMRP–MOV10 complex: a translational regulatory switch modulated by G-quadruplexes. Nucleic Acids Res 48(2):862–878

Kharel P, Balaratnam S, Beals N et al (2020a) The role of RNA G-quadruplexes in human diseases and therapeutic strategies. Wires RNA 11(1):e1568

Kharel P, Becker G, Tsvetkov V et al (2020b) Properties and biological impact of RNA G-quadruplexes: from order to turmoil and back. Nucleic Acids Res 48(22):12534–12555

Kharel P, Fay M, Manasova EV et al (2023) Stress promotes RNA G-quadruplex folding in human cells. Nature Commun 14:205

Khateb S, Weisman-Shomer P, Hershco-Shani I et al (2007) The tetraplex (CGG)n destabilizing proteins hnRNP A2 and CBF-A enhance the in vivo translation of fragile X premutation mRNA. Nucleic Acids Res 35(17):5775–5788

Kikin O, Zappala Z, D'Antonio L et al (2008) GRSDB2 and GRS_UTRdb: databases of quadruplex forming G-rich sequences in pre-mRNAs and mRNAs. Nucleic Acids Res 36:D141-148

Koh WS, Porter JR, Batchelor E (2019) Tuning of mRNA stability through altering 3′-UTR sequences generates distinct output expression in a synthetic circuit driven by p53 oscillations. Sci Rep 9(1):5976

Koukouraki P, Doxakis E (2016) Constitutive translation of human α-synuclein is mediated by the 5'-untranslated region. Open Biol 6(4):160022

Kumari S, Bugaut A, Huppert JL et al (2007) An RNA G-quadruplex in the 5′ UTR of the NRAS proto-oncogene modulates translation. Nat Chem Biol 3(4):218–221

Kumari S, Bugaut A, Balasubramanian S (2008) Position and stability are determining factors for translation repression by an RNA G-quadruplex-forming sequence within the 5′ UTR of the NRAS proto-oncogene. Biochemistry 47(48):12664–12669

Kwok CK, Sahakyan AB, Balasubramanian S (2016) Structural analysis using SHALiPE to reveal RNA G-quadruplex formation in human precursor microRNA. Angew Chem Int Ed Engl 55(31):8958–8961

Lago S, Tosoni E, Nadai M, Palumbo M et al (2017) The cellular protein nucleolin preferentially binds long-looped G-quadruplex nucleic acids. Biochim Biophys Acta Gen Subj, 1861(5 Pt B):1371–1381

Lightfoot HL, Hagen T, Tatum NJ et al (2019) The diverse structural landscape of quadruplexes. FEBS Lett 593(16):2083–2102

Loya CM, Van Vactor D, Fulga TA (2010) Understanding neuronal connectivity through the post-transcriptional toolkit. Genes Dev 24(7):625–635

Lyons SM, Achorn C, Kedersha NL et al (2016) YB-1 regulates tiRNA-induced Stress Granule formation but not translational repression. Nucleic Acids Res 44(14):6949–6960

