Guanine-Quadruplexes and Possible Role in Nutritional Epigenetics and Aging

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Contents

Abstract

Guanine-quadruplexes are secondary DNA structures that have recently gained a lot of interest from the scientific community. These stable tetra-stranded DNA

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structures have been identified as mediators of DNA replication and regulators of gene expression and recombination. During DNA replication, these guanine-quadruplex structures must be resolved by DNA helicases to prevent telomere dysfunction and DNA damage. Premature aging disorders exhibiting elevated DNA damage are characterized by mutation in DNA helicases involved in resolving guanine-quadruplexes. Epigenetic modification mechanisms such as DNA methylation as well as DNA base oxidation have been indicated to play a significant role regarding stability and occurrence of guanine-quadruplexes. Consequently, the stability and presence of guanine-quadruplexes in the genome may be affected by excess or insufficiencies in nutritional factors involved in DNA methylation and oxidation mechanisms. We also discuss the hypothesis that guanine-quadruplex structures may be associated with changes in genome integrity such as DNA damage. Guanine-quadruplexes have recently emerged as having important biological roles in both nutrition and aging, and they require further investigation as to their application in research for new biomarkers of disease.

List of Abbreviations

8-oxo-G	8-oxo-7,8-dihydroguanine
G4	Guanine-quadruplexes
ROS	Reactive oxygen species
TSS	Transcription start site

Introduction

DNA can adopt various alternative structural conformations (Bochman et al. 2012). Guanine-quadruplexes (G4), for instance, are stable tetra-stranded secondary DNA structures that appear at guanine-rich sequences in the genome (Huppert 2010). Within those G4 forming sequences, four guanines can adopt a square planar conformation by Hoogsteen hydrogen bonding which is called a G-quartet. G4 motifs are generated when two or more G-quartets stack together and connect by single-stranded DNA loops. These structures are stabilized by a monovalent cation (e.g., K^+ or Na^+) at their center, which helps lessening the electrostatic repulsion from guanine oxygens (Wong et al. 2009). A representation of a G4 structure is shown in Fig. 1. The topology and stability of G4 vary depending on the size of the connector loops, G4 motifs' sequence composition and length, and the nature of the monovalent cation and can be formed by one or multiple DNA strands (Fig. 2) (Bugaut and Balasubramanian 2008; Hardin et al. 2000).

Recent evidence has verified the existence and functional importance of these secondary structures (Bochman et al. 2012). Numerous potential G4 structure-forming sequences exist within the human genome, and they are particularly found in telomeres and gene promoters. For instance, more than 40% of promoters have
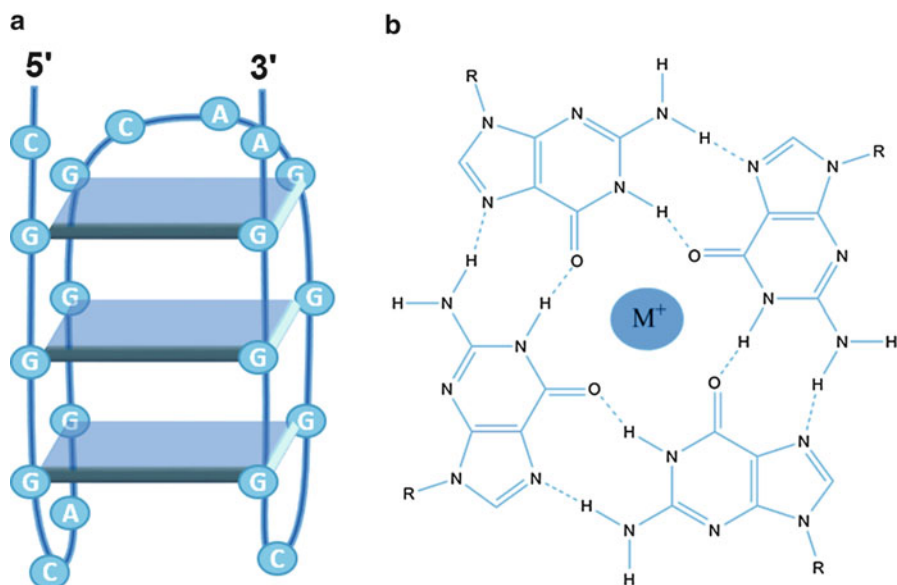
Fig. 1 (**a**) G4 structures are generated when two or more G-quartets stack together. (**b**) Within a guanine-rich region, four guanines can adopt a square planar conformation by Hoogsten hydrogen bonding and subsequently form a guanine-quartet (M^+ symbolizes a monovalent cation, e.g., K^+ or Na^+). Abbreviation: *G4* G-quadruplex (Adapted from François et al. (2015))
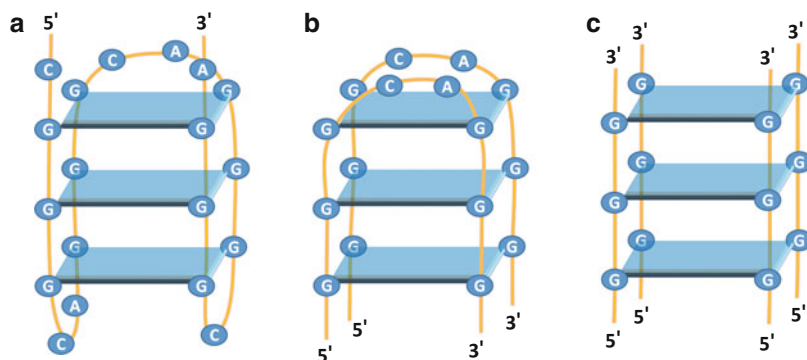

