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Replication stalling and DNA microsatellite instability

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Abstract

Microsatellites are short, tandemly repeated DNA motifs of 1–6 nucleotides, also termed simple sequence repeats (SSRs) or short tandem repeats (STRs). Collectively, these repeats comprise approximately 3% of the human genome Subramanian et al. (2003), Lander and Lander (2001) [1,2], and represent a large reservoir of loci highly prone to mutations Sun et al. (2012), Ellegren (2004) [3,4] that contribute to human evolution and disease. Microsatellites are known to stall and reverse replication forks in model systems Pelletier et al. (2003), Samadashwily et al. (1997), Kerrest et al. (2009) [5–7], and are hotspots of chromosomal double strand breaks (DSBs). We briefly review the relationship of these repeated sequences to replication stalling and genome instability, and present recent data on the impact of replication stress on DNA fragility at microsatellites in vivo.

GRAPHICAL ABSTRACT

1. Methods

Methods are indicated in the figure legends, cited references, or from the authors.

2. Microsatellite DNAs adopt non-Watson-Crick secondary structures

The term “replication stalling” has been used to refer to the slowing of DNA polymerization, for example by nucleotide depletion as assayed by precursor incorporation into DNA fibers, or at hard-to-replicate DNA structures monitored by nascent strand qPCR (reviewed in [8]. Replication stalling also refers to the slowing of the CDC45/MCM2-7/GINS (CMG) replicative helicase, commonly assayed by 2D gel electrophoresis. However, stalling of the DNA polymerase does not guarantee stalling of the helicase, and the CMG helicase may bypass stalling sites [9]. Although dissociation of the helicase from the leading strand

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polymerase can lead to the appearance of ssDNA behind the replication fork, the activation of dormant origins [10,11] downstream of a stalled CMG helicase, and polymerase re-priming after a noncanonical template structure [12] may allow continuation of DNA synthesis.

This discussion will focus primarily on microsatellites most often associated with replication stalling in vitro and in vivo, namely direct repeat tracts (including mono- and dinucleotide repeats), hairpin-forming CNG triplets, repeats of the G quadruplex consensus ($G_3+N_{1-7}G_3+N_{1-7}G_3+N_{1-7}G_3+$) and homopurine-homopyrimidine mirror repeats prone to forming inter- or intra-molecular triplex/ H-DNA [13].

Microsatellite DNA sequences adopt non-B secondary structures including hairpins, slipped strands (i.e. one or more offset hairpins on complementary DNA strands) [14–16], DNA triplexes (H-DNA) [17] and guanine quadruplexes [18,19]. Following in vitro replication, mono-nucleotide and dinucleotide repeats show >10-fold elevated rates of frame shift mutations over control template sequences [20–22]. CNG triplets, and sequences able to form G quadruplex or H-DNA also cause premature termination by DNA polymerases in vitro [23–25]. Especially upstream of hard-to-replicate sequences, polymerase dissociation, partial denaturation of the nascent DNA 3' end, and out-of-register reannealing to the template causes insertions/deletions (indels) in the nascent DNA [26–29] (Fig. 1A). In the case of self-complementary dinucleotides (e.g. $(AT)_n$) and CNG trinucleotide repeats, hairpin formation in the nascent DNA or the template DNA also promotes an integral number of repeat expansions or contractions, respectively, in the nascent strands.

Similar non-B structures formed as a result of collisions between replication and transcription complexes, or during DNA repair synthesis [151,175–182], would also be predicted to result in microsatellite instability.

Noncanonical structures such as hairpins may also form during 5' displacement synthesis, when reannealing of the displaced strand and Okazaki fragment ligation stabilize the expanded nascent DNA structure (Fig. 1B) [30–32]. In theory, contractions also may result from polymerase stalling before a hard-to-replicate hairpin structure (Fig. 1C), followed by 3' end displacement and equilibration of the transient hairpin structure to a new position within the repeated sequence template. Reannealing and ligation of the 3' end would effectively bypass and stabilize the template hairpin precluding, for example, the translesion polymerase Pol eta replicating past the gapped hairpin base [33].

Replication fork reversal occurs at an appreciable frequency during several forms of replication stress [34,35]. Fork reversal may permit hairpin formation in the leading (Fig. 1D, upper panel) or lagging (Fig. 1D, lower panel) strand nascent DNA, resulting in expansions. The homologous recombination proteins RAD51 and BRCA1/2 have been implicated in the formation of reversed forks, and the protection of reversed forks against MRE11-, DNA2- or EXO1-dependent degradation of the nascent DNA [36–39].

