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Contributed Mini Review

Metal–stabilized G–quadruplexes: biological insights and sensing applications

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Repeat sequences account for approximately 45% of the human genome, and can produce noncanonical DNA secondary structures that include G–quadruplexes (G4s). Among these, G4s are unique, in that their formation and stability are largely influenced by metal cations, such as Na^+, K^+, Ca^{2+}, and Mg^{2+}. These cations stabilize G4 structures, while also influencing their folding and biological activities. Interactions between G4s and metal ions affect key cellular processes that include transcription, replication, and genome stability. This review highlights the structural diversity and functional roles of G4s, and further explores how their ion-dependent properties have been harnessed for applications in biosensing and therapeutic development. Future research directions to advance G4-targeted technologies for both diagnostic and clinical use are also discussed. [BMB Reports 2025; 58(9): 397-405]

INTRODUCTION

Repeat sequences are stretches of DNA that occur multiple times, and appear throughout the genome as tandem or dispersed elements (1). The adjacent tandem repeats that occur are termed satellite DNA, with some human diseases being attributed to such repeats. For example, CTG repeats are associated with spinocerebellar ataxia type 8 (SCA8) and Fuchs endothelial corneal dystrophy (FECD) (2, 3). CGG repeats cause Beratela–Scott syndrome (BSS), Fragile X syndrome, and Neuronal intranuclear inclusion disease (NIID) (4-6), while GGGGCC repeats are linked to amyotrophic lateral sclerosis and frontotemporal degeneration (ALS/FTD) (5, 7, 8). These repeat sequences contribute to disease development, while also promoting the formation of noncanonical DNA secondary structures, such as G–quadruplex (G4), hairpin, cruciform, slipped structure, and triple-stranded DNA (H－DNA) (9).

G4 structures were first identified by Gellert *et al*., who observed stable tetrameric helical structures in guanine-rich sequences (10). Since this discovery, biophysical methods, such as circular dichroism (CD) and nuclear magnetic resonance (NMR), along with computational tools, including G4Hunter, have been developed to predict and detect G4 formation (11). Unlike other noncanonical DNA structures, G4s can adopt various conformations depending on environmental conditions, such as the type of cation, DNA sequence, or pH (12-14).

G4 structures have been implicated in genetic instability, primarily due to their interference with DNA replication and repair processes (9). In comparison to other noncanonical structures, they are also enriched in gene promoter regions, suggesting critical epigenetic roles *in vivo*, such as during cell differentiation (15). Zyner *et al*. found that G4 is abundant in human embryonic stem cells, and decreases during neuronal differentiation; conversely, stabilization of G4s with PhenDC3 delayed differentiation and maintained pluripotency, implying that during the transition from pluripotency to neuronal identity, G4s act as key epigenetic regulators (16).

In addition to their biological significance, the metal cation–dependent conformational flexibility of G4s has been used to detect various metal ions, and treat disease. For example, antiparallel G4s bound to iridium(III) complexes exhibit low luminescence; however, upon incubation with Ca^{2+}, G4s transition to a parallel conformation, leading to a significant increase in luminescence from the parallel G4-selective iridium (III) complex. This property has been exploited to develop the Ca^{2+} detection probe (17). In therapeutic contexts, cancer cells with impaired DNA repair pathways are particularly sensitive to G4-stabilizing ligands, such as pyridostatin and RHPS4 (18, 19). Another G4 ligand, MM41, has been reported to reduce tumor growth by 80% (20).

In this review, we discuss structural conformations and biological functions of G4s, as well as recent applications involving their interaction with metal cations in the context of biosensing and disease treatment.

G4 formation and genomic distribution

G4 structures are formed by specific guanine-rich sequences in

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single-stranded DNA or RNA. As the formation of G4s is highly dependent on sequence motif, computational algorithms have been developed to predict putative G4-forming regions in genomic DNA based on patterns such as $G_{\geq 3}N_{1-7}G_{\geq 3}N_{1-7}G_{\geq 3}N_{1-7}G_{\geq 3}N_{1-7}$ (21, 22).

