The effect of elevated dNTPs on DNA replication fidelity

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Abstract

During DNA replication, DNA polymerases duplicate the cell's DNA prior to cell division. DNA polymerases synthesize a new strand of DNA by incorporating deoxyribonuclotides (dNTPs), the building blocks of DNA, opposite the parental strand. Completion of DNA replication requires adequate dNTP levels. However, when dNTP pools are elevated, there is an increase in the number of errors in DNA replication leading to increased mutation rates. Importantly, many human cancer cells exhibit elevated dNTP pools, which may contribute to tumorgenesis and/or cancer progression. We are interested in the way in which elevated dNTP pools affect both the error rate of DNA polymerases and the types of mutations that are generated under these conditions. Several reporters for different mutation types are available in *Saccharomyces cerevisiae*, making this an ideal model system. In *S. cerevisiae*, we have modulated the dNTP pools with mutations in ribonucleotide reductase, which catalyzes the rate-limiting step in dNTP synthesis, leading to elevated dNTP levels. In addition, these yeast strains lack the highly conserved mismatch repair system, which corrects errors in replication. This will provide us a more accurate picture of the extent and type of polymerase error under different dNTP concentrations.

Eukaryotic DNA Replication

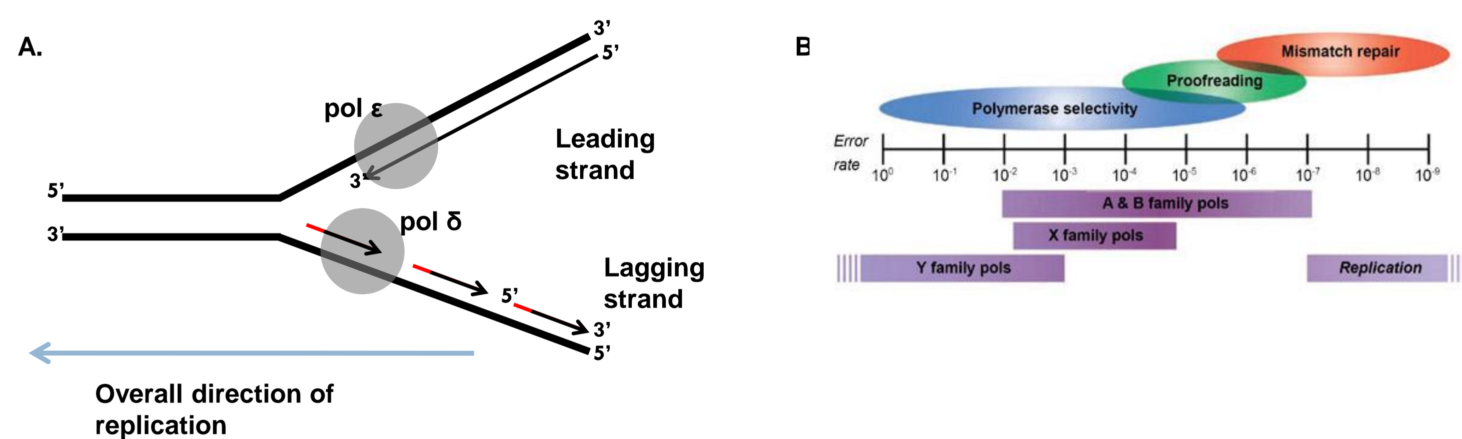


Figure 1. A. DNA replication is a process of copying the cell's DNA before division. Each DNA strand is used as a template for DNA synthesis, which is carried out by DNA polymerases. B. There are many types of DNA polymerases, that are used in different circumstances. Replicative polymerases have high fidelity, resulting in an error rate of 1 in every 10 million bases copied, whereas Y-family polymerases are very error-prone. The mismatch repair (MMR) system is associated with the replication fork and locates any mistakes that remain after the fork has passed. This increases the fidelity of DNA replication an additional 100- to 1000-fold, leading to error rates of 1 in every 1-10 billion bases copied. This extraordinary level of fidelity is critical in avoiding the accumulation of mutations in each subsequent generation.

In *S. cerevisiae*, MSH2-MSH6 and MSH2-MSH3 initiate mismatch repair (MMR).

