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Review Article

The repair of G-quadruplex-induced DNA damage

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ABSTRACT

G4 DNA motifs, which can form stable secondary structures called G-quadruplexes, are ubiquitous in eukaryotic genomes, and have been shown to cause genomic instability. Specialized helicases that unwind G-quadruplexes *in vitro* have been identified, and they have been shown to prevent genetic instability *in vivo*. In the absence of these helicases, G-quadruplexes can persist and cause replication fork stalling and collapse. Translesion synthesis (TLS) and homologous recombination (HR) have been proposed to play a role in the repair of this damage, but recently it was found in the nematode *Caenorhabditis elegans* that G4-induced genome alterations are generated by an error-prone repair mechanism that is dependent on the A-family polymerase Theta (Pol θ). Current data point towards a scenario where DNA replication blocked at G-quadruplexes causes DNA double strand breaks (DSBs), and where the choice of repair pathway that can act on these breaks dictates the nature of genomic alterations that are observed in various organisms.

Contents

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Maintaining the stability of G-quadruplex-forming DNA

A strand of ssDNA with the consensus sequence $G_{3-5}N_{1-7}G_{3-5}N_{1-7}G_{3-5}N_{1-7}G_{3-5}$ can form a stable secondary four-stranded structure. Four guanines can pair by Hoogsteen base pairing, forming a square planar structure, of which multiple can stack on top of each other to form a so-called G-quadruplex. Even though sequences with the potential to adopt G-quadruplexes are ubiquitous in the genome of eukaryotes, the existence of G-quadruplexes *in vivo* has been the subject of discussion, since the structure is not easily demonstrated experimentally, and is not rapidly formed under physiological conditions, and likely very efficiently dealt with by cellular genome maintenance pathways. Most G-quadruplex-forming sequences are found in repetitive sequences such as the telomeres [1]. In the human genome, G-quadruplexes are also enriched at the 5′ UTR and the first intron of genes, as compared to coding regions. Such a distinct non-random distribution suggests biological function for G-quadruplexes, perhaps in the regulation of gene expression [2]. A recent study suggests that the TFIIH helicases XPB and XPD are recruited to a subset of G-quadruplexes to respectively bind to and unwind them [3].

In closed chromatin, there is little opportunity for a G-quadruplex to form: too much energy is required to convert complementary strands held together by Watson–Crick basepairing into a structure with a G4 configuration. However, a window of opportunity for the formation of G-quadruplexes is created under conditions that the DNA is single-stranded, *e.g.* during transcription and replication, and at the telomeres [1,4]. It has been proposed that particularly the lagging strand may be prone to the formation of secondary structures because of the discontinuous manner in which it is replicated [5,6]. However, several studies imply that formation of G-quadruplexes predominates in the leading strand [7,8].

Since the G-quadruplex is highly stable under physiological conditions, it can present a hindrance for the progression of the replication fork, and if not resolved, the replication fork may stall or collapse [9], leaving a single-stranded gap in the newly synthesized DNA. Such gaps may lead to the formation of DSBs, either because of the action of structure-specific endonucleases, because of an intrinsic instability of ssDNA, or upon replication of the gapped DNA in the next round of replication, provided that gapped structures can survive mitosis and are transmitted to daughter cells.

Even though the formation of G-quadruplexes *in vivo* has been disputed, it has been shown in several organisms that G-quadruplex-forming sequences confer genomic instability. In humans, genomic breakage sites where somatic copy number alterations arise are associated with G-quadruplex-forming sequences and the hypomethylation of these sequences. This has been shown in multiple cancer cell lines, originating from different cancer types [10,11]. In yeast it was shown that human GC-rich minisatellites that contain multiple copies of the G-quadruplex consensus site cause gross chromosomal rearrangements (GCRs) in a G-quadruplex-dependent manner [12,13,14]. The *Caenorhabditis elegans* genome contains many sites that have the ability of folding into a G-quadruplex. These were found to trigger the formation of deletions that take out the motif as well as neighboring sequences [5,15,16]. G-quadruplex-induced genome alterations are not all bad: DNA recombination driving antigenic variation in *Neisseria gonorrhoeae* was found to be initiated by a G4-quadruplex [17]. While there are clear similarities in G-quadruplexes and the ways they are dealt with in different organisms – the thermodynamic gain of energy upon folding is likely the same, independent of the host of the DNA molecule – there are also great differences, especially in how different organisms replicate or transcribe through a preformed structure, or how they deal with DNA breaks resulting from the inability to replicate through such structures.

