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Accuracy of DNA Repair During Replication in Saccharomyces Cerevisiae

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ACCURACY OF DNA REPAIR DURING REPLICATION IN *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

DNA repair is a crucial part of organismal survival. The repair process is carried out by DNA polymerases and mismatch repair proteins. Things don't always go as planned in DNA repair, and sometimes DNA repair is inaccurate. Inaccurate DNA repair can potentially lead to the loss of the genes important for cell division and replication. There has been much research into the efficiency of these DNA polymerases, yet there has been no thorough research into how the accuracy of repair is distributed among all of the different types of homologous recombination. The goal of this article is to review the literature on the accuracy of DNA repair during replication in *Saccharomyces cerevisiae*.

INTRODUCTION

Replication and DNA Polymerase Proofreading

In order to make or replace cells of damaged tissues, cells must divide. Mitotic cell division specifically occurs when one cell replicates and divides into two identical daughter cells. In order to divide, the chromosomes must be replicated. Eukaryotic DNA replication begins at the opening of the origin of replication. The next steps take place at the 3' end of RNA-primed DNA. The DNA nucleotides need to be RNA-primed to be synthesized by DNA polymerase α. DNA polymerase α moves quickly, but lacks proofreading activity. Since the genome can be tens of hundreds of thousands of nucleotides long, there is significant room for

error. In order to have the newly synthesized DNA proofread, other DNA polymerases attach to the newly primed ends. For initial replication proofreading, DNA polymerase ε reads first on the leading strand, while similarly, DNA polymerase δ proofreads on the lagging strand (Strathern, Shafer, & McGill, 2006). When mistakes are made there are various ways that DNA can repair itself through a process called *homologous recombination*.

Damaged DNA can be repaired by using an intact homologous DNA region (**Figure 1.**). This occurs when the functional copy of a gene is lost. It begins when the broken or damaged chromosome uses the homologous chromosome next to it to finish replication. This leads to a loss of heterozygosity, as seen when the chromatids reassort. Homologous recombination is important because the cell can lose heterozygosity (LOH) when the functional copy of the gene is lost. A lost gene may render the organism more susceptible to negative consequences; for example, a lost tumor-suppressor gene would make the organism more susceptible to tumor growth.

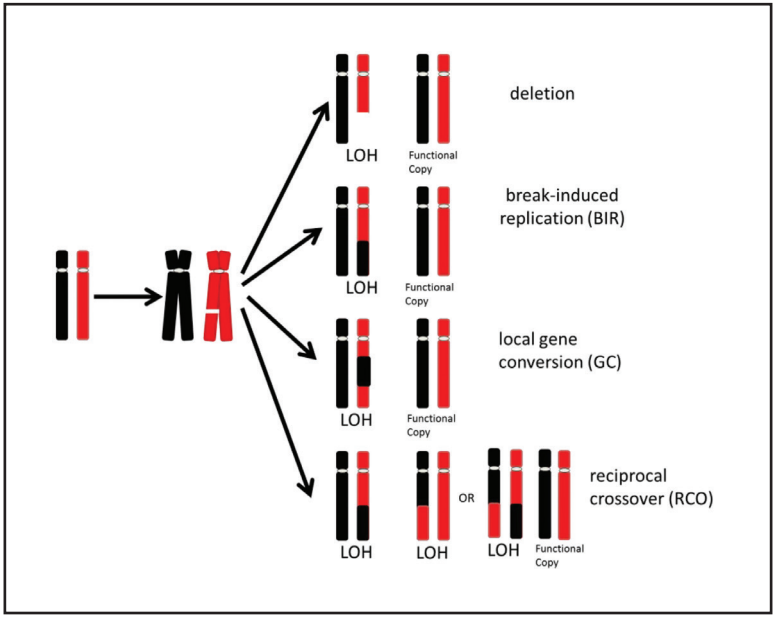

Figure 1. Loss of heterozygosity.

DNA Repair

DNA damage can happen in many different ways, including double-stranded breaks (DSBs; Strathern et al., 2006). In order to repair damaged DNA, DNA repair genes make proteins to repair the damage (Liefshitz et al., 1995). Many errors made by DNA polymerases are proofread and repaired by DNA polymerases, and all DNA polymerase molecules have different levels of efficiency and proofreading capabilities. Replication and DNA polymerase proofreading activity is crucial in the review of the accuracy of DNA repair in the yeast genome *Saccharomyces cerevisiae*. DNA repair may be inaccurate, which means that the DNA is misread, damaged, or repaired in such a way that a functional copy of a gene is lost. Loss of functions in the repair genes leads to higher levels of mutagenesis (Strathern et al., 2006).

