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Genome instabilities arising from ribonucleotides in DNA

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ABSTRACT

Genomic DNA is transiently contaminated with ribonucleotide residues during the process of DNA replication through misincorporation by the replicative DNA polymerases α, δ and ε, and by the normal replication process on the lagging strand, which uses RNA primers. These ribonucleotides are efficiently removed during replication by RNase H enzymes and the lagging strand synthesis machinery. However, when ribonucleotides remain in DNA they can distort the DNA helix, affect machineries for DNA replication, transcription and repair, and can stimulate genomic instabilities which are manifest as increased mutation, recombination and chromosome alterations. The genomic instabilities associated with embedded ribonucleotides are considered here, along with a discussion of the origin of the lesions that stimulate particular classes of instabilities.

1. Introduction

The DNA replication process is highly accurate in terms of choosing the correct base and correct sugar, and discrimination of the sugar moiety of the chosen nucleotide is an intrinsic feature of the replicative DNA polymerases [1–3]. Nonetheless, this discrimination is not perfect and given the additional challenge of higher rNTP pools compared to dNTP pools, ribonucleotides are inserted into DNA during replication at a rate of approximately one per every thousand bases replicated [4,5]. In addition to locally distorting the DNA helix from a B-form to an A-form [6–8], ribonucleotides can hinder replication and transcription machineries and sensitize the DNA backbone to alkali [9,10].

Generally, normal cells with intact RNase H enzymes efficiently recognize and remove ribonucleotides that are in a RNA:DNA hybrid form in a process called RER, ribonucleotide excision repair (Fig. 1). Ribonucleotides that remain in DNA, usually when the RNase H enzymes are defective, ultimately result in genomic instabilities. These form three broad categories: mutagenesis with a signature of small deletions in mononucleotide or dinucleotide tracts, increased homologous recombination, and chromosome rearrangements, loss and truncations. Embedded ribonucleotides can be a target for DNA topoisomerase I (Top1) [11,12] and some ribonucleotide-promoted instabilities are Top1-dependent (Fig. 2). Top1 has a preference for ribonucleotides incorporated by DNA polymerase ε [13], resulting in an emphasis on genome instability from DNA polymerase ε errors of rNTP misincorporation. This review examines the known Top1-dependency and DNA polymerase-misincorporation for stimulation of instability, but also explores the contribution of DNA polymerases α and δ in genome instability arising from rNTP misincorporation on the lagging strand during replication. The nature of instability hotspots in light of apparently random misincorporation of ribonucleotides into DNA is also discussed.

R-loops resulting from transcription/replication collisions have been shown to cause double-strand breaks and genomic instability [14]. R-loops are the target of RNase H1, which recognizes RNA:DNA hybrids of at least three nucleotides and whose action seems to be related to R-loops arising during transcription [15]. Protection against genome instability resulting from transcription-arising R-loops has been recently reviewed [14,16,17] and will not be discussed here. Recent reports on genome instability in mutants lacking both RNase H1 and RNase H2 enzymes and the implications for genome destabilizing lesions will be highlighted.

2. Sources of ribonucleotides in DNA

The most common source of ribonucleotides embedded in genomic DNA comes from the replicative polymerases and the failure to completely discriminate between rNTPs and dNTPs. The discrimination pocket that is a feature of all DNA polymerases ensure high accuracy in selecting the correct nucleotide during replication [3] but nonetheless there is an error rate of approximately of 1/1000 bases replicated [5]. The highest contributor to this is DNA polymerase ε, but DNA polymerases α and δ also misincorporate ribonucleotides. This may be due to a critical residue in the steric gate of the polymerase for sugar

Abbreviations: AGS, Aicardi Goutieres Syndrome; DSB, double-strand break; HR, homologous recombination; Top1, topoisomerase 1; LOH, loss of heterozygosity; ncRNA, non-coding RNA; RER, ribonucleotide excision repair; dNTP, deoxynucleotide triphosphate; rNTP, ribonucleotide triphosphate; rNMP, ribonucleotide monophosphate

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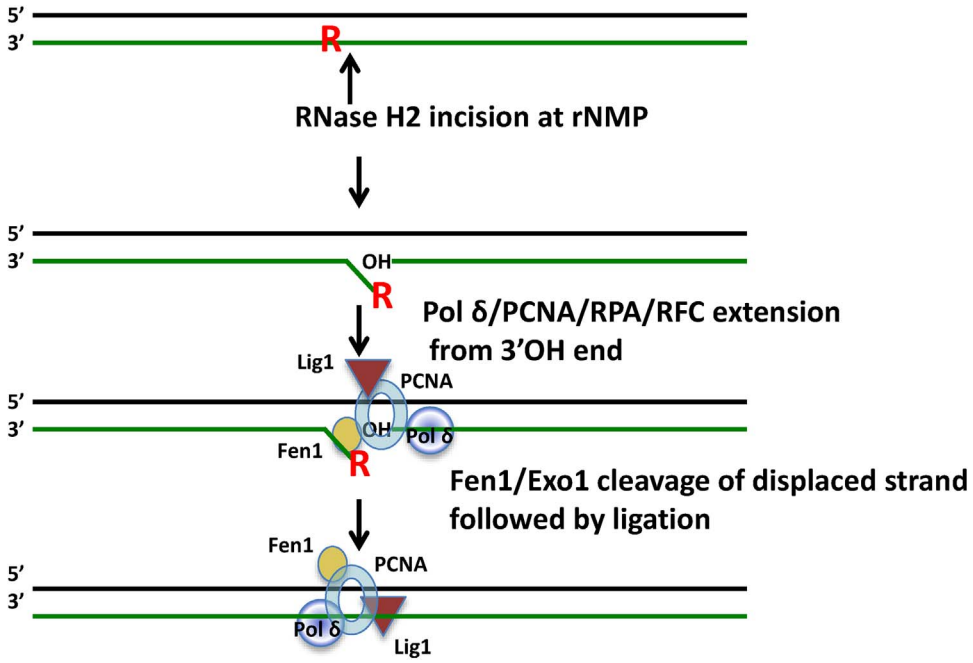

