DNA Repair 56 (2017) 156–165

Contents lists available at ScienceDirect

DNA Repair

journal homepage: www.elsevier.com/locate/dnarepair

Role of recombination and replication fork restart in repeat instability

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ARTICLE INFO

Keywords:
Trinucleotide repeat expansion
Recombination
Replication fork restart
Chromosome fragility
DNA structure

ABSTRACT

Eukaryotic genomes contain many repetitive DNA sequences that exhibit size instability. Some repeat elements have the added complication of being able to form secondary structures, such as hairpin loops, slipped DNA, triplex DNA or G-quadruplexes. Especially when repeat sequences are long, these DNA structures can form a significant impediment to DNA replication and repair, leading to DNA nicks, gaps, and breaks. In turn, repair or replication fork restart attempts within the repeat DNA can lead to addition or removal of repeat elements, which can sometimes lead to disease. One important DNA repair mechanism to maintain genomic integrity is recombination. Though early studies dismissed recombination as a mechanism driving repeat expansion and instability, recent results indicate that mitotic recombination is a key pathway operating within repetitive DNA. The action is two-fold: first, it is an important mechanism to repair nicks, gaps, breaks, or stalled forks to prevent chromosome fragility and protect cell health; second, recombination can cause repeat expansions or contractions, which can be deleterious. In this review, we summarize recent developments that illuminate the role of recombination in maintaining genome stability at DNA repeats.

1. Introduction

Expanded tracts of repetitive DNA sequences are the cause of over 30 genetic diseases and can consist of trinucleotide or larger repetitive units [1–5]. The expandable repeats form stable non-B-form DNA structures which impede normal cellular processes like DNA replication and repair. Expanded trinucleotide repeats (TNRs) and other structure-forming repeats break at a greater frequency than non-repetitive DNA; types of DNA breaks that occur include nicks, gaps and double-stranded breaks (DSBs). These lesions must then be repaired in the context of the repetitive DNA. Much of the time the cell will succeed in repairing DNA damage at structure-forming repeats with fidelity, i.e. with no loss or gain of genetic material, thus preserving genome integrity. However, due to both the repetitive nature of the tract as well as the structure-forming potential, mistakes that lead to repeat expansions or contractions are relatively frequent.

There are multiple pathways that repair DNA damage that occurs within TNRs and other repetitive sequences. For example, nicks and gaps can be repaired by base excision repair (BER), or by transcription-coupled repair (TCR) within transcribed regions, both of which can generate TNR expansions (for recent reviews see [2,5] and the review by Polyzos and McMurray in this issue). Damage that results in DSBs can be repaired by various types of end-joining, by annealing of processed ends, or by recombination-based mechanisms using either a sister chromatid or homolog as the template. In addition, recombination is a primary mechanism used in restarting stalled or collapsed replication forks and in repairing gaps left behind the replication fork. This review will summarize the current knowledge about the role of mitotic recombination in generating genomic changes within repetitive DNA. We will focus on structure-forming triplet repeats, but with comparisons to results found at other biologically relevant repeats and DNA structures.

1.1. DNA damage at expanded trinucleotide repeats is repaired by recombination

Deletion of genes required for recombination results in increased breakage of expanded TNRs, suggesting that recombination is normally required for healing these DNA breaks [6,7]. In replicating yeast cells, homologous recombination (HR) and ligase 4-dependent end joining (EJ) both contribute to the repair of breaks at CAG repeats [6]. Genome-wide studies to identify novel genes preventing DSBs at GAA

Abbreviations: TNR, trinucleotide repeat; HR, homologous recombination; EJ, end joining; DSB, double stranded break; SCR, sister chromatid recombination; NHEJ, non homologous end-joining; MMEJ, microhomology-mediated end-joining; DM1, myotonic Dystrophy type I; HD, Huntington's Disease; SCA, spinocerebellar ataxia; BER, base excision repair; TCR, transcription-coupled repair; TLS, translesion synthesis; PRR, post-replication repair; SSA, single strand annealing; G4, DNA G-quadruplex forming DNA

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http://dx.doi.org/10.1016/j.dnarep.2017.06.018

Available online 09 June 2017

and Alu repeats identified several recombinational repair proteins as important, among them the nuclease Mre11, whose absence increased fragility of both repeats [7,8]. Additionally, dividing cells deficient in replication proteins exhibit cell cycle arrest and gross chromosomal rearrangements at Alu repeats because recombination intermediates cannot be resolved, which results in DSBs [8]. Failure to heal breaks at expanded TNR repeats can have dire consequences for cells. Yeast cells that lack Rad52 or Ligase 4 and have expanded CAG repeat tracts undergo frequent cell cycle arrest and cell death [9].

Traditionally, DNA repair using recombination has been considered to be an error-free form of repair. However, in actuality, recombination can be highly mutagenic and a source of genomic instability [10–13]. Though they are required for repair and cell health, both HR and EJ can be mutagenic when they occur within repetitive DNA, resulting in a loss (contraction) or gain (expansion) of repeat units [14]. This is largely due to the challenges of replicating or aligning DNA across a repetitive region, especially one that has formed DNA secondary structures. These DNA structures are varied and include DNA hairpins (common in CAG/CTG and CGG/GCC repeats or inverted repeats), triplexes (formed by purine-rich repeats such as GAA/TTC) and G quadruplexes (for reviews see [5,15–17]). Though the structures are different, the common theme is that they impede DNA transactions so that replication and repair cannot proceed with fidelity within the repetitive sequence. This inaccurate repair can lead to the incorporation of errors that can range from the aberrant insertion/deletion of DNA bases, as seen in TNR repeat genetic diseases, to genomic rearrangements and loss of heterozygosity, which are commonly seen in cancers. Historically, misalignment of alleles during meiotic crossover was shown to be a mechanism for $(GCN)_n$ repeat expansions that code for polyalanine tracts [18], but discounted as a mechanism for length changes of other TNRs, such as $(CAG)_n$ repeat tracts encoding polyglutamine. However, these early studies focused on meiotic recombination and did not explore mitotic recombination as a potential mechanism for repairing DNA damage at TNRs and causing repeat instability. The following sections will delve into the various roles of recombination during DNA repair, how each contributes to genomic maintenance of repeat sequences, and the current knowledge of how recombination pathways result in repeat instability.