Fig. 2 The topology of G4 structures varies depending on the size of the connector loops, G4 motifs' sequence composition and length, and the nature of the monovalent cation, and they can be formed from (**a**) one (monomolecular), (**b**) two (bimolecular), or (**c**) four (tetramolecular) DNA strands

one or more G4 forming sequence (Maizels and Gray 2013; Huppert and Balasubramanian 2007). The occurrence of G4 motifs at these strategic sites and their enrichment in 5′CpG3′ dinucleotides reinforce their important biological roles in gene regulation (Huppert and Balasubramanian 2007). In addition, treating the cells with compounds that can stabilize G4 motifs results in alteration of the gene expression suggesting an influence of G4 on stimulation or inhibition of DNA transcription (Clark et al. 2012).

Possible roles of G4 in the phenotype of aging have also emerged, with mutations in DNA helicases responsible for the resolution of G4 structures leading to premature aging disorders (e.g., Werner syndrome). The two primary epigenetic regulation mechanisms, DNA methylation and oxidation, are linked with aging and the regulation of gene transcription. Previous chemical and genome-wide studies have verified the respective connection between G4 structures' stability and DNA methylation (Halder et al. 2010; Lin et al. 2013). Furthermore, DNA methylation is correlated with nutrition metabolic pathways, and in order to maintain methylation balance, methyl donor nutrients such as folate are required (Chan et al. 2013). A recent study showed that deficiency in folate or inhibition of DNA methyltransferase, responsible for methylation of DNA, can modulate the occurrence of DNA G4 structures (Francois et al. 2016b). However, the extent to which excess or deficiency of nutritional factors can affect G4 structure levels is still unclear. Since some nutrients can bind to nuclear receptors and lead to the preferential binding of transcription factors to G4 motifs, it has been proposed that certain nutritional factors may have an influence on the mediating function of G4 motifs in gene regulation (Francois et al. 2015). The purpose of this chapter is to provide an overview of the possible role of nutrients in modulating G4 structures and G4-induced DNA damage by affecting oxidation and methylation status and their links to aging disorders such as Werner syndrome.

DNA Helicases and G4

DNA helicases are a group of enzymes essential for breaking the hydrogen bonds of double-stranded DNA or other DNA secondary structures (such as G4 motifs) (Suhasini and Brosh 2013). Formation of G4 structures often occurs when DNA is in its single-stranded form throughout replication and transcription (Maizels and Gray 2013). G4 pose a threat to replication due to their highly stable conformation. They must be recognized and resolved by DNA helicases through an ATP-dependent reaction in a preferred manner (Huber et al. 2002, 2006; Sanders 2010). Therefore, DNA helicases are required in essential cellular activities including DNA repair, transcription, and replication which make them crucial for maintaining genome stability (Suhasini and Brosh 2013).

Mutations in DNA helicase encoding genes are likely contributors resulting in the chromosomal instability and defects that are observed in a number of genetic disorders. For examples, mutations in the RecQ family of DNA helicases are responsible for degenerative disorders such as Werner and Bloom syndromes (Wu and Brosh 2010). While Bloom syndrome is characterized by short stature, developed skin rash, distinctive facial features, and higher emerging risk of cancer, Werner syndrome is characterized by a premature aging phenotype (Suhasini and Brosh 2013). Mutation in WRN gene and subsequent deficiency in WRN helicase can lead to an increase in DNA damage, defects in replication, and genomic instability (Brosh and Bohr 2007; Monnat 2010). A previous study investigated the ability of BLM and WRN helicases to bind and unravel a range of DNA structures (Kamath-Loeb et al. 2012). It was shown that both helicases can unravel several types of DNA structures.

