Ribonucleotides and Transcription-Associated Mutagenesis in Yeast

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Abstract

High levels of transcription stimulate mutation rates in microorganisms, and this occurs primarily through an enhanced accumulation of DNA damage. The major source of transcription-associated damage in yeast is Topoisomerase I (Top1), an enzyme that removes torsional stress that accumulates when DNA strands are separated. Top1 relieves torsional stress by nicking and resealing one DNA strand, and some Top1-dependent mutations are due to trapping and processing of the covalent cleavage intermediate. Most, however, reflect enzyme incision at ribonucleotides, which are the most abundant noncanonical component of DNA. In either case, Top1 generates a distinctive mutation signature composed of short deletions in tandem repeats; in the specific case of ribonucleotide-initiated events, mutations reflect sequential cleavage by the enzyme. Top1-dependent mutations do not require highly activated transcription, but their levels are greatly increased by transcription, which partially reflects an interaction of Top1 with RNA polymerase. Recent studies have demonstrated that Top1-dependent mutations exhibit a strand bias, with the nature of the bias differing depending on the transcriptional status of the underlying DNA. Under low-transcription conditions, most Top1-dependent mutations arise in the context of replication and reflect incision at ribonucleotides incorporated during leading-strand synthesis. Under high-transcription conditions, most Top1-dependent events arise when the enzyme cleaves the non-transcribed strand of DNA. In addition to increasing genetic instability in growing cells, Top1 activity in transcriptionally active regions may be a source of mutations in quiescent cells.

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Mutations are permanent changes to DNA that arise spontaneously or are induced by exogenous DNA-damaging agents. Basic mechanisms of mutagenesis have been intensively studied because of their relevance to evolutionary processes and tumorigenesis. With regard to cancer, mutation patterns can reveal information about the driver(s) of genetic instability, both in terms of primary DNA damage and underlying abnormalities in repair processes [1]. This information, in turn, can inform overall prognosis and tumor management. Mutations can be confined to one or a few base pairs or can affect overall chromosome structure. Small-scale changes include base substitutions, small insertions/deletions, and more complex mutations composed of multiple, closely linked changes. Large-scale alterations such as translocations, inversions, and other types of gross chromosomal rearrangements primarily reflect replication fork breakage/repair and will not be considered here.

Most spontaneous mutations in actively dividing cells arise as errors during DNA replication. Replicative DNA polymerases (DNAPs) are highly accurate but nevertheless insert incorrect nucleotides at low rate, or slip in repetitive regions such as mononucleotide repeats. The resulting base-base mismatches or insertion/deletion loops are efficiently proofread by DNAP exonuclease activity or are removed by the post-replicative mismatch repair (MMR) machinery (reviewed in Ref. [2]). If an error escapes repair, it becomes fixed as a permanent mutation at the next round of replication. In addition to simple mistakes made when replicating "normal" DNA, damaged DNA can force errors by replicative

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DNAPs or engage specialized, but error-prone, translesion synthesis (TLS) DNAPs (reviewed in Ref. [3]). Endogenous sources of DNA damage that contribute to spontaneous mutagenesis include water, reactive oxygen species, reactive nitrogen species, and alkylating agents [4]. For decades, abasic sites and oxidative damage were considered to be the predominant abnormalities in DNA. Recent work has demonstrated, however, that ribonucleotides are the most abundant noncanonical component of DNA (reviewed in Ref. [5]). The effects of ribonucleotides on mutagenesis in *Saccharomyces cerevisiae*, especially in the context of transcription, will be the focus of this review.