In yeast, large expansions of GAA repeats have been proposed to result from a template switching mechanism to bypass DNA hairpins [40]. Depending on which strand retains the hairpin, contractions, expansions or slipped strand structures could result (Fig. 1E). In

essence, the in-vasion of a new template by a nascent DNA3' end bears similarity to the homology dependent initiation of break induced replication (BIR) [41, 42]. Mechanisms involving fork stalling, template switching and microhomology mediated break induced replication (FoSTeS/MMBIR) have been invoked to explain duplications, deletions and other complex nonrecurrent genome rearrangements during the replication of repeated sequences in humans [43–47]. As with stalled forks, homology-dependent template switching between sister chromatids or homeologous chromosome sequences would also be expected to generate aberrant 2D gel structures (17).

CNG repeats have been shown by numerous assays to form hairpin or slipped-strand structures in vitro [48–52]. These imperfectly base paired, transient configurations are thermodynamically favored by negative supercoiling, as are G quadruplexes and DNA triplexes [53–61]. The stabilization of structures with reduced base pairing by negative supercoiling has led to the suggestion that non-B structures would preferentially form on the lagging arm of replication forks in vivo [62,63]. As discussed below, the propensity of microsatellite sequences to exhibit instability in vivo, in many cases in a replication polarity-dependent manner, supports the view that microsatellites adopt unusual structures in bacteria, yeast, and human cells.

The in vivo structures that lead to replication stalling at many of these repeats have yet to be proven, however, in the case of SV40 T antigen-dependent plasmid replication of GAA₉₀/TTC₉₀ tracts in either replication orientation in human cells, the asymmetric homopurine-homopyrimidine sequence caused replication fork stalling and fork reversal as shown by 2D gel electrophoresis, and to form triplex-stabilized DNA loops visible by EM [17].

Towards a direct demonstration of in vivo CTG/CAG hairpin formation we constructed HeLa cell clones carrying an ectopic copy of a (CTG/CAG)₁₀₂ or (CTG/CAG)₄₅ microsatellite in either orientation relative to the c-myc replication origin [64,65]. Following prolonged culture or under acute replication stress (0.2 μ M aphidicolin, 1.0 μ M emetine, knockdown of Fen1), these microsatellites displayed expansions and contractions, irrespective of replication polarity. Zinc finger nucleases (ZFNs) were constructed specifically to target CTG or CAG hairpin structures (Fig. 2A), and were expressed in the cells containing the ectopic (CTG/CAG) microsatellites. The CTG-specific ZFN could cleave both leading or lagging strand template CTG hairpins in a cell division dependent manner, as could the CAG-specific ZFN [64,65], showing that hairpins can form on both arms of the replication fork (Fig. 2B – E). Transfection of (CTG)₇ or (CAG)₇ oligonucleotides eliminated polymerase stalling at these repeats, and specifically blocked formation of lagging strand (CAG) or (CTG) hairpins, respectively. Surprisingly, oligonucleotide blocking of lagging strand CTG or CAG hairpins also eliminated the presence of the sister leading strand CAG or CTG hairpins, suggesting that hairpin formation is coupled on the lagging and leading arms of the fork [64,65].

Slipped-strand structures were also observed at the endogenous myotonic dystrophy type 1 (DM1) locus in the brain and heart DNA of a DM1 patient containing an expanded CTG/CAG repeat [14]. After nondenaturing DNA isolation, restriction digestion and

immunoprecipitation using an anti-DNA junction antibody, the results of PCR or EM suggested that slipped-DNAs are present in these post-mitotic tissues in the absence of replication, or as a result of DNA repair, or by their persistence after replication early in development.

3. Microsatellite repeats are sites of genome stress

Repeated sequences are preferential sites of expansion or contraction in bacterial plasmids [66]. Consistent with the prediction that non-B structures would be more likely to form when situated in the lagging arm of a replication fork, bacterial plasmids showed replication-dependent deletions and 10- to 20-fold increased plasmid loss when a hairpin-prone sequence was in the lagging strand template [63,67]. In SV40 origin plasmids replicating in COS-1 cells, replication stalling was dependent on repeat tract length [62,68]. Similarly, in *S. cerevisiae* contractions of a CTG₁₃₀ tract occurred preferentially in the lagging strand replication orientation and lagging strand CTG or CGG tracts caused length dependent increases in chromosome breakage [69–71].