Lyons SM, Gudanis D, Coyne SM et al (2017) Identification of functional tetramolecular RNA G-quadruplexes derived from transfer RNAs. Nature Commun 8(1):1127
Lyons SM, Kharel P, Akiyama Y et al (2020) eIF4G has intrinsic G-quadruplex binding activity that is required for tiRNA function. Nucleic Acids Res 48(11):6223–6233
Marcel V, Tran PL, Sagne C et al (2011) G-quadruplex structures in TP53 intron 3: role in alternative splicing and in production of p53 mRNA isoforms. Carcinogenesis 32(3):271–278
McRae EKS, Booy EP, Padilla-Meier GP et al (2017) On characterizing the interactions between proteins and guanine quadruplex structures of nucleic acids. J Nucleic Acids 9675348
Mei Y, Deng Z, Vladimirova O et al (2021) TERRA G-quadruplex RNA interaction with TRF2 GAR domain is required for telomere integrity. Sci Rep 11(1):3509
Melko M, Douguet D, Bensaid M et al (2011) Functional characterization of the AFF (AF4/FMR2) family of RNA-binding proteins: insights into the molecular pathology of FRAXE intellectual disability. Human Mol Genet 20(10):1873–1885
Mestre-Fos S, Penev PI, Suttapitugsakul S et al (2019) G-quadruplexes in human ribosomal RNA. J Mol Biol 431(10):1940–1955
Miglietta G, Cogoi S, Marinello J et al (2017) RNA G-quadruplexes in Kirsten Ras (KRAS) oncogene as targets for small molecules inhibiting translation. J Med Chem 60(23):9448–9461
Mirihana Arachchilage G, Dassanayake Arosha C, Basu S (2015) A potassium ion-dependent RNA structural switch regulates human pre-miRNA 92b maturation. Chem Biol 22(2):262–272
Mirihana Arachchilage G, Kharel P, Reid J et al (2018) Targeting of G-quadruplex harboring pre-miRNA 92b by LNA rescues PTEN expression in NSCL cancer cells. ACS Chem Biol 13(4):909–914
Mirihana Arachchilage G, Hetti Arachchilage M, Venkataraman A et al (2019) Stable G-quadruplex enabling sequences are selected against by the context-dependent codon bias. Gene 696:149–161
Mori K, Lammich S, Mackenzie IR et al (2013) hnRNP A3 binds to GGGGCC repeats and is a constituent of p62-positive/TDP43-negative inclusions in the hippocampus of patients with C9orf72 mutations. Acta Neuropathol 125(3):413–423
Morris MJ, Basu S (2009) An unusually stable G-quadruplex within the 5'-UTR of the MT3 matrix metalloproteinase mRNA represses translation in eukaryotic cells. Biochemistry 48(23):5313–5319
Morris MJ, Negishi Y, Pazsint C et al (2010) An RNA G-quadruplex is essential for cap-independent translation initiation in human VEGF IRES. J Am Chem Soc 132(50):17831–17839
Newman M, Sfaxi R, Saha A et al (2017) The G-quadruplex-specific RNA helicase DHX36 regulates p53 pre-mRNA 3′-end processing following UV-induced DNA damage. J Mol Biol 429(21):3121–3131
O'Day E, Le MTN, Imai S et al (2015) An RNA-binding protein, Lin28, recognizes and remodels G-quartets in the microRNAs (miRNAs) and mRNAs it regulates. J Biol Chem 290(29):17909–17922
Oyaghire O, SN, Cherubim CJ, Telmer CA, et al (2016) RNA G-quadruplex invasion and translation inhibition by antisense γ-peptide nucleic acid oligomers. Biochemistry 55(13):1977–1988
Pandey S, Agarwala P, Jayaraj GG et al (2015) The RNA stem–loop to G-quadruplex equilibrium controls mature microRNA production inside the cell. Biochemistry 54(48):7067–7078
Pietras Z, Wojcik MA, Borowski LS et al (2018) Dedicated surveillance mechanism controls G-quadruplex forming non-coding RNAs in human mitochondria. Nat Commun 9(1):2558
Puig LE, Londoño-Vallejo A (2020) A guide to computational methods for G-quadruplex prediction. Nucleic Acids Res 48(1):1–15
Ribeiro de Almeida C, Dhir S, Dhir A (2018) RNA helicase DDX1 converts RNA G-quadruplex structures into R-loops to promote IgH class switch recombination. Mol Cell 70(4):650-662.e658
Rocca R, Talarico C, Moraca F et al (2017) Molecular recognition of a carboxy pyridostatin toward G-quadruplex structures: Why does it prefer RNA? Chem Biol Drug Des 90(5):919–925
Rouleau S, Glouzon JS, Brumwell A et al (2017) 3' UTR G-quadruplexes regulate miRNA binding. RNA 23(8):1172–1179

Russo A, Siciliano G, Catillo M et al (2010) hnRNP H1 and intronic G runs in the splicing control of the human rpL3 gene. Biochim Biophys Acta 1799(5):419–428

Sabharwal NC, Savikhin V, Turek-Herman JR et al (2014) N-methylmesoporphyrin IX fluorescence as a reporter of strand orientation in guanine quadruplexes. FEBS J 281(7):1726–1737

Santos T, Salgado GF, Cabrita EJ et al (2021). G-Quadruplexes and their ligands: biophysical methods to unravel G-quadruplex/ligand interactions. Pharmaceuticals (Basel) 14(8)

Schmidt T, Dabrowska A, Waldron JA et al (2022). Purine-rich RNA sequences in the 5'UTR site-specifically regulate eIF4A1-unwinding through eIF4A1-multimerisation to facilitate translation. bioRxiv 2022.2008.2008.503179.