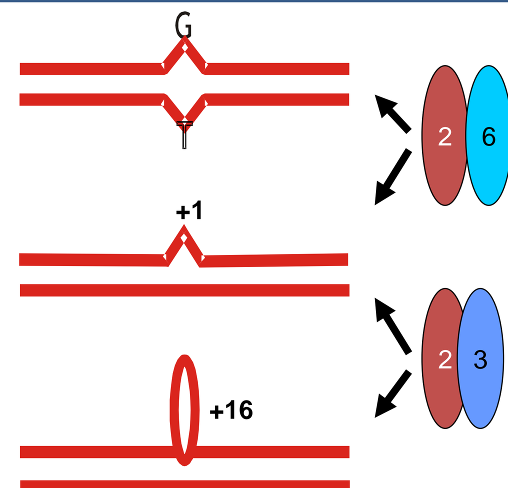


Figure 2. MMR recognizes and binds polymerase errors such as insertion/deletion loops and misincorperations of bases and targets them for repair. MMR is a highly conversed process. Two protein complexes, Msh2-Msh3 and Msh2-Msh6, recognizes replication errors. Msh2-Msh3 recognizes and binds large insertion/deletion mismatches, while Msh2-Msh6 responds to base-base and small insertion/deletion mismatches. Once the mispair is bound by one of these complexes, downstream factors are recruited to initiate unwinding of the newly synthesized DNA strand and its degradation by exonucleases. DNA polymerase then re-synthesizes the nascent strand and ligase seals the nick to generate an intact strand of DNA.

RNR Complex

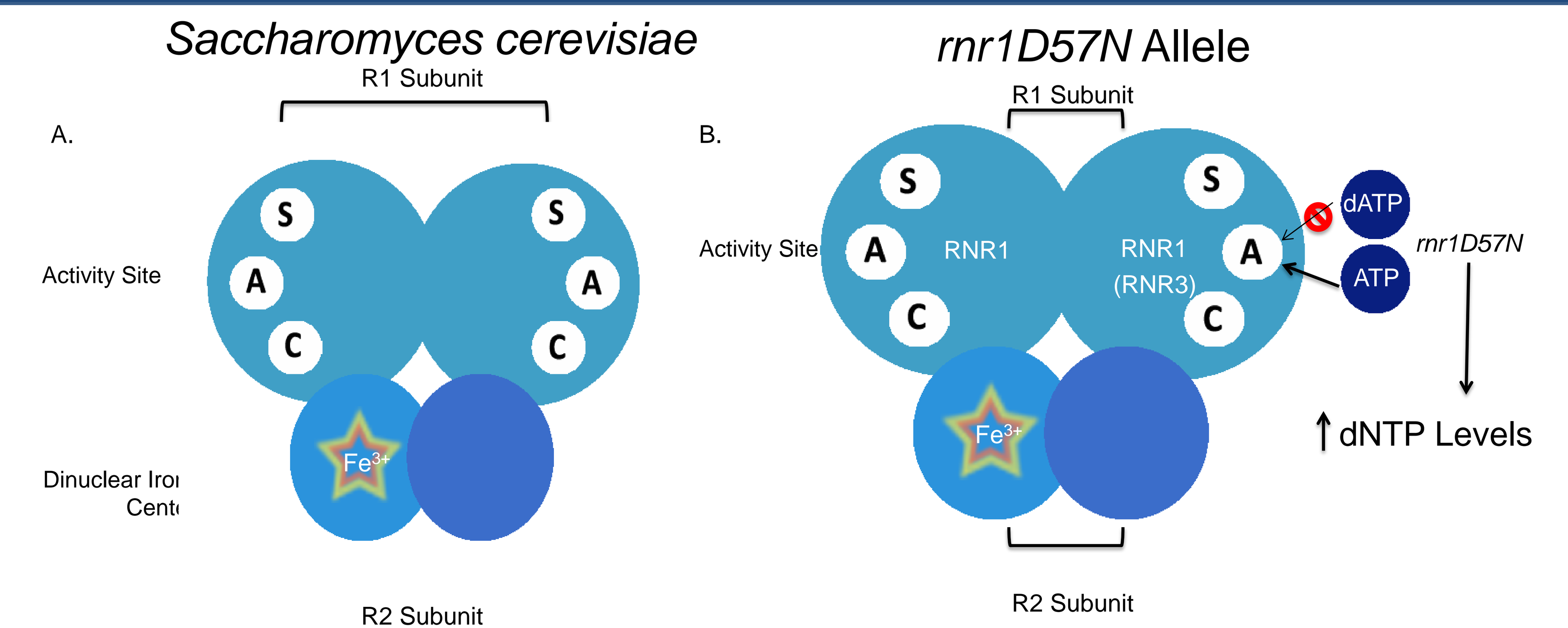
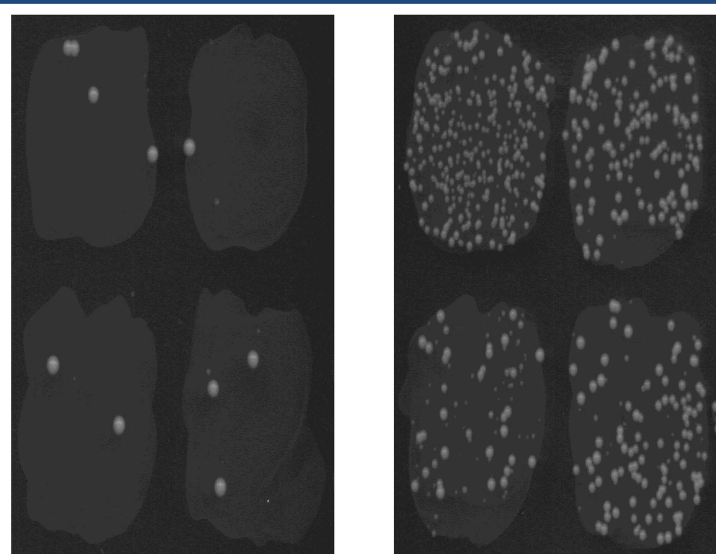


