The Effect of dNTP Alterations on Genome Stability

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Deoxyribonucleoside triphosphates (dNTPs) are the building blocks of DNA. Previous studies showed that unbalanced dNTP levels reduce the fidelity of DNA synthesis, increasing mutation rates. However, DNA mismatch repair (MMR), which targets replication errors for repair, was present in these studies, potentially masking the full effect of the altered dNTP pools. Therefore, using *Saccharomyces cerevisiae*, or budding yeast as a model system, we have combined mutations in *RNR1*, encoding a subunit of ribonucleotide reductase (RNR), that skew the dNTP pools *in vivo* with a deletion of *MSH2*, eliminating MMR. Mutation rates will be determined using the canavanine resistance assay; the *can1* locus of canavanine resistant colonies will be sequenced to determine whether altered dNTP pools change the spectrum and/or location of mutations. Tumor cells often have altered dNTP pools. Therefore knowing their effect on replication fidelity in different genetic backgrounds is critical in understanding processes that contribute to carcinogenesis.

Eukaryotic DNA Replication

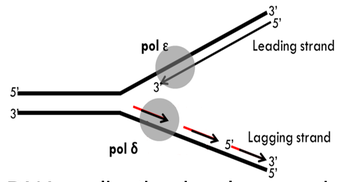


Figure 1. DNA replication involves unwinding the DNA double helix which creates a leading and a lagging strand from which the new strand is synthesized by a DNA polymerase. The building blocks of this new strand are deoxyribonucleoside triphosphates (dNTPs). dNTP levels are tightly regulated to ensure the fidelity of DNA replication.

Mismatch Repair

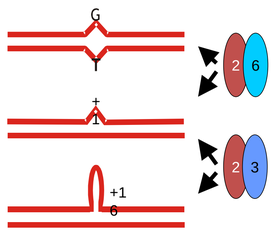
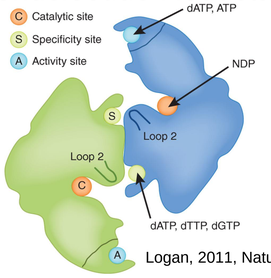


Figure 2. DNA replication errors, either misincorporation events or insertion or deletion events, lead to mismatched DNA structures. The mismatch repair system (MMR) accurately identifies and directs repair of the mismatch. The structures are recognized by protein complexes MSH2/MSH3 or MSH2/MSH6. Recognition initiates downstream steps in repair. MSH2 is present in both complexes and thus is critical to the MMR system.

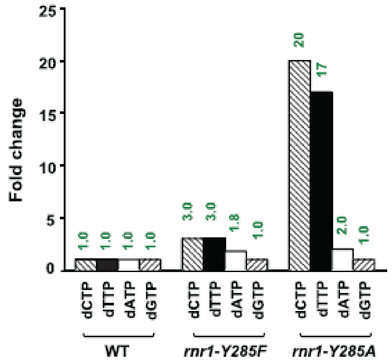
Ribonucleotide Reductase



Logan, 2011, Nature.

Figure 3. Ribonucleotide reductase (RNR) is an enzyme that catalyzes the rate limited step in dNTP production, i.e. the conversion of NDPs to dNDPs. The activity site of Rnr1 is responsible for regulating the total dNTP pool size and the *D57N* mutation leads to elevated, but balanced dNTP pools. Loop 2 connects the activity site to the specificity site and controls the access of each dNTP to the activity site. Mutations in the specificity site lead to an altered ratio of the four dNTPs. Both defects are mutagenic

dNTP pools in different *rnr1* backgrounds



Kumar *et* al., 2011, Nucleic Acids Research

Figure 4. We used *Saccharomyces cerevisiae* as our model system. In order the alter the balance of dNTP pools, I used strains of yeast which have amino acid substitutions in loop 2 of the large subunit of Rnr1. Previous studies have shown that these mutations create unbalanced pools. Both of the mutated strains show a greater increase in the levels of dCTP and dTTP while dATP and dGTP are largely unaffected. I then constructed *msh2* deletion strains in the mutant *rnr1* backgrounds to test the true fidelity of DNA replication in the presence of unbalanced dNTP pools.