Schuster BS, Reed EH, Parthasarathy R et al (2018) Controllable protein phase separation and modular recruitment to form responsive membraneless organelles. Nat Commun 9(1):2985

Sen D, Gilbert W (1988) Formation of parallel four-stranded complexes by guanine-rich motifs in DNA and its implications for meiosis. Nature 334(6180):364–366

Shrestha P, Xiao S, Dhakal S et al (2014) Nascent RNA transcripts facilitate the formation of G-quadruplexes. Nucleic Acids Res 42:7236–7246

Siegel DA, Le Tonqueze O, Biton A et al (2021). Massively parallel analysis of human 3′ UTRs reveals that AU-rich element length and registration predict mRNA destabilization. G3 Genes|Genomes|Genetics 12(1)

Simko EAJ, Liu H, Zhang T et al (2020) G-quadruplexes offer a conserved structural motif for NONO recruitment to NEAT1 architectural lncRNA. Nucleic Acids Res 48(13):7421–7438

Simone R, Balendra R, Moens TG et al (2018) G-quadruplex-binding small molecules ameliorate C9orf72 FTD/ALS pathology in vitro and in vivo. EMBO Mol Med 10(1):22–31

Skourti-Stathaki K, Proudfoot NJ, Gromak N (2011) Human senataxin resolves RNA/DNA hybrids formed at transcriptional pause sites to promote Xrn2-dependent termination. Mol Cell 42(6):794–805

Subramanian M, Rage F, Tabet R et al (2011) G–quadruplex RNA structure as a signal for neurite mRNA targeting. EMBO Rep 12(7):697–704

Thandapani P, Song J, Gandin V et al (2015) Aven recognition of RNA G-quadruplexes regulates translation of the mixed lineage leukemia protooncogenes. eLife 4, e06234

Tippana R, Chen MC, Demeshkina NA et al (2019) RNA G-quadruplex is resolved by repetitive and ATP-dependent mechanism of DHX36. Nat Commun 10(1):1855

Vo T, Brownmiller T, Hall K et al (2022) HNRNPH1 destabilizes the G-quadruplex structures formed by G-rich RNA sequences that regulate the alternative splicing of an oncogenic fusion transcript. Nucleic Acids Res 50(11):6474–6496

Wang Y, Liu J, Huang BO et al (2015) Mechanism of alternative splicing and its regulation. Biomed Rep 3(2):152–158

Westmark CJ, MalterJS, (2007) FMRP mediates mGluR5-dependent translation of amyloid precursor protein. PLoS Biol 5(3):e52

Winnerdy FR, & Phan AT (2020) Quadruplex structure and diversity. Ann Rep Med Chem (Vol. 54, pp. 45–73). Academic Press

Wolfe AL, Singh K, Zhong Y et al (2014) RNA G-quadruplexes cause eIF4A-dependent oncogene translation in cancer. Nature 513(7516):65–70

Wolozin B, Ivanov P (2019) Stress granules and neurodegeneration. Nat Rev Neurosci 20(11):649–666

Xiao S, Zhang JY, Zheng KW et al (2013) Bioinformatic analysis reveals an evolutional selection for DNA:RNA hybrid G-quadruplex structures as putative transcription regulatory elements in warm-blooded animals. Nucleic Acids Res 41(22):10379–10390

Yamasaki S, Ivanov P, Hu GF et al (2009) Angiogenin cleaves tRNA and promotes stress-induced translational repression. J Cell Biol 185(1):35–42

Yang X, Yu H, Duncan S et al (2022) RNA G-quadruplex structure contributes to cold adaptation in plants. Nat Commun 13(1):6224

Zaccaria F, Fonseca Guerra C (2018) RNA versus DNA G-quadruplex: the origin of increased stability. Chemistry 24(61):16315–16322

Zhang DH, Fujimoto T, Saxena S et al (2010) Monomorphic RNA G-quadruplex and polymorphic DNA G-quadruplex structures responding to cellular environmental factors. Biochemistry 49(21):4554–4563

Zhang Y, Yang M, Duncan S et al (2019b) G-quadruplex structures trigger RNA phase separation. Nucleic Acids Res 47(22):11746–11754

Zhang X, Yu L, Ye S et al (2019a) MOV10L1 binds RNA G-quadruplex in a structure-specific manner and resolves it more efficiently than MOV10. iScience 17:36–48.

Zheng KW, Wu RY, He YD et al (2014) A competitive formation of DNA:RNA hybrid G-quadruplex is responsible to the mitochondrial transcription termination at the DNA replication priming site. Nucleic Acids Res 42(16):10832–10844

Zheng AJL, Thermou A, Guixens P et al (2022) The different activities of RNA G-quadruplex structures are controlled by flanking sequences. Life Sci All 5(2):e202101232