Figure 3. DNA polymerases require an adequate pool of dNTPs in order to completely synthesize a copy of the genome. The rate-limiting step in dNTP synthesis is catalyzed by ribonucleotide reductase. **Panel A** is a cartoon of RNR from budding yeast, *Saccharomyces cerevisiae*, is shown here. Rnr1 forms a homodimer, or as a heterodimer with Rnr3 under DNA damaging conditions, that interacts with a heterodimer of Rnr2 and Rnr4 . Rnr2 includes a dinuclear iron-radical center which is essential for the reduction of NDPs, while Rnr4 is necessary for stability and folding of the complex . **Panel B** shows the mutation of aspartic acid in position 57 to asparagine destroys regulation in the allosteric activity site. This mutation results in a 2-fold increase in dNTP pools. We are using this allele as a tool to examine the effects of elevated dNTPs on the fidelity of DNA synthesis. In order to get a true sense of the polymerase-induced mutation rate in the presence of *rnr1D57N*, we have also examined the fidelity of DNA replication in the absence of MMR.

Mutation rates in *Saccharomyces cerevisiae* Canavanine resistance patch assay



wild type MMR defective

Figure 4: We use the canavanine resistance reporter assay to determine the mutation rate in different yeast strain backgrounds. Mutations in the *CAN1* gene allow the cells to grow in the presence of canavanine, a toxic analog of arginine. When the overall mutation rate in the cell is high, there will be more growth in the presence of canavanine. The left panel demonstrates the amount of growth on canavanine in the wild-type background, with intact MMR. On the right, MMR has been disrupted, elevating the cellular mutation rate. In this case, there is significantly higher growth on canavanine plates. We can perform this assay in a quantitative way to obtain mutation rates.

Mutation Rates for Canavanine resistance patch assay

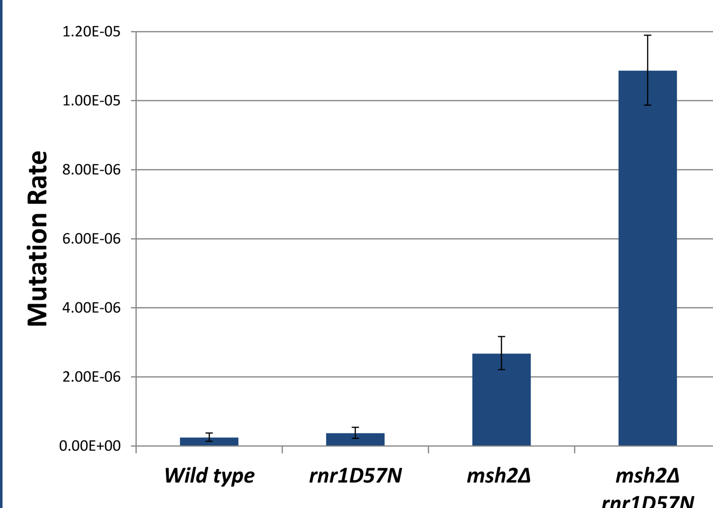


Figure 5. We calculated mutation rates at the *CAN1* locus. We can compare the increase in mutation rates to that of the Wild type. As you can see the msh2Δ has about a 20-fold increase in mutation rate, while the double deletion with the *msh2Δ rnr1D57N* shows synergistic effect. This shows us MSH2 (and MMR) corrects polymerase errors made in the presence of the *rnr1D57N* mutation. When the mutation is combined with the *msh2Δ*, there is no repair mechanism to correct the mistakes being made by the polymerase.