Here, we aim to cover the progress made in multiple species, but zoom in on the nematode *C. elegans*, because the pathway responsible for the repair of G-quadruplex-induced lesions has been recently discovered in this organism, pointing towards a novel way of how cells deal with replication-associated DNA breaks.

The very first indication that G4 motif-containing sequences were mutagenic came from studies in *C. elegans*, when the DOG-1 5′–3′ helicase was identified as a factor required for the maintenance of G-monotracts [4]. In nematodes that had a null mutation in *dog-1*, directional deletions were observed that included a G-monotract at the 3′ junction. It was hypothesized that DOG-1 was responsible for the unwinding of G-quadruplexes that might form in G-rich template strands during DNA replication (Fig. 1). Indeed, FancJ, the human ortholog of DOG-1, has been shown to unwind G-quadruplexes *in vitro* [18,19]. The idea that G-quadruplexes, as opposed to other features of G-rich DNA, was underlying the observed instability at G-monotracts was experimentally supported using array comparative genome hybridization, and later whole genome sequencing of *dog-1* mutant animals: all sequences conforming to the G4 consensus sequence were found to be fragile in *dog-1* nematodes, while G-rich sequences unable to form a G-quadruplex were as stable as other sequences [15,16]. Genetic screens in *C. elegans* for factors preventing G-tract instability thus far only yielded different inactivating mutations in *dog-1*, suggesting *dog-1* is the major player (that is not essential for viability) involved in the maintenance of G-tract stability [14]. Even though *dog-1*, as the *C. elegans* FANCJ ortholog, was found to function in the intrastrand crosslink repair pathway compromised in Fanconi anemia, this function is separated from its function in the maintenance of G-quadruplex-forming DNA. No other factors in the Fanconi pathway were implicated in G-quadruplex maintenance [20]. Other helicases have been shown to unwind G-quadruplex DNA *in vitro*; of these, *wrn-1*, the *C. elegans* ortholog of WRN, which is the helicase inactive in Werner syndrome [21], was found not to be of influence on the stability of G-tracts [22]. However, inactivation of *him-6*, the *C. elegans* ortholog of BLM, which is the helicase inactive in Bloom syndrome [21], has a small enhancing effect on the formation of deletions at G-tracts in a *dog-1*-deficient background, arguing that it may function as a back-up to *dog-1* [22].

Saccharomyces cerevisiae does not encode a FANCJ ortholog, but does encode Pif1, another 5′–3′ helicase with demonstrated ability to bind to and resolve G-quadruplex-forming sequences. Pif1-deficient cells display increased genomic instability at G-quadruplex-forming sequences [12,13,14], are replication-stressed, and accumulate spontaneous mutations at G-quadruplex-forming sites that disturb the ability of these regions to form G-quadruplexes. Together, these data strongly suggest that Pif1 resolves these structures *in vivo* and thus helps to maintain genome stability in yeast [23]. Besides yeast Pif1, Pif1 homologs from other species also prevent G-quadruplex-dependent GCRs when expressed in yeast [24].