Single-stranded DNA (ssDNA) and Hypermutability

Double-stranded breaks (DSBs) can lead to long single stranded DNA (ssDNA) regions (Yang, Sterling, Storici, Resnick, & Gordenin, 2008). Damaged DNA is prone to mutations under conditions of stress (e.g., UV damage, low levels of DNA polymerase) during replication. Long stretches of ssDNA and multiple lesions of DNA are prone to hypermutability (Yang et al., 2008). Hypermutability occurs when there is a significant increase in the mutation rate of a genome. The variation of the 5' end resection in DNA repair can contribute to the hypermutability of the DNA.

These long stretches of DNA and hypermutability play an important role in evolution, encompassing both human evolution and health (Yang et al., 2008). This research has shown that cancer formation and progression is due in part to DNA proofreading errors. Since DNA mutation is a necessity for the formation of tumors and cancer development, this is a good place to look for cancer-causing pathways. By studying the hypermutability of ssDNA, we can learn more about the accuracy of DNA repair.

Proofreading

Proofreading is necessary for DNA synthesis during repair. Mutations that happen to different sequences or regions

have different overall effects and changes in the types of mutations that occur (Strathern et al., 2006). In addition, when DNA polymerase δ was removed, or replaced by a defective mutant compared to another DNA polymerase, DNA polymerase ε changed the frameshift mutation back to wild type less frequently. This indicates that the absence of DNA polymerase δ played a larger role in the proofreading and correction of DNA polymerase α errors than ε. It was also found that when both DNA polymerase δ and ε were removed, the mutation rate was significantly higher than when just one of the DNA polymerases was knocked out (Strathern et al., 2006).

Mismatch repair (MMR)

Mismatch repair (MMR) machinery is an important part of the cell cycle's machinery (**Figure 2.**). The MMR machinery is made of the various enzymes and proteins that are used to repair DNA bases. Mismatch repair proteins fix places along the double-stranded DNA and fix sequences or bases that are out of place. Mismatch repair proteins fix replication errors, much like the DNA polymerase repair proteins. These proteins differ from the DNA polymerases because MMR will repair breaks of a different specificity than DNA polymerases. Mismatch repair, in particular, plays an important role in fixing frameshift (potential reversion) mutations (Greene, 1997). Frameshift mutations occur when a DNA base, or a series of DNA bases, that is deleted or inserted, shifts the sequence out of the reading frame. If this happens near important genes, it could potentially lead to mutations and defective genes or damaged protein production. Frameshift mutations only account for 10% of mutations found in this study. Because the system could only account for a small range on the chromosome, there was a restriction to the total types of events that could be detected, or a location specificity for some events. There could have been repair events that could indicate even more clearly what is happening in these DNA repair pathways.

MMR proteins are necessary for the proper removal of frameshift mutations. In the scope of DNA repair accuracy these frameshift mutations can be crucial. If the frameshift mutation is removed, the mutation is effectively stopped. More specifically,

if the mutation is stopped, then the genes that were displaced (potentially tumor-suppressor genes) are restored to wild type. This proves to be a very effective tool for the removal of potentially harmful mutations, but the question still remains: how accurately are these frameshift mutations repaired?