Altered Mutation Spectrum with *rnr1D57N*

Types of mutation	Wild Type #	Wild Type %	*rnr1D57N* #	*rnr1D57N* %	*msh2Δ* #	*msh2Δ* %	*rnr1D57N msh2Δ* #	*rnr1D57N msh2Δ* %
Base substitution								
GC pair	6	55%	10	63%	4	31%	3	18%
AT pair	3	27%	3	19%	2	15%	0	0%
Frame shift								
Deletion	1	9%	1	6%	6	46%	4	24%
Insertion	0	0%	2	12%	1	8%	10	59%
Large deletion	1	9%	0	0%	0	0%	0	0%
Total	11	100%	16	100%	13	100%	17	100%

Table 1: The *can1* gene from canavanine resistant colonies was PCR amplified and sequenced to determine the mutation spectra that arose in the presence of *rnr1D57N* with or without MMR. (MMR is disrupted in the msh2Δ background.) This allows us to determine what type of mutations (misincorporation or frame shift) the DNA polymerase(s) are prone to incorporating e in these strain backgrounds. In this preliminary data set, it is noteworthy that *rnr1D57N* leads to a substantial increase in insertion mutations, which are rare events for any DNA polymerase. I am currently collecting more canavanine resistant colonies to sequence, since the total in this data is relatively low. (Data in this table is taken from Xu et al, 2008.) An increase in insertion mutations indicates that the polymerase is preferentially adding extra nucleotides when dNTP pools are elevated. Alternatively, an error-prone polymerase is gaining access to the replication fork and making these mistakes.

Gene Disruption by Homologous Recombination

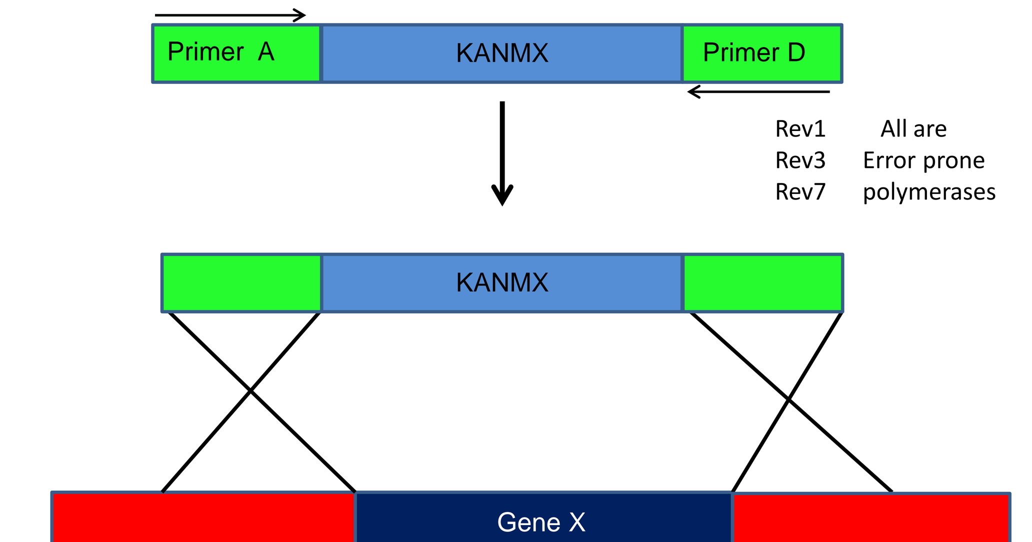


Figure 6: We were interested in which polymerase is responsible for incorporating mutations in response to elevated dNTP pools. We therefore disrupted three genes that encode subunits of error-prone polymerases , polymerases that make more mistakes when replicating the DNA, to determine their contribution to the observed mutation rate. We constructed these deletions in the *rnr1D57N* background, again with or without MMR. To do this, I amplified, by PCR, the appropriate locus of three strains from the yeast deletion collection (constructed to disrupt every non-essential open-reading frame in yeast). These strains had deletions of *rev1*, *rev3* and *rev7*. I used primers specific for the upstream and downstream sequence of each gene, transformed with the PCR product and selected for integration events. We are now determining the mutation rates in these different strain backgrounds.

Future Direction

We have observed that a small increase in dNTP levels has a significant effect on polymerase fidelity. We will next test the effect of deleting error-prone polymerases, to determine their contribution to this mutation rate. We will further characterize the effect on polymerase fidelity by sequencing the *can1* locus of resistant cells in all the strain backgrounds described . A longer term goal will be to look at the effect of even higher dNTP levels on DNA polymerase fidelity, using the same approach described here. This can be done by exposing the *rnr1D57N* mutant strains to DNA damage – this leads to a further 10-fold increase in dNTP levels. However, the DNA damage might make the interpretation of the mutation rates complicated. We can also use a strain that induces higher levels of *RNR1* expression, which also leads to high dNTP pools. An alternative approach will be to use different alleles of RNR1 that lead to elevated and unbalanced dNTP pools. These strains are currently being constructed in the lab [The Surtees lab is funded by NIH-GM87459].