Notably, Chambers *et al.* developed a high-throughput sequencing method known as G4–seq that enables the genome-wide identification and mapping of G4 structures in the human genome using the G4–stabilizing ligand pyridostatin (23). Using this approach, they identified 716,310 distinct G4–forming regions throughout the human genome. These G4s are distributed across various genomic elements, including exons, introns, 5′ and 3′ untranslated regions (UTRs), and promoters. Chromatin state has been shown to influence G4 formation, in particular in promoter regions (24). High G4 density is frequently observed in the regulatory regions of oncogenes and tumor suppressor genes, such as MYC, TERT, CUL7, and FOXA1, suggesting a strong association between G4 formation and genomic instability, somatic copy number alterations, and cancer development (25).

Another G4–binding probe, BG4, exhibits high specificity for both intra- and intermolecular DNA G4s, with no detectable binding to RNA hairpins or single-/double-stranded DNA. Using BG4, Biffi *et al.* demonstrated that G4 formation is cell cycle-dependent, with the highest levels observed during S phase, and the lowest during the G2 and M phases (26). Notably, when DNA replication was blocked, the number of BG4 foci was significantly reduced, indicating that G4s primarily form during DNA replication.

Influence of metal ions and structural classification of G4 structures

Metal cations are key determinants of G4 structure and stability. G4 structures are generally formed by the stacking of multiple G–quartet layers (10). To better understand their structural diversity, G4s can be classified according to three main criteria: the direction of G–quartet stacking, the number of nucleic acid strands involved, and the orientation of guanines within the strands.

Based on the direction of G–quartet stacking, both left-handed and right-handed G4 conformations have been observed using NMR and X-ray crystallography (Fig. 1A) (27). The stacking mode is influenced by both the type and concentration of metal cations. In the presence of monovalent metal

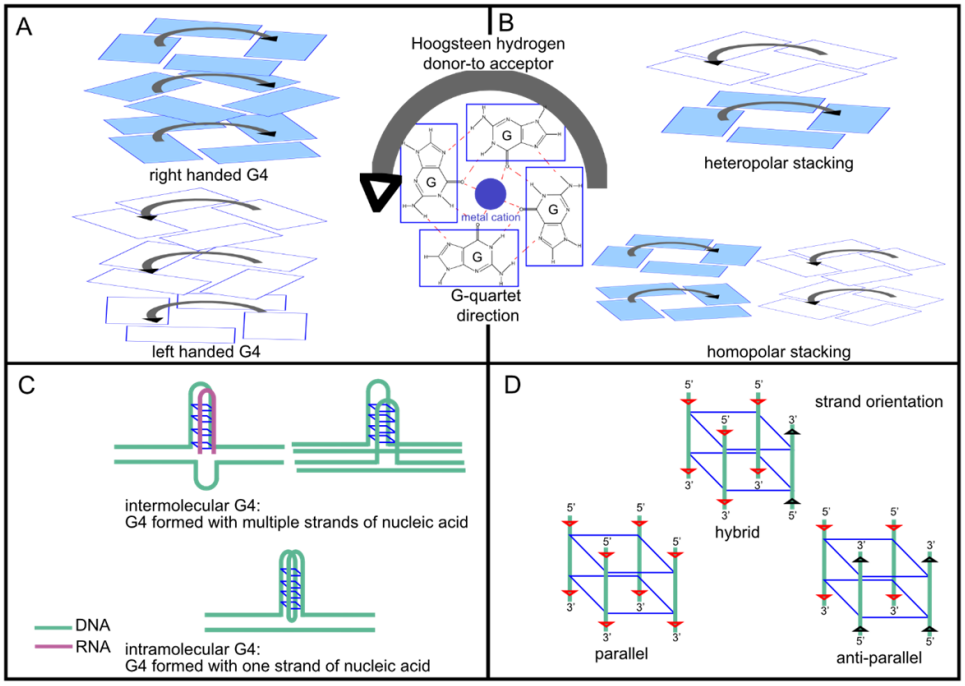

Fig. 1. Diversity of G4 structures. (A) When G-quartets stack in the same direction, the structure is classified as either right-handed (clockwise), or left-handed (counterclockwise). (B) G-quartet stacking in opposite directions is referred to as heteropolar stacking, whereas stacking in the same direction is termed homopolar stacking. Metal cations that bind to G-quartet planes can stabilize the structure via π–π interactions, enabling the complex to function as a metal ion sensor. (C) G4 structures when formed by two or more separate DNA or RNA strands are classified as intermolecular, and when formed by a single strand, intramolecular. (D) Based on strand orientation, G4s are categorized as parallel (all strands aligned in the same direction), antiparallel (two strands in one direction, and two in the other), or hybrid (three strands in the same direction).