Fig. 1. The RER process. RNase H2 recognizes a rNMP residue (red R) in duplex DNA and makes an incision 5′ to the residue, creating a 3′OH end. Extension of this end by DNA Pol δ as shown or DNA pol ε (blue circle) along with associated factors PCNA (light blue open oval ring), the RFC clamp loader of PCNA and the single strand DNA binding protein RPA displaces the rNMP containing end, generating a flap. The flap is removed by the action of Fen1 nuclease as shown (yellow oval) (or Exo1 nuclease), and the nick is then sealed by Lig1 (brown triangle), completely removal of the embedded rNMP residue.

fidelity. DNA polymerase ε has a methionine residue while the DNA polymerases α and δ have a leucine residue in the gate. The methionine residue may be more permissive for incorporation of a sugar with the 2′-OH residue [4,18,19]. The proofreading exonuclease of DNA polymerases ε and δ do not contribute significantly to ribonucleotide removal [4]. Under altered replication conditions, for example when the dNTP pool balance is altered, incorporation of ribonucleotides may be enhanced. Gap repair by the translesion DNA polymerases may be another source of ribonucleotides. Although the replicated tract is a very small fraction of the genome, these polymerases are far more lax in the sugar selection of the base being incorporated and also lack any proofreading editing nuclease. Ribonucleotides may also become incorporated during replication restart, which can use RNA primers for replication priming. Incomplete removal of Okazaki fragment primers is also another potential source of embedded ribonucleotides, although the contribution to the RNA:DNA hybrid load in genomic DNA has not been assessed.

Another potential source of embedded ribonucleotides, particular in

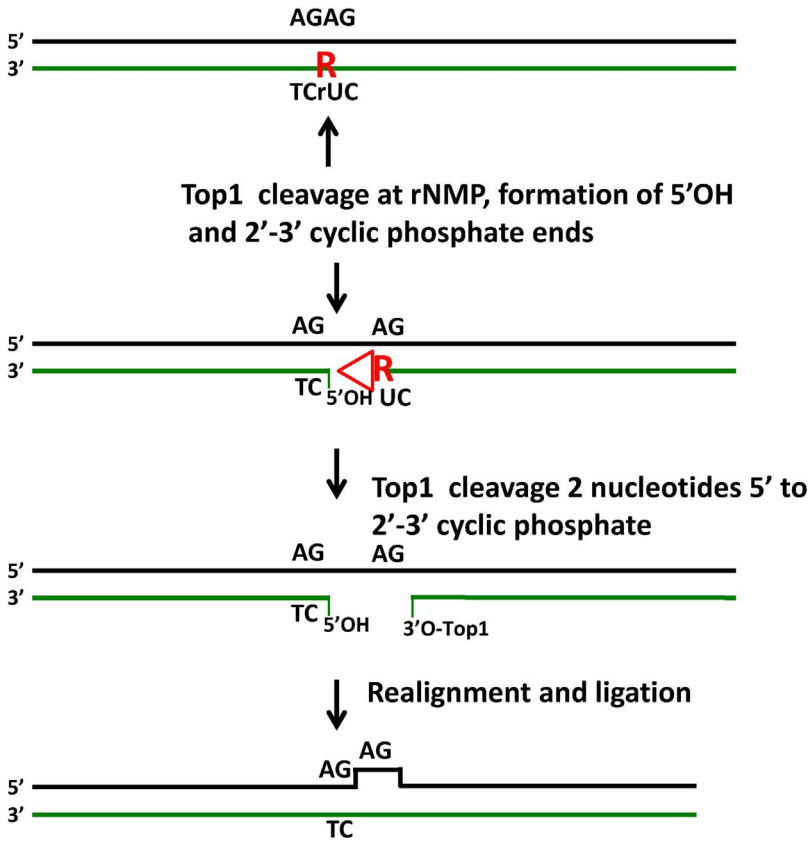

Fig. 2. Top1 processing of rNMP residues. An embedded rNMP residue (red R) is shown in a repeated sequence AGAG:TCrUC. In the absence of the RER process Top1 can cleave at rNMP residues in duplex DNA. Top1 cleaves 3′ to the residue, and in completeting the Top1 cleavage cycle, generates a 5′OH end and a 2′-3′ cyclic phosphate end (red triangle) through nucleophilic attack by the 2′OH group of the ribose sugar. A second cleavage by Top1 2 nucleotides from the 2′-3′ cyclic phosphate end releases this blocked end and the ribose residue. If the resulting gap is in a repeated sequence as shown, slippage alignment can occur during completion of the Top1 reaction and the gap is resealed by Top1, leading to a −2 deletion on the strand that originally contained the rNMP residue.