The replication perturbation caused by a deficiency in *dog-1*/*fancJ* or another G-quadruplex-unwinding helicase could also

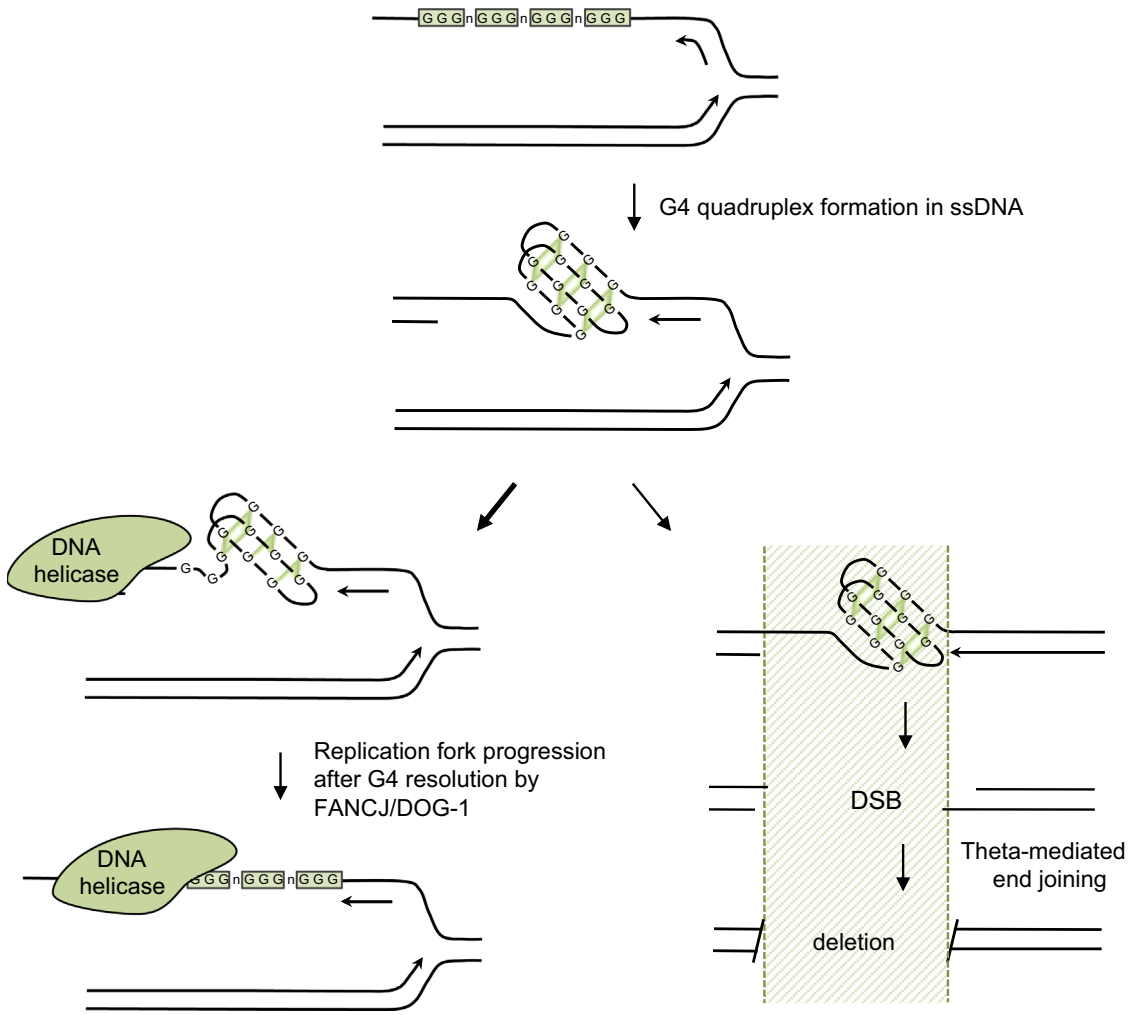

Fig. 1 – G-quadruplexes constitute replication-blocking DNA structures. Specialized DNA helicases are required for their solvation and for replication to proceed. In their absence, genome instability will manifest itself. Here, a model is displayed that can account for the non-symmetric deletions that occur in *C. elegans* strains that lack DOG-1/FancJ. For purpose of clarity the G4 structure is drawn in the lagging strand, while it should be noted that the only available data on this issue suggest that particularly leading strand G-quadruplexes hamper replication.

have consequences for the correct propagation of the epigenetic state of cells; recently, epigenetic instability has been reported in *fancJ*-deficient chicken DT40 cells, but also in cells that lack the translesion synthesis polymerase REV1 [7,25].