However, WRN helicase demonstrated a more profound ability to unwind all structures tested in this study, while the BLM only resolved parallel G4 structures (Kamath-Loeb et al. 2012). In addition, another study measuring mRNA levels showed a strong association between the expression of genes containing potential G4-forming sequences and the upregulation of these genes in fibroblasts deficient in either BLM or WRN (Johnson et al. 2010). This evidence suggests a role of G4 structures in Werner syndrome disorder and emphasizes the special function of WRN helicase to release the replication fork from blocking G4 structures (Rossi et al. 2010).

Another type of helicase called FANCJ, which in contrast with WRN and BLM is a 5′ helicase, was also discovered to play a role in resolving G4 structures (Bharti et al. 2013). Mutation in this helicase, also known as BRIP1, causes Fanconi anemia which is characterized by bone marrow failure, high risk of cancer, and genome instability at the cellular level (Wu and Brosh 2010). It has also been suggested that FANCJ cooperates with both WRN and BLM helicases in order to unravel G4 structures (Sarkies et al. 2012). The role of FANCJ in resolving G4 motifs was further proven in a study where a precise murine monoclonal antibody named "1H6" was utilized to detect the abundance of G4 in cells by immunofluorescence. As measured by the 1H6 signal, elevated levels in the intensity and number of G4 structures were detected in FANCJ-depleted cells following treatment with a ligand that stabilizes G4 structures (i.e., telomestatin) (Henderson et al. 2014).

As mentioned before, telomeres have an elevated tendency to form G4 motifs as a result of their 3′ single-stranded overhang and their high content in guanines. A study showed that WRN helicase can inhibit the deletion of human telomeric sequences during replication which may happen as a result of unresolved G4 structures (Damerla et al. 2012). Furthermore, it has been indicated that WRN helicase more favorably binds to damaged D-loops located at the 3′ end of telomeres which often contains more 8-oxoguanine, one of the common DNA lesions (Ghosh et al. 2009). These findings suggest that WRN and BLM helicases are essential for resolving G4 structures, maintaining telomere replication, and preserving genome integrity. Genomic instability can also be induced by deficiencies in zinc, a crucial component of the human diet (Sharif et al. 2012b). Zinc is a cofactor of many proteins that are involved in DNA repair, antioxidant response, and methionine metabolism (Sharif et al. 2012a). For instance, zinc is an essential element of zinc-finger domain of DNA helicases such as RecQ helicases. Thus, deficiencies in zinc could have an effect on the capacity of DNA helicases to resolve G4 (Bish and Myers 2007; Liu et al. 2004).

DNA Methylation, Nutrients, and G4

DNA Methylation

DNA methylation is a chemical process in which a methyl group is covalently added to a cytosine in order to form methylcytosine (Fig. 3) (Robertson 2005). DNA methylation is an epigenetic modification method that has a significant function in gene regulation, genomic imprinting, inactivation of X chromosome, transcription, genome stability, and aging (Robertson 2005). In mammalian cells,
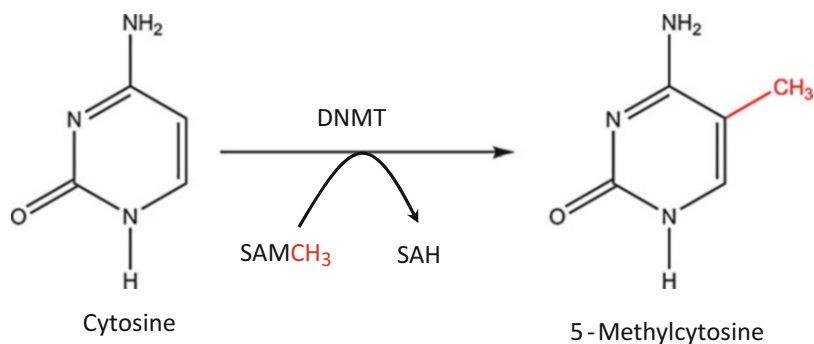
Fig. 3 DNA methylation is a process of covalently adding a methyl group to a cytosine in order to form methylcytosine. In mammalian cells, this occurs mainly at cytosine 5 within high CpG content sequences and catalyzed by the activity of DNMT enzyme. Abbreviation: *DNMT* DNA methyltransferase, *SAH* S-adenosylhomocysteine, *SAM* S-adenosylmethionine

this epigenetic modification mechanism occurs mainly at cytosine 5 within high CpG content sequences, named CpG dinucleotide islands (Bird 2002). These CpG islands exist in 50% of genes in the human genome, and they are frequently correlated with genes' transcription start site (Jones 2012). Methylation of CpG-rich regions located at gene promoters can inhibit the binding of transcriptional elements resulting in the transcriptional suppression of the gene (Robertson 2002). However, it has also been demonstrated that methylation of the CpG islands which are situated downstream of gene transcription start site is often related to activation of the gene (Jones 1999).

