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Transcription-associated mutagenesis in yeast is directly proportional to the level of gene expression and influenced by the direction of DNA replication

Nayun Kim[a,e], *Amy L. Abdulovic*[b], *Regan Gealy*[c], *Malcolm J. Lippert*[d], *Sue Jinks-Robertson*[a,b,c,e,*]

[a] *Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC 27710, United States*
[b] *Graduate program in Biochemistry, Cell and Developmental Biology, Emory University, Atlanta, GA 30322, United States*
[c] *Graduate program in Genetics and Molecular Biology, Emory University, Atlanta, GA 30322, United States*
[d] *Department of Biology, Saint Michael's College, Colchester, VT 05439, United States*
[e] *Department of Biology, Emory University, Atlanta, GA 30322, United States*

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ABSTRACT

A high level of transcription has been associated with elevated spontaneous mutation and recombination rates in eukaryotic organisms. To determine whether the transcription level is directly correlated with the degree of genomic instability, we have developed a tetracycline-regulated *LYS2* reporter system to modulate the transcription level over a broad range in *Saccharomyces cerevisiae*. We find that spontaneous mutation rate is directly proportional to the transcription level, suggesting that movement of RNA polymerase through the target initiates a mutagenic process(es). Using this system, we also investigated two hypotheses that have been proposed to explain transcription-associated mutagenesis (TAM): (1) transcription impairs replication fork progression in a directional manner and (2) DNA lesions accumulate under high-transcription conditions. The effect of replication fork progression was probed by comparing the mutational rates and spectra in yeast strains with the reporter gene placed in two different orientations near a well-characterized replication origin. The effect of endogenous DNA damage accumulation was investigated by studying TAM in strains defective in nucleotide excision repair or in lesion bypass by the translesion polymerase Polζ. Our results suggest that both replication orientation and endogenous lesion accumulation play significant roles in TAM, particularly in terms of mutation spectra.

1. Introduction

Mutagenesis is an important contributor to both evolutionary processes and carcinogenesis. While the primary DNA sequence (e.g., homopolymer tracts, di- or tri-nucleotide repeats, palindromes) accounts for the presence of certain mutational hotspots within the genome, transcription is also among the factors that can lead to increased mutagenesis [1]. An effect of transcription on genetic stability in a eukaryotic system was first observed using a segment of the RNA polymerase I transcribed ribosomal DNA locus – denoted *HOT1* – that functioned as a *cis*-acting enhancer of recombination

* *Corresponding author at*: Department of Molecular Genetics and Microbiology, Duke University Medical Center, Research Drive, Jones Bldg., Box 3020, Durham, NC 27710, United States. Tel.: +1 919 681 7273; fax: +1 919 684 2790.
E-mail address: sue.robertson@duke.edu (S. Jinks-Robertson).
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in *Saccharomyces cerevisiae* [2]. A similar increase in mitotic recombination and an additional elevation of spontaneous mutation rates in yeast has been associated with high levels of transcription carried out by RNA polymerase II [3–7]. The effect of such transcription-associated mutagenesis (TAM) and recombination (TAR) would be expected to be generally deleterious (e.g., the loss of a tumor suppressor), but may also be advantageous, promoting processes such as in immunoglobulin somatic hypermutation [8].

TAM and TAR in yeast have been attributed both to indirect and direct effects of transcription ([6]; reviewed in [1]). Evidence for a direct role of transcription in stimulating recombination has come from the analysis of *HPR1*-deficient yeast strains, where up to 1000-fold increases in recombination between direct repeats have been observed [9]. This hyper-recombination phenotype in *HPR1*-deficient strains has been shown to be directly related to the movement of RNA polymerase II (RNAP) complexes through the recombination substrate; when transcription elongation was inhibited by the insertion of a transcription termination sequence between the two direct repeats, the hyper-recombination phenotype was abolished [10]. Subsequently, Hpr1 was shown to be a component of the THO/TREX complex, inactivation of which leads to the elongation and stabilization of RNA:DNA hybrids (R-loops) formed during transcription [11]. Such persistent R-loops can induce frequent arrest of transcription complexes and, therefore, impede the processive movement of RNAP holoenzymes.