cations, such as K^+ and Na^+, G4 structures exhibit both heteropolar and homopolar stacking, regardless of ion concentration. However, in the presence of divalent cations, such as Sr^{2+} and Ba^{2+}, the stacking mode becomes ion concentration–dependent: as the concentration increases, the structure shifts from a mixture of heteropolar and homopolar stacking to exclusively heteropolar stacking. This indicates that excess divalent metal cations confine the heteropolar stacking mode. Interestingly, Ca^{2+}–induced G4 structures exclusively adopt heteropolar stacking, regardless of ion concentration. These cation–specific effects strongly influence the overall topology of the G4s. For example, uniform stacking promoted by Ca^{2+} and Ba^{2+} leads to the formation of a left-handed twist in the G4 helix (Fig. 1B) (12).

G4 structures are also categorized based on the number of nucleic acid strands. When formed by a single nucleic acid strand, they are referred to as intramolecular G4s. In contrast, structures formed by two or more strands are considered intermolecular G4s (Fig. 1C) (28). Lastly, G4s are classified as parallel, antiparallel, or hybrid, according to the orientation of the guanine bases (Fig. 1D), where parallel G4s contain strands with identical polarity, antiparallel G4s include strands with opposing polarities, and hybrid G4s comprise a mixture of orientations (29). Antiparallel G4s have subtypes named 'chair-type' and 'basket-type'. In the chair-type antiparallel G4, the two strands running in the same direction are adjacent. While in the basket-type, they are positioned diagonally across from one another (30, 31).

G4 structures in genome architecture and stability

G4 structures contribute to various aspects of genome architecture, including the formation of topologically associated domains (TADs) and heterochromatin. In *Plasmodium falciparum*, the G-strand binding protein2 (PfGBP2) specifically binds to telomere repeats in G4 conformation *in vitro*, and associates with G−rich RNA. *In vivo*, PfGBP2 partially colocalizes with the telomeric protein HP1α (32). HP1α is involved in the formation and maintenance of heterochromatin through its interaction with trimethylated histone H3 at lysine 9 (H3K9me3) (33). Notably, HP1α recognizes parallel G4s and assemblies formed by telomeric repeat-containing RNAs (TERRA) that are associated with chromatin (Fig. 2A) (34).

Another key player in genome architecture is CTCF, a multifunctional DNA-binding protein that coordinates with the cohesin complex to organize chromatin loops and gene expression (35). G4 formation, often accompanied by R–loops, has been shown to enhance weak CTCF binding at sites located upstream of G4s (Fig. 2B) (36). In the human genome, G4s frequently colocalize with CTCF–bound CpG islands (CGIs) (37).

Beyond chromatin organization, G4 structures also participate in transcriptional regulation through interactions with transcription factors (TFs). For example, AP−1 and SP1, two master transcription factors, are able to bind their recognition sites in the presence of folded G4 structures, thereby contributing to cell type–specific transcriptional programs (38). For this reason, G4s are considered epigenetic regulatory elements in transcription (Fig. 2C).