Repair of G-quadruplex-induced lesions

The aforementioned helicases are thought to prevent genome rearrangements at G4 sites, by allowing replication to proceed through stable secondary structures. However, the mere fact that in their absence cells survive and produce genetically altered daughter cells argues for parallel routes that resolve blocked replication *via* other means, albeit at the cost of deletions or other types of genome rearrangements (Fig. 1). Out of register HR has been suggested to account for G4-induced repeat length alterations observed in yeast [8]. Involvement of HR in the repair of a G-quadruplex-induced lesion has been identified in the prokaryote *N. gonorrhoeae*. In this pathogen, G4-induced and HR-mediated DNA rearrangement has a biological purpose: antigenic variation of the pilus is determined by the expression of the *pilE* gene of which there are many slightly variant copies present as silenced loci in the bacterial chromosome. By gene conversion, different variants can be expressed, ensuring a heterozygous population, which optimizes the chance of bacteria escaping the human immune system. A G-quadruplex-forming sequence was identified and found to be the source of the nick that is essential for gene conversion at this site. Stabilization of the G-quadruplex-forming sequence by addition of a G4-binding compound decreased antigenic variation, and removal or disruption of the G-quadruplex-forming sequence abolished antigenic variation completely [17].

In *C. elegans*, the role of HR in G-quadruplex-induced genome rearrangements is less prominent. When using the sister chromatid at non-repetitive loci, HR is predominantly error-free and a possible involvement of this pathway in repairing breaks at such sites is thus difficult to uncover. A small increase in the amount of the deletions typical for a *dog-1*-deficient background was reported for nematodes deficient for both *dog-1* and components of HR (*rad-51*, *brd-1* or *xpf-1*/*him-9*). In addition, *rfs-1*, a paralog of

Rad51 involved in HR-mediated repair of DNA damage caused by stalled replication forks, was also shown to play a part in the repair of deletions formed when *dog-1* is not present [26], together suggesting that HR participates in the error-free repair of G-quadruplex-induced damage.

TLS has also been implicated in the error-free bypass of G-quadruplexes when RNAi-mediated knockdown of the TLS polymerases *polh-1* and *polk-1* resulted in elevated G4 instability in animals that were already compromised for *dog-1* [22]. However, a recent analysis using genetic null alleles of *polh-1* and *polk-1* did not support this conclusion [16]. Whether the cytidyltransferase TLS polymerase REV1 may help to resolve G-quadruplexes through incorporating cytosines across G-quadruplexed guanines remains to be addressed.

A role for TLS involvement in replicating G-quadruplex-forming sequences has been clearly shown in chicken DT40 cells, where deletion of *rev1* caused epigenetic perturbation at the G-quadruplex-containing ρ-globin gene. While the G-quadruplex-containing sequences were not deleted, REV1 was found to be required for the timely replication of the G-quadruplex-forming sequences. Hampering replication of these sequences by deletion of *rev1* disrupts correct histone recycling, which leads to changes in the epigenetic state of the locus [27]. Further analysis revealed that in chicken cells, *fancJ* works together with either *wrn* and *blm* or *rev1* to maintain the epigenetic status quo, by ensuring the rapid unwinding of G-quadruplexes to allow the replication fork to pass and proceed [7].

Polymerase Theta-mediated end joining repairs G4-induced DNA breaks in *C. elegans*