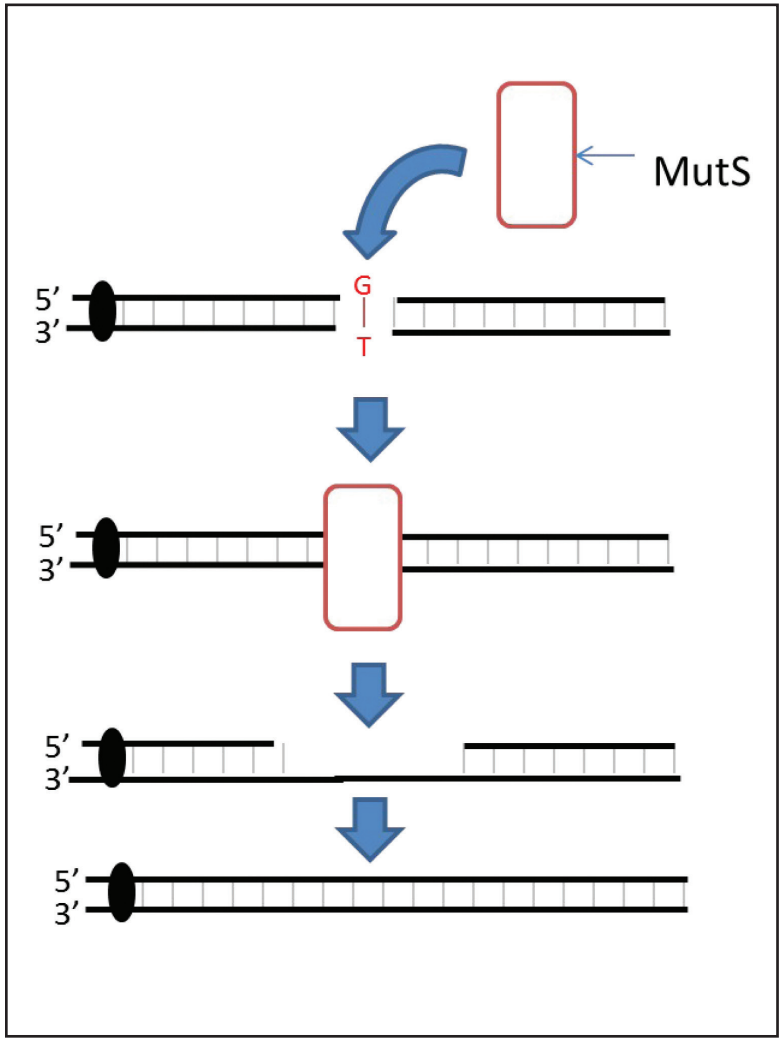

Figure 2. The pathway that was used to study MMR enzymes. MutS cuts around the mutated area, and at the final step DNA polymerase δ synthesizes and proofreads the DNA.

Homologous Recombination and Accuracy of DNA Synthesis During Repair

Stretches of ssDNA are necessary in order for a process called "homologous recombination" to occur (Chung, Zhu, Papusha, Malkova, & Ira, 2010). Homologous recombination occurs when a damaged strand of DNA is mediated by a protein called *Rad51* to invade a homologous template sequence in order to complete replication of the damaged strand of DNA. The 5' end resection plays an important role in the fidelity of the repair (**Figure 3.**). The variability of the 5' end resection could directly contribute to the repair pathways that are used, in addition to the molecular mechanisms of repair for those pathways.

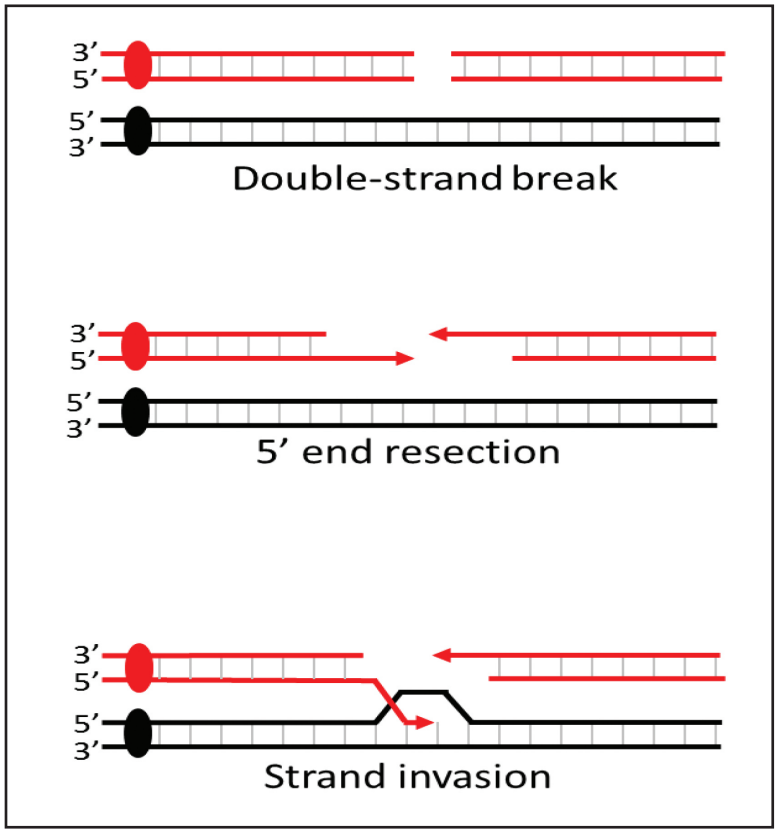

Figure 3. Initiation of DNA repair.

Longer stretches of ssDNA allow for greater fidelity of the repair. This was evidenced by the resection's longer length in BIR repair pathways than gene conversion pathways. The BIR stretches' longer length could arise because the resection continued after the strand invasion continued, while during gene conversions, the strand invasion did not (Chung et al., 2010).