Expanded CGG repeats are sites of chromosome breakage at human folate sensitive fragile loci [72,73], however, other trinucleotide repeats have not shown cytogenetic evidence of chromosome breaks, possibly because the standard cytological fragile site assay does not include replication restart. Nevertheless, enhanced loss of the expanded chromosome 19 in DM1 patients suggests the occurrence of chromosome breakage [74], as does the frequency of chromosome translocations at non-B structure-prone repeats [75,76].

Mutation of the checkpoint response proteins MEC1, DDC2, RAD9 or RAD53 [77–79] significantly increased the frequency of contractions [29,80] and double strand breaks [7] at expanded CNG tracts, supporting the conclusion that the DNA damage response is important for preventing genome instability at trinucleotide repeats. Similarly, deletion of the SGS1 or SRS2 helicases generally increased length instability of CNG repeats, although the effects of SRS2 mutants were smaller, and the quantitative effects of these mutations were dependent on initial repeat tract length and chromosome environment [7,80]. Interestingly, the human RTEL helicase, which is functionally analogous to yeast SRS2, also actively unwinds CNG repeats and suppresses CNG trinucleotide expansions and DSBs [81].

In yeast plasmids GAA repeat-mediated fork stalling was strongly orientation dependent, and the rates of expansion increased dramatically with repeat length [40]. In the yeast chromosome, replication stalling and length instability at GAA repeats were strikingly orientation dependent, and apparent only when the homopurine strand was the lagging strand template [71]. Stalling was not due to abnormal transcription, but was proposed to occur by replication-induced H-DNA triplex formation [82]. In addition to expansions and contractions, GAA-dependent mutations included point substitutions and indels distant to the repeat tract [40], consistent with a template switch mechanism of stalling bypass. GAA-induced fragility was correlated with tract length, replication polarity, the propensity to adopt H-DNA secondary structure, and its extent of fork stalling.

Human microsatellite DNAs are clearly implicated in replication-dependent genome instability [75,76,83–86]. Thus, non-B DNA forming microsatellites co-localize with hotspots of double strand breaks (DSBs), indels, and rearrangements [83,87–91], chromosome translocation junctions map to non-B DNA sites [92], and rare fragile sites co-localize with CTG microsatellites at the SCA1, 6, 7, 8, 12, and 17 loci [93–96]. In yeast and human cells expanded CNG tracts partially slow replication forks [5,64], and engage the DNA damage checkpoint machinery [77,97,98]. Replication-induced DSBs have also been attributed to AT-repeat sequences at the ATXN 10 locus [92,99], common fragile site 16D [89,100], and H-DNA from the BCL-2, Friedrich's ataxia and PKD1 loci [82,96,101].

Similar to the effects of GAA repeats on replication stalling in yeast chromosomes [40,71], an ectopic chromosomal integrant of the PKD1 asymmetric homopurine-homopyrimidine tract stalled replication forks when the GA-rich repeat was the lagging strand replication template [101]. In the fork stalling orientation, this mirror repeat recruited RPA, ATR and RAD1, and elicited a constitutive ATR/CHK1 checkpoint response [101]. Exclusively in the lagging strand orientation, the homopurine repeat DNA shows spontaneous instability in vivo, and is hypersensitive to DSBs in the presence of the G quadruplex stabilizing ligand telomestatin (TMS) (R. Gadgil, unpublished). Moreover, HeLa cells containing ectopic copies of G quadruplex-prone DNA showed increased histone H2AX phosphorylation when depleted of the Pol eta or Pol kappa translesion polymerases [102]. These data suggest that a naturally occurring triplex or G quadruplex structure, or an induced G quadruplex structure, causes replication stress and site specific DNA damage. In contrast, telomestatin treatment of chicken DT40 cells did not cause global replication fork stalling unless the cells were deficient in the FANCD1 helicase [103], whereas FANCD1 depletion in *Xenopus* egg extracts caused persistent replication stalling [104].

The PIF1, FANCD1, dog-1 (*C. elegans*) and RTEL helicases are able to unwind G quadruplex structures, and cells mutant in these helicases exhibit genetic instability at G quadruplex motifs [18,105–113]. Further, compounds similar to telomestatin that selectively bind to G quadruplex structures can induce genetic instability at G quadruplex motifs [110,114]. G quadruplex-directed monoclonal antibodies recognize punctate nuclear foci in human and murine cells [115,116]. The sites of G quadruplex antibody binding are nominally increased in the presence of the G quadruplex ligand telomestatin and further increased approximately 10-fold by telomestatin treatment of FANCD1 null cells [116].