Ever since the first observation of genomic instability at potential G-quadruplex forming sequences in *C. elegans*, the directional nature of the mutations has fueled mechanistic models and the search for a pathway underlying them. Deletions that range in size of about 50–300 base pairs and include the G-rich tract very near to its 3′ junction triggered the idea that the position of one of the junctions is determined by replication stalling at the site of the G-quadruplex (Fig. 1). Importantly, this typical deletion profile is not confined to G4 motifs in animals defective for DOG-1/FANCJ; also genomes of *C. elegans* strains that were isolated from different parts of the globe bear the scars of G-quadruplex-induced deletion formation [16]. This observation argues that G4 motifs are also mutagenic in *dog-1*-proficient animals, and that the error-prone pathway that generates deletions at sites of blocked replication is functional in otherwise wild type animals. The notion that canonical non-homologous end-joining (NHEJ) (*lig-4* and *cku-80*) was found not to influence G-quadruplex-induced deletions in *dog-1* deficient nematodes [22] was surprising and argued for an alternative error-prone pathway. In depth analysis of deletion spectra revealed two observations that hinted towards a mechanism involving polymerase action: the deletion junctions showed 1) minimal microhomology of one base, and 2) insertions with sequences that were apparently copied from the flanks of the break site [16]. A search for the responsible polymerase lead to the identification of the A-family polymerase θ, the absence of which leads to profound loss of sequences surrounding G4s: in a *dog-1 polq-1* deficient background, the small 50–300 bp deletions typical of *dog-1* deficiency were transformed into large deletions spanning several kilobases [16]. *In vitro* studies have revealed that mammalian Pol θ is a low-fidelity polymerase capable of extending mismatched termini and DNA sequences with a minimal overlap [28]. It is also capable of bypassing a variety of base damages [28], and hence Pol θ has been regarded a TLS polymerase. However, recent work in *Drosophila melanogaster* identified the *Drosophila* Pol θ homolog Mus308 in an end-joining pathway that could repair DSBs that were created by transposon excision or *I-SceI* digestion. Similar to the deletions found at G-quadruplexes, Mus308-dependent DSB repair junctions showed a high incidence of templated insertions whose sequences originated from the flanks. Furthermore, microhomology was identified at the break site—single basepair homology was not investigated [29]. Pol θ possesses – at least *in vitro* – all the requirements for the functions of which evidence is seen in the break junctions. It has been proposed that Pol θ can use a one base primer as a starting point to extend from to explain the single nucleotide homology. Flanking insertions are explained by extension of the one basepair homology, followed by separation of the two strands and a second round of extension. Mutation of a conserved amino acid in the polymerase domain of *polq-1* leads to a decrease of 50% in deletion formation at G4 sites, suggesting that polymerase action is required for its function in G-quadruplex-induced deletion formation [16]. It remains to be tested whether the helicase domain is required too.

Evidently, Pol θ is of critical importance in preventing large deletions at the site of G-quadruplex-induced DNA breaks, and the name Theta-mediated end joining (TMEJ) was proposed for this alternative DNA double-strand break repair pathway to set it apart from canonical NHEJ. The suggested mechanism is highly reminiscent of the DSB repair performed by the *Drosophila* Pol θ which was termed synthesis-dependent microhomology-mediated end joining (SDMMEJ) [30] but with one conceptual difference that is reflected within the name SDMMEJ: in SDMMEJ, microhomology was proposed to mediate repair (MM in SDMMEJ) through providing thermodynamic stability to annealed DNA tails. In contrast, TMEJ is characterized by only a single nucleotide of homology, unlikely to provide any stability for keeping the DSB ends together. Instead this single basepair homology is thought to reflect the preference of Pol θ to prime extension from at least 1 base pair. Also because this feature may be specific to Pol θ, a more specific and less generic name was chosen.

Importantly, TMEJ is not confined to repair of G-quadruplex-induced breaks; *C. elegans* POLQ-1 is also required for the repair of replication-associated breaks that are elevated in a TLS-deficient background, and result from endogenous DNA damage [31]. Although randomly distributed throughout the genome, these deletions accumulating in TLS-deficient backgrounds have similar characteristics to those seen at G4 motifs in *dog-1*-deficient animals: similar size distribution, single nucleotide homology at the junctions and the occasional presence of templated inserts [31]. The parallels between G-quadruplex-induced deletions and those that are induced by endogenous damage under TLS-compromised conditions provide further support for G-quadruplexes being replication fork impediments. In-depth analysis of deletion junctions also provided clues as to the substrate of TMEJ: a DSB resulting from the local inability to replicate DNA, a conclusion further supported by higher levels of foci containing the DSB marker RAD-51 in animals with increased levels of deletion formation [15]. At this stage it is, however, not known how these breaks arise. A provocative hypothesis is to assume that very low levels of ssDNA gaps across a G-quadruplex can survive mitotic cell division and that replication of such a gap in the next S-phase leads to a DSB, with across it a sister