Based on the observed arrest of RNAP complexes in *HPR1*-deficient strains, three possible explanations for the initiating steps of TAR and/or TAM have been suggested. First, replication blockage arising from the collision between RNAP and the replication fork may result in collapse of the fork and its subsequent rescue by recombination. Second, arrested or paused RNAP itself might recruit the DNA repair machinery, triggering a process similar to transcription-coupled DNA repair [11,12]. Finally, the extended single-stranded character of the non-transcribed strand of DNA during highly activated transcription might enhance the accessibility of DNA to endogenous damaging agents, leading to an increase in mutation- or recombination-initiating lesions. Evidence consistent with increased lesions arising from the enhanced accessibility of the highly transcribed region to DNA damaging agents has been reported [13]. Also, at least for TAR, there is evidence that chromatin remodeling associated with the activation of transcription may be a contributing factor [14]. Finally, the stress resulting from transcription-associated changes in DNA topology/superhelicity may lead to nicks or breaks in DNA [10,15,16].

With regard to a possible link between transcription and replication in genome instability, differences in the accumulation of mutagenic or recombinogenic DNA damage might lead to a disparity in mutagenesis when highly activated transcription is oriented toward versus away from an approaching replication fork. A recent report has demonstrated that the relative orientation of the replication fork with respect to the direction of transcription indeed plays a significant role in transcription-associated recombination in a plasmid-based assay system [17]. This effect has been attributed to an enhanced replication block when the transcription machinery is directed toward an oncoming replication fork. Such transcription-dependent replication pause/stall sites have been observed in yeast [17,18] as well as in bacteria [19]. In addition, in *E. coli*, it has been observed that the manner in which a given DNA lesion is dealt with depends on the direction of replication [20]. When a plasmid containing a defined DNA lesion was transformed into *E. coli* cells, the resulting mutation frequency was distinctly different depending on whether the lesion was located on the leading-strand template versus the lagging-strand template [20].

Previously, we have studied the role of transcription in mutation and recombination in yeast using a chromosomal *LYS2* reporter system in which the low-level *LYS2* promoter (*pLYS*) was replaced with the highly inducible *GAL1-10* promoter (*pGAL*) [5–7]. When *pGAL* was highly induced, the rate of spontaneous frameshift mutation was elevated 10- to 20-fold relative to that observed when *pGAL* was not induced [7]. We have observed a similar stimulatory effect of transcription when examining *LYS2* forward mutations [21] or homologous recombination between two *lys2* alleles positioned either on non-homologous chromosomes or as direct repeats [5,6,12]. The question remains, however, whether there is a direct and quantitative relationship between the level of transcription and the degree of genome instability. The *pGAL*-mediated *LYS2* reporter system so far used to study TAM allows the reliable comparison of only the two extremes of transcription (very high in Gal80$^-$ strains and very low in Gal80$^+$ strains), and is not suitable for the question posed above.

In the current study, we employed a tetracycline-regulated system that allows the level of transcription of a chromosomal mutational target to be varied over a broad range. Because our previous analysis of *LYS2* forward mutations revealed that 2-nt deletion events are a distinctive signature of TAM [21], we examined reversion of the *lys2ΔA746+1* frameshift allele, a target that can specifically detect −2 events. We found that there was a linear and proportional relationship between the level of transcription and the rate of reversion, suggesting a direct involvement of transcription in the induction of mutagenesis. In addition, the question of whether convergence between the RNAP machinery and the replication fork contribute to TAM was addressed by assaying mutation rates and examining mutation spectra when the reporter gene was placed in both orientations relative to a defined replication origin on chromosome III. Although the overall mutation rate was not affected by the direction of fork movement through the highly transcribed region, there were clear effects on the mutation spectrum.

2. Materials and methods

2.1. Construction of yeast strains

All yeast strains used in this study were derived from YPH45 (*MATα ura3-52 ade2-101$_{oc}$ trp1Δ1*). Plasmids pCM225 and pCM244 used in the construction of the *pTET* reporter system were acquired from Euroscarf and are described in Belli et al. [22]. The strains with the doxycycline (Dox)-regulated *lys2ΔA746* allele (*pTET-lys2ΔA746*) in both orientations relative to an origin of replication on Chromosome III, *ARS306* [23], were constructed by the following steps (specific details