2. Recombination during replication results in repeat instability

2.1. Homology-dependent recombinational repair of forks stalled by DNA structures

Addition of repeat units by definition involves DNA synthesis. Incorporation of additional bases might arise as a result of strand slippage either during replication [19] or during fork restart [3]. DNA structures formed by repetitive DNA sequences are impediments for DNA synthesis and can cause fork stalling, or gaps behind the replication fork if bypassed. GAA/TCC triplexes and GGC/CCG repeats strongly interfere with replication progression, acting as site-specific barriers [20–22]. CAG/CTG repeats are much weaker barriers [23–26] but their replication generates joint molecules that likely represent both reversed fork and sister chromatid recombination intermediates [27,28]. Single stranded gaps occur when leading and lagging strand synthesis becomes uncoupled (reviewed in [29]), and pre-existing DNA nicks or gaps can become DSBs if replicated [5,30,31].

After a replication fork stalls at a DNA repeat structure, several types of fork restart can be envisioned (see [32,33] for reviews on fork restart). First, unwinding of the DNA structure by a helicase may allow replication to continue without replisome dissociation, which would not lead to repeat instability unless slippage occurred (Fig. 1C). Second, a fork reversal or template switch mechanism could be used to replicate through the DNA structure (Fig. 1A, B). The outcome in terms of repeat contraction or expansion will vary depending on where the un-excised hairpin forms (template or nascent strand) and which hairpins are resolved. There are several possibilities for hairpin formation or misalignments during the fork restart process, which would likely involve the HR machinery (Fig. 1A). Third, a break in the DNA could lead to an HR-dependent stand invasion, either on the same DNA template (broken fork repair (BFR), similar to what is drawn in Fig. 1A but initiated from a break) or on a different template (ectopic break-induced replication (BIR; Fig. 2)). BIR is kn wn to be a mutagenic process [10,34,35]. Finally, repeat expansions are also known to occur due to hairpin impairment of Okazaki flap processing by the FEN1 endonuclease (Fig. 1D; [36]).

Recently it was shown that expanded CAG repeats, which are natural replication fork barriers, result in the transient localization of chromosomes to the nuclear pore during S-phase [37]. This relocation was dependent on replication, occurred in late S phase and was resolved by G2, and prevented repeat fragility. Yeast chromosomes exposed to both the alkylating agent MMS and the fork stalling drug hydroxyurea similarly relocate to the nuclear periphery [38]. Interestingly, failure to relocate to the pore led to increased Rad52-dependent CAG repeat expansions and contractions. Taken together, these results suggest that relocation to the nuclear pore facilitates fork restart, and this may protect against DSBs and mutagenic Rad52-dependent repair [37,39]. Posttranslational modification of key repair proteins by sumoylation may be important in the re-localization and fork restart process, as deletion of the Slx5/8 SUMO-dependent ubiquitin ligase resulted in an increase in repeat instability and a decrease in nuclear localization of the expanded CAG repeat.

Recombination-mediated repeat instability at the replication fork is not unique to TNR repeats. In *S. pombe*, Swi1 promotes replication fork progression through telomeric repeats and prevents telomeric instability and aberrant recombination at telomeres [40]. Additionally, in human cells, impaired replication of telomeric repeats results in fragile telomeres [41] and efficient replication requires the telomeric binding protein TRF1 and the helicases BLM and WRN to unwind G4 structures that can impede replication machineries [41,42]. Interestingly, replication in the context of HR repair or HR-dependent fork restart proceeds with less fidelity and more mutations than normal replication, even without the complication of copying DNA repeats [43–45]. Recently, GAA repeats have been shown to induce mutagenesis up to 8 kb away from the repeat site in yeast, presumably through an HR-mediated repair event [22,46–48]. The authors hypothesize that a barrier to replication caused by a GAA secondary structure recruits the low-fidelity Polζ polymerase. DSB formation or fork stalling at the repeat leads to strand invasion of the homolog, where synthesis with Polζ leads to mutagenesis [46,48]. This repeat-induced mutagenesis (RIM) has also been observed for H-DNA and Z-DNA forming sequences introduced into mammalian cells [49–51]. Taken together, expanded TNRs and other structure-forming repeat sequences are sites of replication fork collapses that are repaired by HR, and this repair may result in an increase in the mutation rate.

2.2. The role of helicases in replication of structure-forming repeats

Helicases have been shown to be important in preventing replication-associated repeat instability. One important helicase that helps to resolve repeat-induced replication fork stalls in yeast is the helicase Srs2 (Fig. 1C). Using direct visualization of fork stalling *in vivo* by 2D gel electrophoresis, Srs2 has been shown to facilitate replication past a $(CGG)_{45}$ repeat that causes a barrier to replication via hairpin formation [52]. Srs2 had no activity on replication barriers due to G-quadruplex structures or protein binding, thus it is specific to DNA hairpins. Srs2 function at stalled forks was unique among the helicases tested (Sgs1, Pif1, Rrm3), and was dependent on its helicase activity and its ability to interact with PCNA, but not on its Rad51 displacement motif. Srs2 can also unwind CAG hairpins *in vitro* and prevent expansions that occur during template switch [53,54] and during sister-chromatid recombination [27]. Recently, separation of function alleles were used to