DNA methylation deficiency can result in an inadequate control of cell growth, improper recombination, and deregulation of gene transcription which further suggests that proper methylation of DNA is crucial for maintaining genome stability (Robertson 2005). In addition, defects in the preservation and establishment of DNA methylation status have been linked to several human disorders (Brown and Strathdee 2002). For instance, DNA methylation abnormalities have been identified as a causal factor in different types of cancers (Kulis and Esteller 2010). Both hypermethylation and hypomethylation have been shown to contribute to genomic instability and tumor development by inactivating tumor suppressor genes or activating oncogenes (Kulis and Esteller 2010). Furthermore, bioinformatic and genome-wide analyses have demonstrated that the unstable genome is a hallmark and risk enhancer of various cancers (De and Michor 2011; Tang et al. 2012). This increase in genome instability may, for example, be caused by unraveled G4 structures or alteration in gene's methylation status (De and Michor 2011; Tang et al. 2012). DNA breakpoint analysis investigating the somatic copy-number alterations of genome data from samples collected from 26 different kinds of cancer showed a remarkable G4 motif enrichment and hypomethylation in the nearby area suggesting that G4 elements can act as mutagenic factors (De and Michor 2011).

Interestingly, DNA methylation and specifically 5-methylcytosine levels gradually decrease with the aging process (Wilson et al. 1987). Recently, in a genome-

wide association analysis conducted by Heyn et al. (Heyn et al. 2013), the alteration of DNA methylation function on premature aging disorders has been confirmed through methylation profiling of lymphocytes collected from patients affected with Werner syndrome (Heyn et al. 2013). DNA methylation profile of Werner B lymphocytes exhibited profound epigenomic differences when compared with control lymphocytes (Heyn et al. 2013). Based on this information, it is plausible to consider DNA methylation as an important element of the expression of an accelerated aging phenotype in Werner syndrome.

DNA Methylation and G4

Epigenetic control of the transcriptome in humans is generally achieved by DNA methylation mechanisms. However, secondary DNA structures also affect transcription, recombination, and replication (Halder et al. 2010). In a genome-wide study, Halder et al. (Halder et al. 2010) analyzed the cytosine methylation status of more than 20,000 promoters and have found that CpG dinucleotides were considerably less methylated within potential G4-forming regions (Halder et al. 2010). In contrast, non-G4 forming regions were found to be prone to high methylation status (Halder et al. 2010). A connection between G4 and DNA methylation has further been demonstrated by circular dichroism methods suggesting that cytosine methylation within a G4 forming sequence can induce an increase in G4 stability and a conformational shift toward a parallel structure. These methylated G4 motifs can trigger replication stress if unresolved during DNA synthesis (Lin et al. 2013; Hardin et al. 1993). These results advocate that repression of transcription due to stabilized G4 structures may be induced by hypermethylation within the gene promoter. However, contradictory to what previous studies have shown, methylation of O6 within a G-quartet specifically when situated at the middle guanine established a disrupting effect on G4 and caused a reduction in their thermal stability (Mekmaysy et al. 2008). Substitution of O6-methylguanine instead of guanine in telomeres appeared to weaken the Hoogsteen hydrogen bonding, thus decreasing the G4 structures' stability. O6-methylation also deteriorated the cations' interaction, which is required to alleviate the negative electrostatic charge and resulted in unstable G4 motifs (Mekmaysy et al. 2008). Although studies showed discrepancies in the effects of DNA methylation on G4 structures, it remains clear that DNA methylation influences G4 stability. In addition, these opposing results are likely the outcomes of investigating different types of methylation happening at the various locations in the context of G4 (Francois et al. 2015).