chromatid that still contains the G4-quadruplex. The unavailability of the preferred repair donor – the sister, which still contains the G4-quadruplex – may explain why HR cannot operate to repair these replication-associated DNA breaks, hence providing a biological *raison d'être* of TMEJ, being an alternative to HR in cases where the sister chromatid cannot be used.

Future perspectives

Considering the fact that Pol θ is well-conserved between species, it is not unreasonable to assume that also TMEJ is also a conserved repair pathway. In humans, nothing is known about the repair mechanisms that operate on G-quadruplex-induced breaks. What is known is that Pol θ expression is upregulated in certain tumors, and that its upregulation is associated with a poor diagnosis [32]. In addition, and of clinical relevance, knocking down Pol θ has been shown to radiosensitize tumour cells, while hardly affecting normal cells [33]. This indicates that Pol θ – and possibly any other factors involved in TMEJ – may be a suitable target for the development of cancer therapeutics. TMEJ, especially if it constitutes an alternative to repair DSBs in situations where a substrate for HR is unavailable, could potentially be a very relevant mechanism in genome instability and tumorigenesis: on the upside by preventing large, possibly lethal, deletions, but on the downside by giving cells the opportunity to repair replication associated breaks in an error-prone fashion, thus possibly providing cancer cells with the ability to deal with increased replication stress.