In spite of the knowledge we have accumulated regarding DNA repair during replication, there are still knowledge gaps regarding the accuracy of DNA repair during replication. In particular, we know very little about the accuracy of gene conversion mutations. The purpose of Chung's study is to take a critical look at cancer and its relation to DNA proofreading errors. Chung et al. (2010) showed that break-induced replication is a result of the inaccuracy of DNA synthesis during repair. DNA synthesis is inaccurate according to the lower amount of overhang that was created during the 5' resection step of DNA repair (Chung et al., 2010). It was also discovered that the fidelity of the resection allowed monitoring of ssDNA size and preference for repair pathways. There are different preferred repair pathways, depending on the size of the ssDNA stretch. The size of the ssDNA varies the hypermutability of the damaged DNA and could cause a preference of the pathway, depending on the size and the hypermutability of the ssDNA. These comparisons have been made in the case of BIR; however, there are many different types of homologous recombination in which we can test the accuracy of DNA synthesis during repair.

DNA Polymerase δ and ε Proofreading Efficiency in DNA Repair

DNA Polymerase ε

Human colorectal and endometrial cells can lose the proofreading of DNA polymerase ε by mismatch repair of the protein's amino acid substitutions, mutating the protein's function (Kane & Shcherbakova, 2014). DNA polymerase ε is essential for initially reading the DNA on the leading strand. This makes DNA synthesis and repair less accurate. Kane et al. (2014) also used a strong mutator and got a phenotype comparable to complete

mismatch repair protein deficiency in a yeast model system. This showed the possibility that DNA polymerase ε plays a crucial role in mutations that are repaired by using MMR proteins.

It was observed that LOH doesn't necessarily occur for DNA polymerase ε mutant human tumors (Kane & Shcherbakova 2014), because tumorigenesis can be caused by mismatch repair protein malfunction (Kane & Shcherbakova 2014). This study did find that mutations occurred that affected mutant yeast diploids with defective DNA polymerase δ and DNA polymerase ε. This finding corresponded to other findings of DNA polymerase δ and ε in the field (Strathern et al., 2006; Kennedy et al., 2015; St. Charles, Liberti, Williams, Lujan, & Kunkel, 2015).

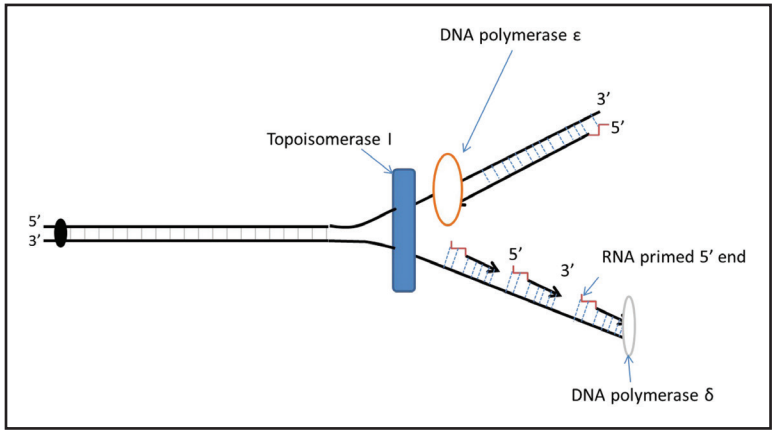

Figure 4. DNA polymerase δ and ε during replication.

DNA Polymerase δ and DNA Polymerase ε

In a similar study, a sensitive mutant was used in order to check error corrections by DNA polymerase δ (Flood et al., 2015). Data showed that DNA polymerase δ was as active as DNA polymerase ε in repair of short homonucleotide runs, and DNA polymerase ε was necessary for longer runs (Flood et al., 2015). The authors found further evidence that the DNA polymerases that repair the leading and lagging strain are different, as was suggested above. To further support this, it has been found that under circumstances when DNA polymerase ε or DNA

polymerase δ is mutant or defective, its proofreading errors are corrected by DNA polymerase δ; while DNA polymerase ε cannot correct mistakes by a mutant or defective DNA polymerase ε (Flood et al., 2015). These findings were inconsistent with the current model of DNA synthesis during repair, which suggests that DNA polymerase ε and DNA polymerase δ fix synthesis errors at different rates. The model shows DNA polymerase ε and δ working on their strands, respectively, but upon error caused by DNA polymerase ε, the strand is repaired by DNA polymerase δ. This model differs from the current model because it indicates that we can have DNA polymerase δ accompanying DNA polymerase ε, which allowing the lower mutation rates, shows that leading strand synthesis is inaccurate. This model does a better job of describing the higher mutation rates that are observed in defective DNA polymerase δ and ε when defective mutants halt the activity of each, respectively. It follows that higher mutation rates occur in proofreading defective DNA polymerase δ mutants.