Human FANCD1 unwinds G quadruplex and DNA triplex structures (24–26). G quadruplexes have been observed to form in human cells [115] and human cells in which mutant FANCD1 protein is deficient in G quadruplex unwinding accumulate deletions at or near G quadruplex consensus sequences [105]. FANCD1 patient cells are hypersensitive to interstrand crosslinking reagents such as mitomycin C (MMC) and other DNA polymerase inhibitors, supporting a direct role for FANCD1 in stabilization of DNA replication forks [106,117–122]. Moreover, mutation of the *C. elegans* dog-1 helicase results in loss of DNA sequences flanking long runs of guanines [107,108].

The FANCD1 helicase interacts with multiple proteins implicated in cellular responses to non-canonical forms of DNA and replication stress [113,123–126]. To test the role of the FANCD1

Transgenic mouse models of microsatellite-dependent disease show that repeat expansions and contractions are not limited to actively proliferating cells, indicating that mitotic DNA replication is not the only contributor to instability [128–136]. In accord with this view, the intensity of aberrant replication intermediate structures on 2D gels of yeast DNA does not track with the incidence of microsatellite length instability [5,40,100,137], and replication orientation may not correlate with microsatellite-dependent chromosome breakage [78].

Although the sensitivity of the 2D gel assay to fork stalling is not proven, the absence of a direct relationship with the extent of DSBs or length instability supports the proposal that the escape from stalling is related to instability or breaks [5], and that faithful DNA repair is required to mitigate DSBs that occur before or after the appearance of non-B structures and atypical replicative intermediates [78,138].

In yeast and human cells, protection of stalled replication forks against irreversible inactivation (fork collapse) requires the ATR kinase-initiated damage checkpoint response [139], however, retention of the major replisome components is not dependent on ATR [139–141], and the exact kinase substrates that permit fork restart are unclear [139]. Consistent with the view that stabilization of stalled replisomes by DNA damage response pathways protects microsatellites against DSBs, expanded CNG repeats provoked checkpoint responses in yeast [97], and chromosome breaks at CNG repeats were increased in strains containing checkpoint-defective MEC1, DDC2, RAD9 and RAD53 [77]. Further, mutation of RAD51, RAD52, MRE11, and SAE2 increased length instability and chromosome breaks at a CAG₇₀ and CAG₁₅₅ repeats, suggesting that double strand break repair pathways are important for stabilization of microsatellite length in yeast [138]. Nevertheless, the relationship between microsatellite stability and homologous recombination is complex, inasmuch as mutation of the proposed *anti*-recombinogenic helicases SRS2 and SGS1 increased chromosome fragility at CAG₇₀ and CTG₇₀ repeats, and SRS2 deletion could be over-come by deletion of RAD 51, whereas deletion of RAD51 was less protective in the SGS1 deletion mutant [7]. Aberrant replicative intermediates were observed at the CAG₅₅ repeats in the *rad51*, *sgs1* and the *rad51*, *sgs1* mutants, but not in the *srs2* or *rad51*, *srs2* mutants [7], highlighting the view that fork stalling is separable from chromosome fragility, and that aberrant replicative intermediates may reflect nuclease-sensitive structures [37,38,142–144] on their way to either replication restart or fork collapse [7,97,127,138].

Recent work implicates the nucleotide excision repair (NER), base excision repair (BER) and mismatch repair (MMR) pathways in micro-satellite instability. Displacement synthesis by Pol delta or Pol epsilon during nucleotide excision repair (NER) may lead to microsatellite instability through the formation of 5' flap structures. Indeed, knockdown of the NER proteins CSB, ERCC1, or XPG decreased microsatellite contraction in a human cell model system [145,146].

The BER polymerase beta has been shown to expand nascent strand CTG trinucleotide repeats at gaps generated by OGG1/APE1, similar to the structure of gaps expected from repeat-induced stalling and dissociation of the lagging strand polymerase [147–149]. Microsatellite expansion catalyzed by OGG1/APE1/Pol beta has engendered the “toxic

oxidation cycle” model to account for microsatellite expansion induced by reactive oxygen species [147]. Alternatively, displacement synthesis by Pol beta or Pol delta in conjunction with other DNA repair proteins may stabilize expanded hairpin-containing 5' flaps that resist FEN1 cleavage [64,150–153]. Recent work has shown that recombinant MutS beta (MSH2/MSH3 heterodimer) binds to CAG imperfect hairpins in vitro [154], physically interacts with DNA Pol beta, and stimulates CAG or CTG hairpin retention catalyzed by Pol beta [155]. MutS beta and Pol beta also interact with each other in vivo, and co-localize to ectopic (CTG/CAG)₄₅ repeats during DNA replication [155].