Folate, a Methyl Donor, and Its Possible Association with G4

In order for DNA methylation to occur, cells require the presence of nutritional factors such as folate to act as a methyl donor. Folate (vitamin B9) cannot be produced de novo in humans, and the structure mainly consists of a folic acid core, and it is predominantly decorated with several glutamates connected together,

usually referred to as polyglutamate tail (Chan et al. 2013). Folate plays a noteworthy role in nucleotide biosynthesis of thymidines and purines and substantially in DNA repair and methionine synthesis. Methionine is produced from the remethylation of homocysteine involved in the formation of S-adenosylmethionine, which is the main methyl donor in humans (Chan et al. 2013; Crider et al. 2012). Evidence gathered by in vitro and in vivo analyses indicates that chromosomal instability, defected DNA repair, DNA strand breaks, and increases in mutation can be the result of folate deficiency (Chan et al. 2013). Hypomethylation and incorporation of uracil can also be caused by folate deficiency as well as vitamin B12 deficiency since B12 is a vital cofactor in methionine synthesis. Deficiency of these two vitamins results in a reduced DNA methylation status, an increase in DNA damage, and chromosomal instability (Fenech 2012).

Recently, a study carried out by François et al. (Francois et al. 2016b) established evidence for the links between methylation, G4 structures, folate deficiency, and DNA damage. In this particular study, HeLa cells were grown at different concentrations of folate and in the absence or presence of 5-aza-2′-deoxycytidine, a compound which inhibits the DNA-methyltransferase activity. Using an automated quantitative imaging microscope, the results showed an increase in G4 structure frequency in 20 nM folate medium (representing deficient physiological levels of folate) compared with 2,000 nM folate. Similarly, HeLa cells that were exposed to the methyltransferase inhibitor exhibited an increase in their nuclei area and G4 frequency (Francois et al. 2016b). Within the same study, further analyses indicated that the changes observed in the cells grown in the low folate condition were increased when pyridostatin, a ligand that stabilizes G4, was added to the culture medium. Additionally, γH2AX, which is a biomarker of DNA double-strand breaks (DSBs), was quantified by immunofluorescence measurements and resulted in a positive correlation ($r = 0.71$) between G4 levels and DNA damage (Fig. 4)
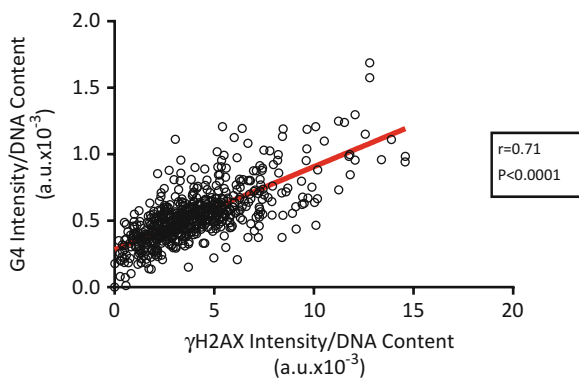

Fig. 4 The connection between DNA damage (γH2AX) and G4 frequency in HeLa cells. Hela cells were grown in low folate condition with pyridostatin. Each circle symbolizes the data from a single cell's nucleus. Data are reported as mean ± standard error of the mean (Data are from François et al. (2016b) with permission from publisher. Abbreviations: *a.u.* arbitrary unit, *G4* guanine-quadruplex. With permission from Oxford University Press)

(Francois et al. 2016b). This study was the first to establish a connection between folate deficiencies and the occurrence of G4 which may arise as a result of DNA hypomethylation. These findings further imply that genome integrity may be sustained by utilizing nutritional factors which are engaged in epigenetic modification pathways. Nutrients could thus be an avenue to control or modulate the balance of G4 structure frequency at the cellular level.