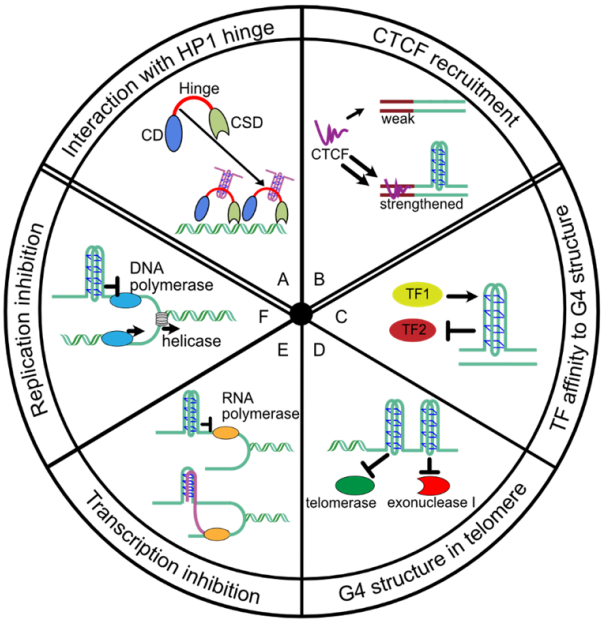

Fig. 2. Physiological functions of G4 structures and their effects on chromatin architecture. (A) The hinge region of HP1 recognizes parallel G4 structures in TERRA RNA. (B) G4 structures enhance weak CTCF binding, and contribute to the formation of topologically associated domains (TADs). (C) Some transcription factors bind more efficiently to G4 structures than to canonical helical DNA, while others exhibit the opposite preference. (D) In telomere regions, G4 formation is favored, due to the repetitive G-rich sequences. Telomeric G4 structures hinder the activity of telomerase and exonuclease I. (E) G4 structures suppress RNA synthesis by interfering with RNA polymerase activity. (F) G4 structures also inhibit DNA synthesis by blocking DNA polymerase activity. The two double lines separate chromatin architecture-related functions from physiological functions.

G4 structures can also impact genome stability by promoting DNA damage and interfering with repair processes. They can stall replication forks, induce double-strand breaks, and hinder the recognition or removal of oxidative lesions (39). At telomeres, G4 structures play an additional regulatory role. Due to the repetitive TTAGGG sequence, telomeres are hotspots for G4 formation. They are involved in regulating telomere shortening, which is associated with exonuclease I activity and cell proliferation (Fig. 2D) (40).

Beyond their structural roles, G4s exert functional impacts on gene regulation and DNA replication. Telomestatin, a telomerase inhibitor, binds to promoter G4s within the proto-oncogene *c-Myb*, and suppress its expression in glioma stem cells (41). G4 formation in the human *c-myb* gene has also been shown to arrest transcription by blocking RNA polymerase (Fig. 2E) (42).

Genomic regions with high G4-forming potential, including telomeres, often require the homologous recombination activities of BRCA1 and BRCA2 for efficient replication (43). These proteins help prevent genomic instability caused by impaired replication through G4-rich regions. As a result, G4-stabilizing compounds reduced the viability of cells lacking BRCA1, BRCA2, or RAD51. G4 structures also interfere with DNA replication at specific loci. ARID1A, a member of the SWI/SNF chromatin remodeling complex, plays an essential role in modulating chromatin structure and gene expression. G4s were found to be required for ARID1A promoter activity, but inhibited DNA replication within the same region (44). More broadly, the blocking of DNA synthesis by G4s occurs regardless of polymerase type, and depends on the thermodynamic stability of the G4 itself (Fig. 2F) (45).

In addition to their role in genome stability, G4s have been implicated in regulating cellular differentiation. Braco-19 is a well-known G4-binding compound. Interestingly, G4 levels increase during adipogenic differentiation, but treatment with Braco-19 reduces their abundance in adipose cells, suggesting that G4s may influence cell fate determination (46).

Molecular interactors of metal ion-dependent G4 structures

A variety of G4-specific ligands and proteins have been identified. For example, L-Apt 12-6 is one of the L-RNA aptamers that specifically recognize parallel G4 structures both *in vitro*, and in cells (47). Meanwhile, SiR-PyPDS, a G4-specific fluorescent probe, enables real-time, single-molecule detection of individual G4 structures in living cells (48).

In addition to these chemical ligands, several helicases play critical roles in resolving G4 structures to maintain genomic stability. Members of the RecQ helicase family, such as BLM and WRN, are notable examples. BLM helicase exhibits high specificity for G4 structures, and Chatterjee *et al.* elucidated its mechanism of interaction with intra-stranded G4 using single-molecule FRET (49). WRN, another RecQ family helicase, selectively unwinds specific tetra-helical structures, such as $d(CGG)_7$, but is unable to resolve telomeric G4s (50).

DHX36, a member of the DEAH/RHA helicase family, binds both DNA and RNA G4s with exceptionally high affinity (51). DHX9 helicase is capable of unwinding various DNA and RNA secondary structures, including replication forks, D-loops, R-loops, and G4s (52). Paeschke *et al.* demonstrated that both *S. cerevisiae* PIF1 and human PIF1 can bind and unwind G4 structures *in vitro*. Loss of PIF1 activity results in G4-associated genetic and epigenetic instability, whereas expression of human PIF1 in yeast suppresses G4-induced DNA damage and telomere elongation (53).