In yeast, mutation of the orthologs of the human MMR proteins MSH2, MSH3, MLH1 and PMS1 decreased large contractions, increased small contractions, and decreased chromosome breaks at GAA repeats [71], suggesting that MMR contributes to microsatellite-induced chromosome instability. This is in accord with extensive data from mouse model systems containing expanded CNG transgenes (CTG₅₅, CTG_{>300}) flanked by sizeable amounts of human chromosomal DNA [128,156], which mimicked the age- length- and sex-dependent variations and physiological characteristics of CTG expansions in human DM1 families. In the (CTG)_{>300} mice, inactivation of proteins in the nonhomologous end joining (NHEJ) or homologous recombination (HR) pathways did not appreciably affect CTG stability. However, the absence of MSH2 shifted the CTG instability towards contractions rather than expansions [157]. The switch from expansions to contractions may possibly reflect a change in the stabilization of template vs. nascent strand non-B structures, or misalignment during single strand annealing, BIR, or other forms of homology-directed repair. In contrast, crossing (CAG)_{111–117} mice with *msh2*^{-/-} mice blocked CAG expansion in mouse models of Huntington's disease [158,159].

Although these results hint at the complexities of the transgenic mouse model systems of microsatellite expansion diseases [132,160, 161] depending on chromosome environment, flanking sequences, microsatellite sequence, length and secondary structure, collectively these results indicate that the MMR pathway contributes to CNG microsatellite instability in vivo [162–168]. Both the MutS alpha (MSH2, MSH6) and MutS beta (MSH2, MSH3) complexes bind hairpin DNA in vitro. In (CTG)_{>300} mice mutation of MSH2 or MSH3 but not MSH6 inhibited repeat expansion [157,169]. In addition, expansion required active MSH2 ATPase activity [163]. Interestingly, the inhibition of wild type MSH2 ATPase activity can vary depending on the specific sequence and hairpin conformation to which it is bound [170,171].

In human cells, both MUTS α and MUTS β are bound at stalled replication forks [140], and MUTS β binding to hairpins recruits ATRIP/ATR and activates ATR [172]. Knocking down MSH2 or MSH6 in cells derived from a Friedreich's Ataxia (GAA/TTC) patient [173] or MSH6 in DM1 (CTG/CAG) patient cells [173] blocked microsatellite expansions. Similarly, in replication-competent human cell free extracts deficient in MutS alpha or MutS beta, slipped strand heteroduplex DNAs were produced by SV40 origin plasmids or CTG slip-out substrates [16,174].

5. Perspective/discussion

Microsatellite DNA repeats exhibit unique biophysical properties that lead to replication stalling, noncanonical secondary structural aberrations, length instability, and chromosome breaks. These characteristics are likely modulated by chromosome environment, epigenetic modifications to DNA and chromatin, damage from endogenous metabolites, nucleotide pool levels, and a staggering array of replisome-associated and checkpoint response proteins. Among many questions that remain to be answered are the *in vivo* roles of the translesion DNA polymerases in microsatellite metabolism, the mechanisms of structural remodeling that occur at sites of non-B structures, and polymerase or helicase stalling, the contributions of replication and repair proteins to repeat instability, and the molecular, genomic and cellular consequences of individual or concurrent DSBs at microsatellite loci.

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HIGHLIGHTS

- Microsatellite DNA repeat sequences are prone to adopt noncanonical DNA structures.
- Noncanonical microsatellite DNA structures are sites of polymerase stalling.
- Stalling at microsatellites is associated with repeat length instability and DNA double strand breaks.
- The FANCI protein is necessary to protect microsatellite repeats against DSBs during replication stress.

Fig. 1.