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REFERENCES

[1] H.J. Lipps, D. Rhodes, G-quadruplex structures: *in vivo* evidence and function, Trends Cell Biol. 19 (8) (2009) 414–422, http://dx.doi.org/10.1016/j.tcb.2009.05.002.
[2] J. Eddy, N. Maizels, Selection for the G4 DNA motif at the 5′ end of human genes, Mol. Carcinog. 48 (4) (2009) 319–325, http://dx.doi.org/10.1002/mc.20496.
[3] L.T. Gray, A.C. Vallur, J. Eddy, N. Maizels, G quadruplexes are genomewide targets of transcriptional helicases XPB and XPD, Nat. Chem. Biol. 10 (4) (2014) 313–318, http://dx.doi.org/10.1038/nchembio.1475.
[4] M.L. Bochman, K. Paeschke, V.A. Zakian, DNA secondary structures: stability and function of G-quadruplex structures, Nat. Rev. Genet. *13* (11) (2012) 770–780, http://dx.doi.org/10.1038/nrg3296.
[5] I. Cheung, M. Schertzer, A. Rose, P.M. Lansdorp, Disruption of dog-1 in Caenorhabditis elegans triggers deletions upstream of guanine-rich DNA, Nat. Genet. 31 (4) (2002) 405–409, http://dx.doi.org/10.1038/ng928.
[6] E.V. Mirkin, S.M. Mirkin, Replication fork stalling at natural impediments, Microbiol. Mol. Biol. Rev. 71 (1) (2007) 13–35, http://dx.doi.org/10.1128/MMBR.00030-06.
[7] P. Sarkies, P. Murat, L.G. Phillips, K.J. Patel, S. Balasubramanian, J.E. Sale, FANCJ coordinates two pathways that maintain epigenetic stability at G-quadruplex DNA, Nucleic Acids Res. 40 (4) (2012) 1485–1498, http://dx.doi.org/10.1093/nar/gkr868.
[8] J. Lopes, A. Piazza, R. Bermejo, B. Kriegsman, A. Colosio, M.-P. Teulade-Fichou, A. Nicolas, G-quadruplex-induced instability during leading-strand replication, EMBO J. *30* (19) (2011) 4033–4046, http://dx.doi.org/10.1038/emboj.2011.316.
[9] S. Lambert, A.M. Carr, Impediments to replication fork movement: stabilization, reactivation and genome instability, Chromosoma 122 (1–2) (2013) 33–45, http://dx.doi.org/10.1007/s00412-013-0398-9.
[10] S. De, F. Michor, DNA secondary structures and epigenetic determinants of cancer genome evolution, Nat. Struct. Mol. Biol. 18 (8) (2011) 950–955, http://dx.doi.org/10.1038/nsmb.2089.
[11] L. Aghili, J. Foo, J. DeGregori, S. De, Patterns of somatically acquired amplifications and deletions in apparently normal tissues of ovarian cancer patients, Cell Rep. *7* (4) (2014) 1310–1319, http://dx.doi.org/10.1016/j.celrep.2014.03.071.
[12] C. Ribeyre, J. Lopes, J.-B. Boulé, A. Piazza, A. Guédin, V.A. Zakian, J.L.N. Mergny, A. Nicolas, The yeast Pif1 helicase prevents genomic instability caused by G-quadruplex-forming CEB1 sequences *in vivo*, PLoS Genet. 5 (5) (2009) e1000475, http://dx.doi.org/10.1371/journal.pgen.1000475.
[13] A. Piazza, J.-B. Boulé, J. Lopes, K. Mingo, E. Largy, M.-P. Teulade-Fichou, A. Nicolas, Genetic instability triggered by G-quadruplex interacting Phen-DC compounds in Saccharomyces cerevisiae, Nucleic Acids Res. 38 (13) (2010) 4337–4348, http://dx.doi.org/10.1093/nar/gkq136.
[14] A. Piazza, A. Serero, J.-B. Boulé, P. Legoix-Né, J. Lopes, A. Nicolas, Stimulation of gross chromosomal rearrangements by the human CEB1 and CEB25 minisatellites in Saccharomyces cerevisiae depends on G-quadruplexes or Cdc13, PLoS Genet. 8 (11) (2012) e1003033, http://dx.doi.org/10.1371/journal.pgen.1003033.
[15] E. Kruisselbrink, V. Guryev, K. Brouwer, D.B. Pontier, E. Cuppen, M. Tijsterman, Mutagenic capacity of endogenous G4 DNA underlies genome instability in FANCJ-defective C. elegans, Curr. Biol. 18 (12) (2008) 900–905, http://dx.doi.org/10.1016/j.cub.2008.05.013.
[16] W. Koole, R. van Schendel, A.E. Karambelas, J.T. van Heteren, K.L. Okihara, M. Tijsterman, A polymerase Theta-dependent repair pathway suppresses extensive genomic instability at endogenous G4 DNA sites, Nat. Commun. *5* (2014) 3216, http://dx.doi.org/10.1038/ncomms 4216.
[17] L.A. Cahoon, H.S. Seifert, An alternative DNA structure is necessary for pilin antigenic variation in Neisseria gonorrhoeae, Science 325 (5941) (2009) 764–767, http://dx.doi.org/10.1126/science.1175653.
[18] S.K. Bharti, J.A. Sommers, F. George, J. Kuper, F. Hamon, K. Shin-ya, M.P. Teulade-Fichou, C. Kisker, R.M. Brosh, Specialization among iron-sulfur cluster helicases to resolve G-quadruplex DNA structures that threaten genomic stability, J. Biol. Chem. *288* (39) (2013) 28217–28229, http://dx.doi.org/10.1074/jbc.M113.496463.
[19] Y. Wu, K. Shin-ya, R.M. Brosh, FANCJ helicase defective in Fanconia anemia and breast cancer unwinds G-quadruplex DNA to defend genomic stability, Mol. Cell. Biol. 28 (12) (2008) 4116–4128, http://dx.doi.org/10.1128/MCB.02210-07.
[20] J.L. Youds, L.J. Barber, J.D. Ward, S.J. Collis, N.J. O'Neil, S.J. Boulton, A.M. Rose, DOG-1 is the Caenorhabditis elegans BRIP1/FANCJ homologue and functions in interstrand cross-link repair, Mol. Cell. Biol. 28 (5) (2008) 1470–1479, http://dx.doi.org/10.1128/MCB.01641-07.
[21] Y. Wu, R.M. Brosh, G-quadruplex nucleic acids and human disease, FEBS J. 277 (17) (2010) 3470–3488, http://dx.doi.org/10.1111/j.1742-4658.2010.07760.x.
[22] J.L. Youds, N.J. O'Neil, A.M. Rose, Homologous recombination is required for genome stability in the absence of DOG-1 in Caenorhabditis elegans, Genetics 173 (2) (2006) 697–708, http://dx.doi.org/10.1534/genetics.106.056879.