Oxidation, Nutritional Factors, and G4

Oxidation of DNA is a mechanism in which DNA base pairs undergo the loss of one electron (Berquist and Wilson 2012; Morikawa et al. 2014). Reactive oxygen species (ROS) originated from endogenous or exogenous sources can induce oxidative stress and DNA damage with potential deleterious effects on genome integrity (Berquist and Wilson 2012). For instance, foods containing heavy metals, chemicals, and chemotherapeutic drugs can generate exogenous ROS, while cellular pathways such as mitochondrial oxidative phosphorylation can cause endogenous ROS (Francois et al. 2015). Interestingly, many studies have suggested that ROS generation and oxidative damage to DNA increase as a response to aging in multiple tissues and species (Gemma et al. 2007; Finkel and Holbrook 2000). For instance, it has previously been shown that the expression levels of necessary genes for neural function in the frontal cortex of human brains were significantly reduced with aging and were also associated with increased susceptibility to guanine oxidation (Lu et al. 2004). Among the four DNA bases, guanine shows the highest susceptibility to oxidation as a result of its lowest redox potential (Clark et al. 2012), therefore indicating that G-rich regions such as G4 forming sequences have the highest susceptibility to oxidative damage (Delaney and Barton 2003). In addition, it has been shown that guanine oxidation in G4 structures is about twofold faster in comparison with that of duplex telomeric DNA showing the higher propensity of G4 structures to oxidation (Szalai et al. 2002). Guanine oxidation results in the production of 8-oxo-7,8-dihydroguanine (8-oxo-G), as shown in Fig. 5, which has been shown to have increasing effect on thermal stability of G4 motifs depending on the oxidized guanine location (Gros et al. 2007). For instance, the oxidation of 5′G at telomeres can induce the folding of G4 structures at telomeric sequences, while substitution of a middle guanine of the guanine triplet with an 8-oxo-G can disrupt the formation of G4 (Szalai et al. 2002). According to the position of 8-oxo-G within a telomeric sequence, the telomerase activity and its access to the binding site may possibly be altered due to G4 structural changes, thus affecting telomeres maintenance (Szalai et al. 2002; Biffi et al. 2012). In addition, a study investigating G4 structures at promoter regions of c-MYC and c-Kit using molecular dynamic stimulation has shown the dissociative effect of oxidation on G4 motifs (Stebbeds et al. 2012). Those findings suggest that DNA oxidation has an influence on the stability of G4 structures and may possibly contribute to stabilizing them. Furthermore, since the influence of nutritional factors such as antioxidants has been verified to reduce levels of oxidative stress in vivo (Vetrani et al. 2013;
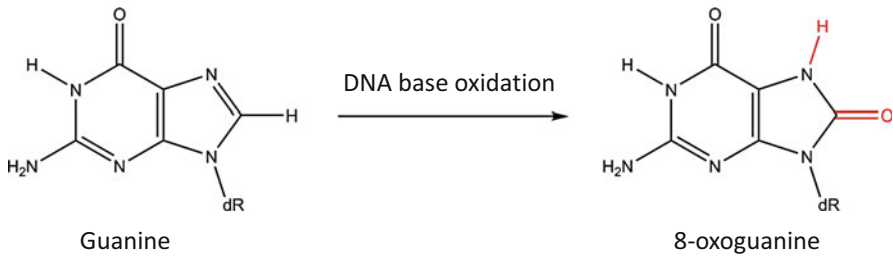
Fig. 5 Guanine is the most susceptible DNA base pair to oxidation and, when oxidized, results in the production of 8-oxo-G. Oxidation, induced by endogenous or exogenous ROS, can result in deleterious effect on genome integrity and DNA damage if remained unrepaired. Abbreviations: 8-oxo-G, 8-oxo-7,8-dihydroguanine; ROS, reactive oxygen species

Benzie and Choi 2014), it is plausible that nutrients important in cellular oxidation pathways may also assist in modulating G4 motifs.

Werner Syndrome, Nutritional Factors, and G4

Possible roles of G4 in the phenotype of aging have emerged, with mutations in DNA helicases responsible for the resolution of G4 motifs leading to premature aging disorders (e.g., Werner syndrome). Furthermore, the two primary epigenetic regulation mechanisms, DNA methylation and oxidation, which are linked with aging can also affect G4 stability. Recently, it has been suggested that G4 structures are also involved in mild cognitive impairment, an aging disorder which is characterized by the loss of memory and reflects early onset of Alzheimer's disease (Francois et al. 2016a; Burns et al. 2002). A recent study showed an increase in the level of G4 in lymphocytes obtained from individuals with mild cognitive impairment when compared to controls and also demonstrated a strong positive correlation between γH2AX intensity and G4 frequency (Francois et al. 2016a). Likewise, in another study comparing fibroblasts collected from Werner syndrome patients and healthy individuals, the data showed an increase in γH2AX foci in Werner syndrome cells suggesting increased DNA damage in Werner syndrome (Sedelnikova et al. 2008). Thus, these findings suggest that an association may exist between G4 increase and induction of DNA damage in aging.

The connection between Werner syndrome and nutritional factors such as vitamin C, an antioxidant that can only be obtained through our diet, has been formerly investigated. For instance, Werner syndrome mouse models which were given ascorbate (vitamin C) in their diet exhibited a decrease in oxidative stress level within the liver and heart tissues as well as a reduction in fat weight. Moreover, the supplementation of those mice with ascorbate also resulted in reversing some of the premature aging phenotypes, such as hypertriglyceridemia and increased blood insulin levels. Importantly, vitamin C supplementation did not have an effect on the metabolic pathways of the control mouse (Lebel et al. 2010; Massip et al. 2010).