In another study, enriched mutations in the first half of the replicon and termination zones were found. This shows that genome replication events may be more volatile than thought and can give us much more to learn about mutations and their evolution with the genome (Kennedy et al., 2015), by showing that mutations happen most often at termination zones and at the first half of the replicon. This furthers the evidence of the importance of 5' end resection. The 5' end resection makes repair in higher fidelity than if repair was done with no resection. In addition to the higher fidelity of repair, ssDNA is known to have increase hypermutativity. The researchers found that volatility of the mutator leads to different phenotypes. This means that depending on how aggressively the mutator that is used mutates the genes observed, we can view different phenotypes arise.

Mismatch Repair Proteins and Proofreading Activity

Greene et al. (1997) used a reversion assay where the reversion spectra of the types of insertions and deletions that are generated during replication were analyzed. They found that the mismatch repair proteins (MMR) preferentially correct

frameshift mutations. Frameshift mutations move the open reading frame (ORF) from what is supposed to be read, to make potentially nonsensical nucleotide sequences (**Figure 5.**). Figure 5. shows an example of a +1 frameshift mutation. This is indicated by the nucleotide "C" highlighted in red, being added and shifting the reading frame of the sequence downstream from the mutation. This makes a non-homologous sequence, which is caused by the +1 frameshift mutation. Note that the three letter code for the amino acids on the changed strand are different from the top non-mutated blue row of amino acids. The study also found that the MMR machinery tracked the progression of DNA replication and repairs along the way. Upon varying the sizes of insertions and deletions, it was found that MMR effectively removed the frameshift mutations.

It was observed that a mutation in DNA polymerase ε caused there to be an increase in frameshift mutations (Kirchner, Tran, & Resnick, 2000). This increase in frameshift mutations means that they can be corrected by functioning MMR machinery. The relation of DNA polymerase ε and MMR enzymes show that there is a relationship between the connectedness of most of the DNA repair proteins and enzymes.

It appears that replication of the two DNA strands results in a variable balance between error prevention, proofreading, and mismatch repair proteins (St. Charles et al., 2015). It was also observed that base selectivity is 10 times higher in vivo than in vitro (St. Charles et al., 2015). Since there was a tenfold increase in base selectivity in vivo, this shows we cannot completely know the action of these DNA polymerases and repair machinery in vitro with equal clarity. This also indicates how particular these repair proteins and enzymes are. The selectivity suggests the very intricate nature of our DNA repair. The study also found that the mutations that occur in the DNA polymerases

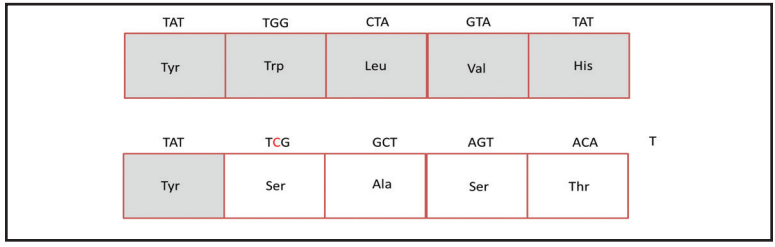

Figure 5. Example of a +1 Frameshift mutation

are much more crucial in the fidelity of the overall process of DNA repair than does the MMR machinery.

CONCLUSIONS

The problem remains that we lack data on the accuracy of genetic instability caused by homologous recombination in cells under replication stress. Homologous recombination, even break-induced replication, is 1,000 times higher in cells under replication stress than cells under normal replication conditions (Chung et al., 2010). These homologous recombination events can result in LOH, which have great consequences affecting human health, such as cancer. Altering levels of DNA polymerase will allow us to investigate other forms of homologous recombination. This would allow for better measurements and a full range of activity of DNA polymerases.

SsDNA is highly prone to mutations. Moreover, ssDNA is so prone to mutations that it is considered hypermutable (Yang et al., 2008). This hypermutability has steep consequences; one of them is frameshift mutations and inaccurate repair of damaged DNA (Chung et al., 2010).

Expanding on the work detailed in this review, we can further examine the gaps of DNA replication that we can fill, including whether we can get a better understanding of the accuracy of these reversions by homologous recombination. Investigating homologous recombination events through selectable markers and altering levels of DNA polymerase would also shed more light on the variation of cell repair pathways and allow us to develop a whole picture of the accuracy reversions of homologous recombination events.

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