[23] K. Paeschke, J.A. Capra, V.A. Zakian, DNA replication through G-quadruplex motifs is promoted by the Saccharomyces cerevisiae Pif1 DNA helicase, Cell 145 (5) (2011) 678–691, http://dx.doi.org/10.1016/j.cell.2011.04.015.
[24] K. Paeschke, M.L. Bochman, P.D. Garcia, P. Cejka, K.L. Friedman, S.C. Kowalczykowski, V.A. Zakian, Pif1 family helicases suppress genome instability at G-quadruplex motifs, Nature 497 (2013) 458–462, http://dx.doi.org/10.1038/nature12149.
[25] R.A. Schwab, J. Nieminuszczy, K. Shin-Ya, W. Niedzwiedz, FANCJ couples replication past natural fork barriers with maintenance of chromatin structure, J. Cell Biol. 201 (1) (2013) 33–48, http://dx.doi.org/10.1083/jcb.201208009.
[26] J.D. Ward, L.J. Barber, M.I. Petalcorin, J. Yanowitz, S.J. Boulton, Replication blocking lesions present a unique substrate for homologous recombination, EMBO J. 26 (14) (2007) 3384–3396, http://dx.doi.org/10.1038/sj.emboj.7601766.
[27] P. Sarkies, C. Reams, L.J. Simpson, J.E. Sale, Epigenetic instability due to defective replication of structured DNA, Mol. Cell 40 (5) (2010) 703–713, http://dx.doi.org/10.1016/j.molcel.2010.11.009.
[28] M. Hogg, A.E. Sauer-Eriksson, E. Johansson, Promiscuous DNA synthesis by human DNA polymerase θ, Nucleic Acids Res. 40 (6) (2012) 2611–2622, http://dx.doi.org/10.1093/nar/gkr1102.
[29] S.H. Chan, A.M. Yu, M. McVey, Dual roles for DNA polymerase theta in alternative end-joining repair of double-strand breaks in Drosophila, PLoS Genet. 6 (7) (2010) e1001005, http://dx.doi.org/10.1371/journal.pgen.1001005.
[30] A.M. Yu, M. McVey, Synthesis-dependent microhomology-mediated end joining accounts for multiple types of repair junctions, Nucleic Acids Res. 38 (17) (2010) 5706–5717, http://dx.doi.org/10.1093/nar/gkq379.
[31] S.F. Roerink, R. van Schendel, M. Tijsterman, Polymerase theta-mediated end joining of replication-associated DNA breaks in C. elegans, Genome Res. 24 (6) (2014) 954–962, http://dx.doi.org/10.1101/gr.170431.113.
[32] M.J. Yousefzadeh, R.D. Wood, DNA polymerase POLQ and cellular defense against DNA damage, DNA Repair 12 (1) (2013) 1–9, http://dx.doi.org/10.1016/j.dnarep.2012.10.004.
[33] G.S. Higgins, R. Prevo, Y.-F. Lee, T. Helleday, R.J. Muschel, S. Taylor, M. Yoshimura, I.D. Hickson, E.J. Bernhard, W.G. McKenna, A small interfering RNA screen of genes involved in DNA repair identifies tumor-specific radiosensitization by POLQ knockdown, Cancer Res. 70 (7) (2010) 2984–2993, http://dx.doi.org/10.1158/0008-5472.CAN-09-4040.