However, it should be kept in mind that mice, unlike humans, are capable of synthesizing vitamin C endogenously (Linster and Van Schaftingen 2007). Therefore, future investigations aiming at testing the beneficial effects of antioxidants on Werner syndrome in human cell lines would be valuable. Another study also demonstrated the association of replicative cellular life span with oxidative stress produced by aerobic metabolic pathways. This study showed that the cell senescence rate is reduced when treating both healthy control and Werner syndrome fibroblasts with ascorbic acid phosphoric ester magnesium salt (APM), a low toxic form of ascorbate, with a more pronounced response in Werner syndrome cells (Kashino et al. 2003). Interestingly, further analyses in this study exhibited a reduction in the telomere shortening's rate of cells under APM treatment, possibly caused by a fall in 8-oxo-7,8-dihydroguanine (8-oxo-G) levels (Kashino et al. 2003). Since 8-oxo-G levels affects the formation and stability of G4 (Ghosh et al. 2009; Kashino et al. 2003), these results suggest that a decrease in 8-oxo-G may be correlated with a decrease in G4 motif formation at telomeres, subsequently lessening the reduction in telomere length and increasing the cell's replicative life span. Therefore, it may be possible that treating Werner syndrome cells with APM moderates their susceptibility to oxidative stress and potentially lowering genome instability (Francois et al. 2015). Cell cultures obtained from people affected with Werner syndrome would thus provide a valuable opportunity to study G4 motif function and to analyze the effect of methyl donor nutrients such as folate on this disease in the future.

Conclusion and Future Prospects

Only recently, investigations have aimed at understanding some of the biological functions of G4, mostly through *in vitro* studies. The current limited findings support the plausibility that G4 structures may be important contributing factors of genome instability and aging processes. Recently, it has been shown that the frequency of nuclear G4 foci in HeLa cells can be affected by deficiency in folate and/or by treatment with a DNA-methyltransferase inhibitor (Francois et al. 2016b). These findings suggest that the formation of G4 motifs can be modulated by nutritional factors, possibly via alterations in DNA methylation. Nutrients obtained from the diet can directly affect genome integrity; thus, from a nutrigenomic point of view, understanding the association of nutrient deficiency or excess and the stability of G4 motifs and their occurrence is essential. The possible links between G4, nutrients, epigenetic modification mechanisms, and aging are proposed in Fig. 6. However, the extent to which nutritional elements can influence DNA helicase activity, G4 formation frequency, and epigenetic modifications such as methylation is not yet clear. Therefore, investigating the influence of different concentrations of nutrients on the susceptibility of appearance of G4 motif-induced DNA lesions within the genome is a significant gap that needs to be addressed. In particular, personalized nutrition can emerge as a tool for managing G4-related disorders such as Werner syndrome by comprehensive understanding of
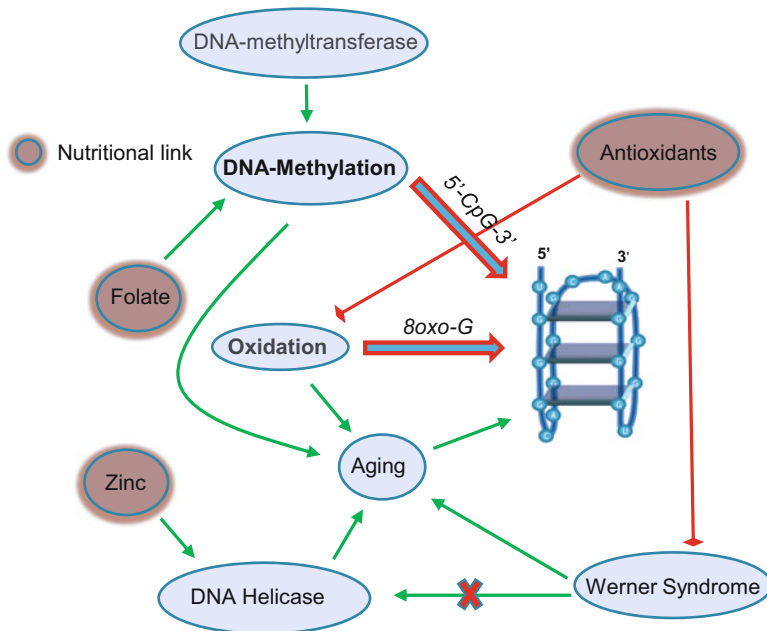
Fig. 6 G4 structures have important roles in inhibiting or stimulating gene transcription. DNA helicases (e.g., WRN) are required in order to resolve these structures since unresolved G4 structures can stall the replication fork and may trigger DNA double-strand breaks and telomere dysfunction. On the other hand, DNA methylation and oxidation can have significant influence on G4 stability, which subsequently mediates gene expression. In addition, DNA methylation and oxidation levels decrease with aging, and it is an important factor in the expression of accelerated aging phenotypes in Werner syndrome. Werner syndrome is an outcome of mutations in the WRN helicase gene; therefore, G4 structures are considered to be a causal element in this disorder. In order for DNA methylation to occur, cells require the presence of nutritional factors such as folate to act as a methyl donor. Accordingly, nutrients such as antioxidants are essential for balancing the redox homeostasis and oxidation level and protecting macromolecules from oxidative damage. Therefore, G4 frequency may also be affected by excess or insufficiencies in certain nutrients supporting the hypothesis that nutritional approaches could be used for minimizing genome instability