FANCJ helicase can also resolve G4s in an ATPase-dependent manner. Its activity is critical to maintain genomic stability, particularly in telomere metabolism. When FANCJ-depleted cells were treated with a G4-stabilizing compound, they showed impaired proliferation, increased apoptosis, and elevated levels of DNA damage, highlighting the essential role of FANCJ in mitigating G4-induced stress (54-56).

Another important G4-resolving factor is CNBP, which promotes G4 unfolding, and regulates gene expression in promoter regions (57). Nucleolin also contributes to G4 metabolism by binding to the long loops of G4s, irrespective of their conformation or sequence (58).

To summarize, these G4-interacting ligands and proteins play diverse and complementary roles in recognizing, binding, resolving, and visualizing G4 structures. Notably, many of these interactions are influenced by the presence and type of metal ions, which are critical for G4 folding and stability. Their coordinated actions are essential to preserve genome integrity and regulate gene expression in the presence of G4s.

APPLICATIONS FOR METAL SENSOR

Metal cations have been extensively studied for their roles in forming and stabilizing G4s, which spontaneously form in guanine-rich sequences in the presence of specific cations (59). For example, Na^+ can enter the central channel of a G4 structure, preventing its collapse, and promoting the formation of a stable antiparallel conformation (23, 59). An NMR study has shown that G4s preferentially bind K^+ over Na^+ ions due to the lower hydration free energy of K^+, making K^+ energetically favorable (60). Wong *et al.* also demonstrated that Rb^+ binds strongly to G4s using NMR analysis (61). Also, other monovalent (e.g., Li^+, Cs^+) and divalent cations, such as Mg^{2+}, were found to stabilize G4s (62). Overall, metal cations significantly influence the structural stability and conformational dynamics of G4s.

Recently, several studies have highlighted the potential of metal cations to serve as functional probes by interacting with G4s (63-66). This section has focused on how the type and concentration of metal cations affect G4 properties, particularly in the context of their application as sensing elements. Understanding G4-metal cation interactions provides valuable insights to develop both diagnostic sensors for harmful metal ions, and therapeutic strategies targeting G4-mediated pathways.

Metal ion-stabilized G4 structures and G4 in therapeutic applications

Metal cations play a critical role in enhancing the affinity between G4 structures and therapeutic ligands. Gama *et al.* showed that platinum (Pt) and copper (Cu) ions significantly increase the binding affinity of anthracene-containing terpyridine ligands for G4s (67). This metal-ligand-G4 complex can inhibit telomerase activity, a key enzyme involved in cell immortalization that is overexpressed in (85-90)% of cancer cells (Fig. 3A).

Similarly, Cai *et al.* reported that Thioflavin T (ThT), a selective G4 ligand, induces a conformational stabilization of G4s under high concentrations of K^+, using single-molecule nanopore technology (68). This ThT-G4 complex is also a potential therapeutic agent that suppresses telomerase activity (Fig. 3A). As well, two types of ruthenium complexes have