Canavanine Resistance Assay

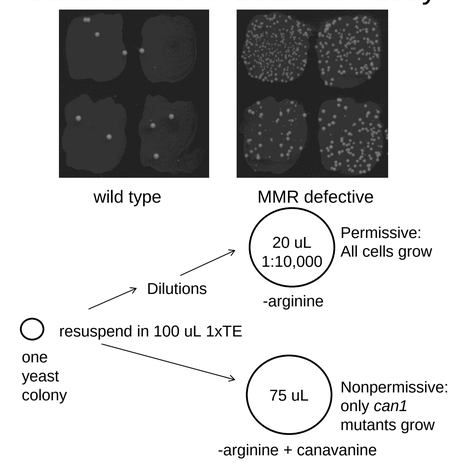
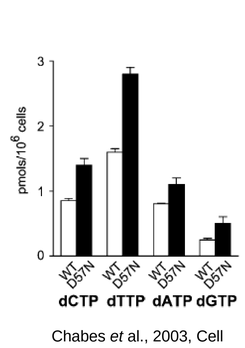
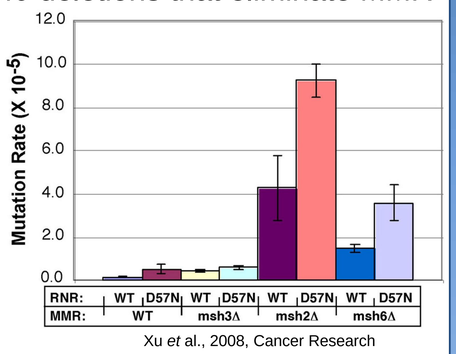


Figure 5. In order to measure the fidelity of these strains, we performed canavanine resistance assays and calculated mutation rates at the endogenous *CAN1* locus. Canavanine is a toxic arginine analog and yeast normally don't grow in the presence of this drug. However, inactivating mutations at the *CAN1* locus yield a nonfunctional protein which allows cells to grow in the presence of canavanine. The mutation rates are determined by calculating the median number of canavanine resistant colonies and the mean number of permissive colonies. The mutation rate indicates the number of mutations per generation.

The mutator phenotype of *rnr1D57N* is synergistic with *msh2* and *msh6* deletions that eliminate MMR



Chabes *et* al., 2003, Cell



Xu *et* al., 2008, Cancer Research

Figure 6. **Left panel.** *rnr1D57N* leads to a balanced 2-fold increase in dNTP pools. **Right panel.** *rnr1D57N* exhibited a mild mutator phenotype alone. There was a synergistic increase in mutation rate when MMR was eliminated or compromised.

Elevated mutation rates in *rnr1* mutants

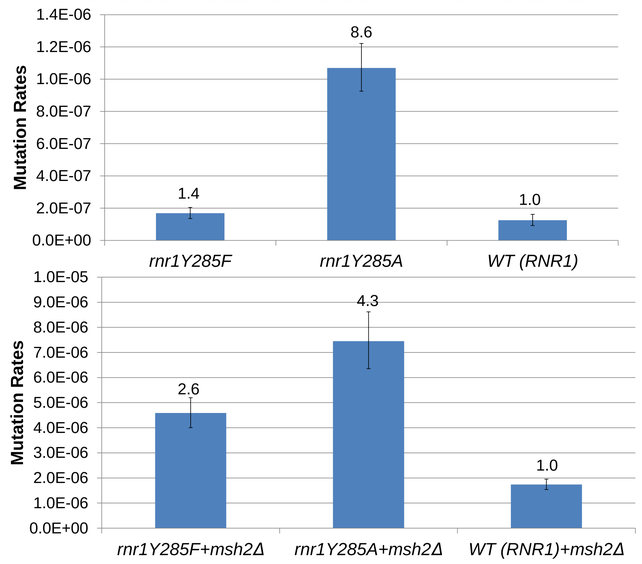


Figure 7. The mutation rates were measured for the *rnr1* mutant strains and the MMR deficient strains. **Top panel.** *rnr1Y285F* exhibited a small ~2-fold increase in mutation rate, compared to the wild-type *RNR1* strain. In contrast, *rnr1Y285A* which has much higher dCTP and dTTP levels, exhibited a larger ~ 10-fold increase in mutation. **Bottom panel.** We also measured the mutation rates in these *rnr1* backgrounds in the absence of MMR. MMR will normally repair DNA polymerase errors therefore these strains give a better idea of the fidelity of DNA polymerase in the presence of skewed dNTP pools. Note the differences in scale between the graphs; the overall mutation rate is significantly higher in the absence of MMR, i.e. the *msh2Δ* background. Error bars indicate 95% confidence intervals for these assays.

Conclusions

- There is a higher mutation rate when tyrosine is mutated to alanine versus phenylalanine in *rnr1*
- There is an increase in mutation rate in both *rnr1* mutant strains when MMR is absent
- We see similar increases in mutation rate when the dNTP pools are balanced (*rnr1D57N*) or skewed (*rnr1Y285F/A*).

Future Directions

- *rnr1D57N* increases the cell's resistance to DNA damage. We will test the effect of *rnr1Y285F/A* on DNA damage sensitivity.
- The sequence of the *CAN1* locus of the canavanine resistant colonies will be used to determine the mutation spectrum in each of the strain backgrounds.

Funding

- American Cancer Society Research Scholar Grant #RSG-14-235-01-DMC
- CURCA Undergraduate Research Award

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