how G4 structures and DNA damage can be balanced by nutrients and their intrinsic effect on DNA oxidation and methylation.

Dictionary of Terms

- **Circular dichroism methods** – is a technique usually used for analyzing the secondary structure or conformational transition of biological macromolecules. This method distinguishes the different absorption levels of left-handed and right-handed polarized light over a range of wavelength.

- **CpG dinucleotide (or sites)** – are the DNA regions in which a cytosine is situated at the 5′, and it is followed by a guanine with a phosphate molecule in between in a linear sequence.
- **Immunofluorescence staining and microscopy** – are common techniques for the detection and quantification of proteins or any other biological and non-biological molecules using fluorescent-labeled antibodies which specifically bind to cellular antigens. This technique is generally used on cultured cells or tissue sections.
- **Progeroid syndromes** – are a group of rare monogenetic disorders featured by the appearance of premature physiological aging phenotypes including Werner syndrome and Bloom syndrome.
- **Telomeres** – are explicit DNA regions comprising of G-rich repeat sequences (TTAGGG) which are positioned at the 3′ end of unduplicated chromosomes. Telomeres are vital to inhibit the DNA repair machinery from identifying telomere ends as DNA breaks, thus protecting them from degradation.
- **γH2AX** – one of the primary known cellular reactions to the DNA double-strand breaks is the phosphorylation of the conserved C-terminus of the core histone protein H2AX, termed γH2AX when phosphorylated.
- **8-Oxo-7,8-dihydroguanine (8-oxo-G)** – is one of the oxidative forms of deoxyguanosine which forms as a result of DNA oxidation and represents the cellular level of oxidative stress.
- **8-Oxoguanine** – is one of the common DNA lesions caused by reactive oxygen. This lesion results in mismatch with adenine and triggers the substitution of G to T and C to A in the genome.

Key Facts of G4

- A tetra-stranded DNA structure was first observed to be formed within a guanine-rich single-stranded DNA in 1988 at physiological salt concentrations.
- In 1989, these structures were observed in the oligonucleotides comprising of telomeric repeat sequences from Oxytricha and Tetrahymena using non-denaturing gels containing Na^+ and K^+.
- Soon after the expanded observation of these structures in poly(dG) runs, immunoglobulin switch region, and telomeric DNA repeats, it was suggested that these structures might play a role in chromosomal alignment during meiosis.

Key Facts of Werner Syndrome

- This progeroid syndrome is a rare autosomal recessive disease featured by premature aging phenotypes.
- It can usually be diagnosed around the age of 24 due to the lack of growth spurt and hypogonadism.

- Affected individuals reach their final height at the age of 13 while experiencing loss and graying of hair.
- Over a short period of time, these individuals experience severe loss of muscles and bone density, hardening of soft tissues such as tendons, skin abnormalities, and so on.
- The leading cause of death is cancer and/or cardiovascular disease, and it usually occurs around the age of 48.

Summary Points

- G4 structures are stable tetra-stranded DNA structures which can be vastly found in telomeric sequences and promoter regions.
- These structures must be resolved by RecQ helicase family proteins during replication.
- Premature aging syndromes such as Werner syndrome are associated with mutation in RecQ helicase genes.
- Defects in resolving these structures can lead to blockage of DNA replication, telomere dysfunction, and DNA damage.
- This further implicates the relevance of G4 structures with aging and expression of premature aging disorders phenotypes.
- In addition, DNA methylation and DNA oxidation have been suggested to play a significant role regarding stability and occurrence of G4, which subsequently may mediate gene expression.
- Consequently, G4 frequency, DNA methylation, and oxidation mechanisms may be affected by excess or insufficiencies in certain nutritional factors.
- Therefore, it is plausible that nutritional approaches can be used for minimizing G4 induced genome instability in progeroid syndrome patients.

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