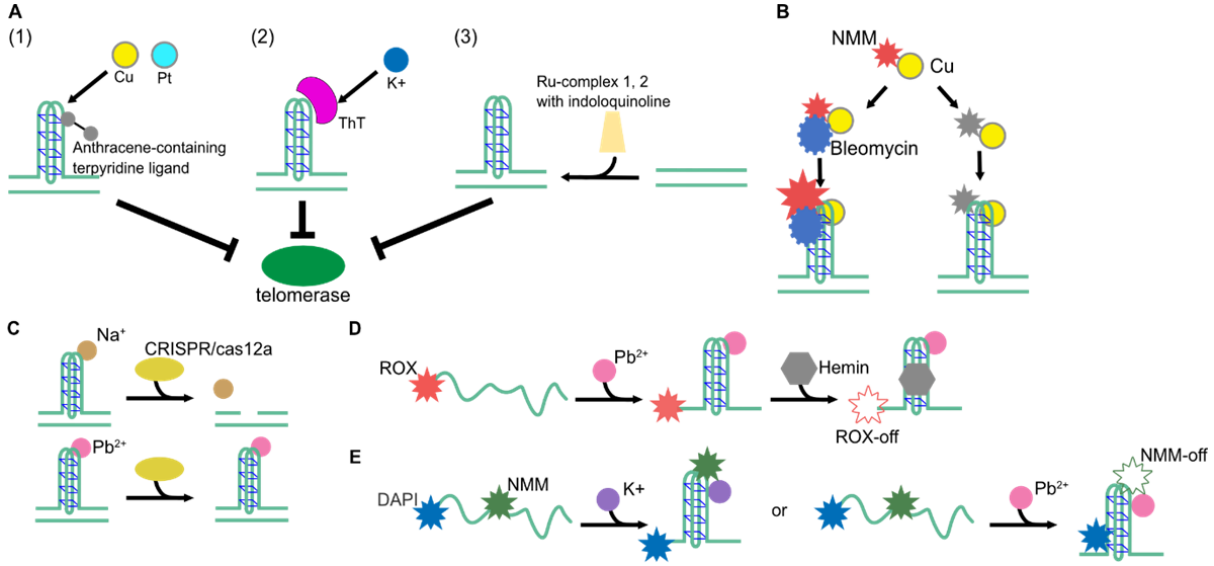

Fig. 3. Applications of G4–metal cation interactions in sensing and telomerase inhibition. (A) Three strategies for telomerase inhibition: (1) inhibition via G4 binding of Cu- and Pt-based anthracene–containing terpyridine ligands; (2) G4 stabilization and telomerase inhibition using Thioflavin T (ThT) and K^+ ions; and (3) stabilization of human telomeric G4 and inhibition of telomerase activity by Ru–complexes 1 and 2 containing indoloquinoline. (B) Bleomycin reduces the fluorescence quenching ability of metal cations. This leads to increased fluorescence intensity of the NMM−G4 complex, enabling bleomycin detection. (C) In the absence of Pb^{2+}, Na^+–induced G4 is cleaved by CRISPR−Cas12a. However, in the presence of Pb^{2+}, G4 undergoes a conformational change that prevents cleavage. (D) Hemin released from trypsin-degraded hemoglobin binds to fluorescently labeled G-rich oligonucleotides to form a G4–hemin complex. Upon Pb^{2+} exposure, the complex undergoes photoinduced electron transfer (PET), resulting in quenching of ROX fluorescence ("ROX-off"). (E) K^+–induced G4 structures can bind both DAPI and NMM (left), while Pb^{2+}–induced G4s fail to bind NMM, leading to signal loss (NMM–off, right).

been shown to stabilize human telomeric G4s (69). These ruthenium complexes provide enhanced selectivity of indoloquinoline ligand for human telomeric G4s. The resulting indoloquinoline–G4 complex can repress telomerase activity in human cells (Fig. 3A) (70).

Beyond cancer–related applications, G4–based sensors have also been explored for toxic compounds. Bleomycin, a chemotherapeutic agent associated with lung toxicity, disrupts lung architecture, and leads to rapid pulmonary dysfunction and death (71). Qin *et al.* developed a G4–based sensor for Bleomycin detection (72). In this system, interactions between Bleomycin and metal cations diminish the cations' ability to quench fluorescence, resulting in a detectable signal change. Furthermore, bleomycin can be detected by a G4-based sensor, in which the G4 structure binds to N-methylmesoporphyrin (NMM). This binding markedly enhances the fluorescence intensity of NMM by reducing the Cu^{2+}-mediated quenching effect. This sensor enables the detection of Bleomycin in complex samples, such as serum and wastewater (Fig. 3B).

G4–based probes for heavy metal sensing and diagnostic applications

Some of the heavy metal cations can have harmful effects on the human body. Many studies have developed heavy metal cation sensors with G4 to prevent this. Lin *et al.* demonstrated that hypericin (Hyp) functions as a dispersion-induced fluorophore (DIF), exhibiting red fluorescence upon binding to Ba^{2+}–stabilized G4 structures (73). Based on this mechanism, they developed a highly selective G4-based fluorescent sensor for Ba^{2+} detection. Ba^{2+} also binds to human telomeric G4s, even in the presence of a 15,000–fold excess of K^+, which is highly abundant in physiological environments. This indicates that the Hyp–G4 complex exhibits strong K^+ tolerance, and remains functional under such conditions (73-75).