Ribonucleotide incorporation into DNA

DNAPs discriminate efficiently between ribonucleotide triphosphates (rNTPs) and deoxyribonucleotide triphospahtes (dNTPs), but the high concentration of rNTPs relative to dNTPs in the nucleotide pool leads to frequent ribonucleotide insertion during DNA replication [6–8]. In yeast, it is estimated that DNAPs introduce 10,000–15,000 ribonucleotides during each duplication of the 12-Mb genome [8]. A similar density of genomic ribonucleotides has been inferred in cultured mouse cells [9,10]. In budding yeast, mutant DNAPs that insert either fewer or more ribonucleotides than wild-type have been particularly useful for studying physiological consequences of ribonucleotides embedded in DNA [7,11]. In addition to ribonucleotide insertion by replicative DNAPs during genome duplication, primase-generated ribonucleotide chains are required to prime the Okazaki fragments that characterize lagging-strand synthesis. If not completely removed during Okazaki fragment maturation, ligase can inefficiently seal a nick flanked by a single 5′ ribonucleotide. Such ligation is frequently aborted to generate a highly toxic 5′-adenlyated ribonucleotide that is reversed by aprataxin [12]. Ribonucleotides that persist as nicks are converted into genome-destabilizing, double-strand breaks at the next round of DNA replication. In addition to their insertion during genome replication, ribonucleotides are likely introduced even more frequently during repair that occurs outside of S phase, when rNTP:dNTP ratios are elevated. Finally, RNA transcripts can be used to prime DNA synthesis [13] or as a template to repair double-strand breaks [14], providing additional potential sources of ribonucleotides in DNA.

Error-free removal of ribonucleotides from DNA

One or a few ribonucleotides embedded in duplex DNA are efficiently removed by the ribonucleotide excision repair (RER) pathway, which has been reconstituted *in vitro* (Fig. 1) [15]. RER initiates with incision at the phosphodiester bond 5′ of the ribonucleotide by the trimeric RNase H2 complex, which is composed of the catalytic Rnh201 and accessory Rnh202 and Rnh203 proteins in yeast [16]. *In vitro*, RNase H2 incision provides a 3' hydroxyl (3'-OH) to prime DNA synthesis, resulting in Pol δ or Pol ε displacement of a ribonucleotide-containing flap that is subsequently removed by the flap endonuclease Rad27/Fen1 or by the Exo1 5′ > 3′ exonuclease. Ligation of the remaining nick completes the repair process. Given their abundance in genomic DNA, it has been speculated that ribonucleotides have important physiological functions. In fission yeast, for example, a site-specific di-ribonucleotide is used to initiate mating-type switching [17]. In budding yeast, ribonucleotide-associated nicks provide a very weak strand-discrimination signal for MMR [18,19] and may be relevant to relieving torsional stress in the wake of the replication fork [20].

Ribonucleotides may be important in some circumstances, but seminal studies in yeast showed that in the absence of RNase H2, their persistence is highly mutagenic and is associated with a distinctive mutation signature. This signature is composed of 2–5 bp deletions that reflect the loss of a repeat unit from a low copy-number tandem repeat [7]. These events were originally proposed to reflect the stalling of DNAP at template ribonucleotides, but the lack of intermediate removal by MMR suggested a replication-independent deletion mechanism [6]. As will be elaborated below, ribonucleotide-dependent deletions require the activity of Topoisomerase I (Top1), an enzyme that removes both replication- and transcription-associated supercoils [21].

Transcription as a source of genetic instability

Using mutation accumulation as an evolutionary time clock makes the simplifying assumption that genomic DNA mutates at a constant rate. Early studies in bacteria and budding yeast, however, demonstrated that induction of gene expression affects induced mutation rates in the corresponding gene [22,23]. Subsequent studies using reporters fused to highly inducible promoters established a firm connection between transcription and elevated mutagenesis of the underlying DNA template [24–26]. This phenomenon is referred to as transcription-associated mutagenesis (TAM; reviewed in Ref. [27]). In *Escherichia coli* and yeast, transcription increases multiple types of base substitutions [28,29]. Using a yeast-based frameshift reversion assay, it was demonstrated that TAM is reduced in the absence of the primary, error-prone TLS synthesis DNAP and is elevated in the absence of error-free repair/bypass pathways [25,30]. These genetic studies suggested that TAM is primarily a consequence of damage to

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