Hypothetical models of replication-dependent instability. Red arrows indicate nascent DNA repeat sequences. A. 3' end slippage. Dissociation of the DNA polymerase, partial denaturation of the nascent DNA 3' end, hairpin formation, and out-of-register reannealing to the template may lead to expansion after another round of replication. B. 5' flap hairpin. Displacement synthesis can allow hairpin formation in the nascent DNA, reannealing, and ligation to the upstream Okazaki fragment. C. Hairpin isomerization. A template strand hairpin causes 3' end slippage and may allow equilibration of the hairpin. Reannealing of

the nascent 3' end and Okazaki fragment ligation stabilize the contraction in the nascent DNA. D. Fork reversal. Polymerase stalling in repetitive DNA may allow fork reversal and hairpin formation in leading strand nascent DNA (upper panel) or lagging strand nascent DNA after Okazaki fragment dissociation and repriming (lower panel). E. Template switching. Polymerase stalling at a non-B structure causes the leading strand polymerase to use nearby lagging strand nascent DNA as a template. Reannealing of the nascent strand to the leading strand template can lead to hairpins in the template or nascent DNA. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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Fig. 2. Oligodeoxynucleotide inhibition of hairpin formation. A. Schematic of the CTG-directed zinc finger nuclease (ZFN_{CTG}) homodimer binding to a CTG hairpin. Note that dimerization of the FOK1 nuclease domain (large oval) is required for cleavage, therefore a ZFN_{CTG} homodimer can cleave a CTG hairpin but not CTG/CAG dsDNA [64]. B. Inhibition of hairpin formation by (CAG)₇ in cells containing the ectopic c-myc core origin and (CTG)₄₅ in the lagging strand template. Lanes 1 to 4, mock ODN treatment of cells expressing ZFP_{CTG}; lanes 5 to 8, mock ODN treatment of cells expressing ZFN_{CTG}; lanes 9 to 12, (CTG)₇ treatment of cells expressing ZFP_{CTG}; lanes 13 to 16, (CAG)₇ treatment of cells

expressing ZFN_{CTG}. The 270 bpPCR product [(CTG/ CAG)₄₅ progenitor allele] is indicated with an arrow. The lower-mobility shadow bands observed above the 270 bp amplification product of the (CTG/CAG)₄₅ progenitor sequence are slipped-strand structures formed in vitro during PCR reannealing. Bands migrating faster than the progenitor product are the results of in vivo ZFN_{CTG} cleavage. C. Model for hairpin inhibition by the (CAG)₇ ODNs. D. Inhibition of hairpin formation by (CTG)₇ in (CAG)₄₅ cells. Lanes 1 to 4, mock ODN treatment of cells expressing ZFP_{CAG}; lanes 5 to 8, mock ODN treatment of cells expressing ZFN_{CAG}; lanes 9 to 12, (CTG)₇ treatment of cells expressing ZFP_{CAG}; lanes 13 to 16, (CTG)₇ treatment of cells expressing ZFN_{CAG}. E. Model for hairpin inhibition by (CTG)₇ in (CAG)₄₅ cells. In panels C and E, ODNs are depicted as multiple short bars, repeated CTG or CAG sequences are depicted in red, and ZFNs are depicted in blue. Reprinted with permission. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 3.

FANCJ knockdown leads to loss of ectopic CTG/CAG microsatellite signals in (CTG/CAG)₁₀₂ cells under replication stress. A. Western blots. Whole cell extracts were isolated after treatment of cells with siControl or siFANCJ for a total of five transfections, and 0.2 μ M aphidicolin, or parallel untreated cultures and immunoblotted for FANCJ. B. Duplex small pool PCR with primers spanning a non-repeat internal PCR control site and the ectopic (CAG)₁₀₂ repeats (left) or (CTG)₁₀₂ repeats (right). Notice the loss of the ectopic site repeat PCR band in cells knocked down for FANCJ and treated with APH. Reprinted with permission.

Fig. 4. FANCI null patient cells treated with aphidicolin are prone to microsatellite signal loss at multiple endogenous sites. A–L. Duplex spPCR across endogenous repeated sequences in DNA from FANCI null patient fibroblasts with or without aphidicolin (0.2 μ M) treatment. Reprinted with permission.

Fig. 5.

Chromosome fragility at an ectopic CTG microsatellite. A. DF myc-CTG cells are a clonal HeLa cell line containing a single copy integrant of the c-myc core replication origin [183] next to a CTG/CAG microsatellite, flanked by red (Tomato) and green (eGFP) marker genes. B, D, F. Flow cytometry of untreated DF2 myc, DF2 myc.CTG₂₃, and DF2 myc.CTG₁₀₀ cells. C, E, G. Flow cytometry of untreated DF2 myc, DF2 myc.CTG₂₃, and DF2 myc.CTG₁₀₀ cells treated with 0.2 mM HU for six days and allowed to recover in drug-free medium for

four days. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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