In addition to Ba^{2+} detection, G4 structures have been extensively used in designing fluorescent sensors for divalent lead ions (Pb^{2+}). Here, we highlight three representative G4-based strategies for Pb^{2+} detection. One approach employs a G4 structure in combination with a CRISPR−Cas12a system (Fig. 3C) (76). In the absence of Pb^{2+}, the Na^+–induced G4 can be cleaved by Cas12a. However, in the presence of Pb^{2+}, it competitively binds to the G4, inducing a conformational change that renders it resistant to the Cas12a–mediated cleavage. Based on this principle, the DNA probes labeled with fluorophores at both ends enable fluorescent detection of Pb^{2+} in food samples.

Another strategy involves the G4–hemin complex, which functions as an aptasensor with sophisticated molecular recog-

nition capabilities (Fig. 3D) (77-80). Upon trypsin–mediated cleavage of hemoglobin, the released heme binds to a G-rich oligonucleotide labeled with a fluorescent dye, forming the G4–hemin complex. In the presence of Pb^{2+}, the G-rich sequence folds into a G4 structure that facilitates hemin binding, and the resulting complex acts as an electron acceptor. This triggers photo-induced electron transfer (PET) from the dye to the complex, resulting in fluorescent quenching.

A third strategy involves two dyes: DAPI and NMM, which preferentially bind to double-stranded DNA and G4 structures, respectively (Fig. 3E) (81). G4s exhibit stronger binding affinity for Pb^{2+} than for K^{+}, and while K^{+}–induced G4s can bind both DAPI and NMM, Pb^{2+}–induced G4s selectively bind only DAPI (82). As a result, the fluorescent signals at 450 nm and 610 nm (DAPI and NMM, respectively) shift upon Pb^{2+} exposure, enabling sensitive detection of Pb^{2+} in food samples.

Beyond heavy metal sensing, G4 structures have been explored as diagnostic tools for disease-related targets. A notable example is an aptasensor developed by Mizunuma *et al.*, which used an ion-responsive DNA aptamer (IRDAptamer) library designed based on G4 structure (83). This has been employed to screen for Mn^{2+} ions that are implicated in neurodegenerative diseases, such as Parkinson's and Alzheimer's.

Perspective

This review provides an overview of various G4 structural conformations and their functional implications. G4 structures influence key physiological processes, such as DNA replication, transcription, and telomere extension. Moreover, G4 structures contribute to the regulation of higher-order chromatin architecture by modulating interaction with proteins such as HP1 and CTCF. Due to these important effects, many physiological roles of G4 have been identified. However, the role of G4 conformation in shaping DNA architecture and dynamics remains largely unexplored.

Future research could address how G4 structures influence genome organization, particularly in relation to TADs and DNA loop formation. If G4s affect genome architecture, they may interact with structural proteins, such as CTCF and cohesin complex. These potential roles suggest that G4s could act as critical epigenetic factors that are involved in the formation and regulation of TADs and chromatin loops.

This review also highlights applications based on the interaction between metal cations and G4s. Metal cations can stabilize G4 structures by promoting specific conformations and enhancing binding with G4–interactive ligands. Leveraging this property, researchers have developed sensors that are capable of detecting toxic metal cations in biological samples. These studies indicate promising potential for innovative G4-based metal sensing technologies. However, several limitations remain. Current G4-based sensors often suffer from poor selectivity among different metal cations, and face challenges in achieving accurate quantitative analysis. Furthermore, their sensitivity is often insufficient to detect metal ions at low concentrations. Future research should aim to improve both the sensitivity and precision of these sensors, and enable quantitative detection.

Efforts could also be made to develop sensors that are able to detect not only external sources of heavy metals, but also biologically relevant concentrations within the human body. This could contribute to faster diagnosis of heavy metal toxicity, and potentially support the development of new therapeutic agents.

As most current applications focus on food safety, expanding their use to environmental samples, such as river water or air, would broaden their utility. In summary, advancing sensor technologies based on the interaction between G4 and metal ions would lead to practical applications across medicine, environmental monitoring, and public health.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

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