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DETERMINING THE ROLE OF SUB1 IN THE REPAIR OF DNA DAMAGE IN *SACCHAROMYCES CEREVISIAE* THROUGH A TRP5 REVERSION ASSAY

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ABSTRACT

Understanding the mechanisms involved in DNA repair is an important step in understanding and possibly controlling numerous human diseases. Previously the *S. cerevisiae* gene SUB1 and its human ortholog PC4 have been shown to play an important role in the repair of DNA lesions resulting from oxidative stress. In this project I use a novel TRP5 reversion system to study the mutational changes in sub1 deletion strains. Canavanine mutagenesis was also utilized to test spontaneous mutation frequency.

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ACKNOWLEDGEMENTS

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BACKGROUND

Importance of DNA Repair

DNA damage in all forms plays an important role in numerous human diseases including many cancers (Loft and Poulsen, 1996). Deficiencies in DNA repair mechanisms have been implicated as the cause of various disorders including Xeroderma pigmentosum (XP) and Cockayne syndrome (CS). XP results from a mutation in one of eight genes (XPA-G and XPV) and can result in extreme sensitivity to sunlight and increased incidence of skin cancer. CS results from mutations in either CSA or CSB, and can result in serious developmental disorders among other things (Lehmann, 2003). Below is a chart containing 15 known diseases caused by problems in DNA repair mechanisms. Obviously, the mechanisms of DNA repair are vitally important to maintaining normal cell function.

Disorder	Cancer	Genes	Repair process
XP	Yes	8	NER or translesion synthesis
CS	No	2–5	NER (TCR)
TTD	No	3	NER
HNPCC	Yes	3	Mismatch repair
Ataxia-telangiectasia (AT)	Yes	1	Radiation damage and checkpoints
AT-like disorder	Yes	1	Radiation damage and checkpoints
Nijmegen breakage	Yes	1	Radiation damage and checkpoints
Fanconi anaemia	Yes	7	DNA cross-links
Bloom's syndrome	Yes	1	Stalled forks
Werner's syndrome	Yes	1	Stalled forks
Rothmund–Thompson syndrome	Yes	1	Stalled forks
Familial breast cancer	Yes	2	Homologous recombination and checkpoints
Severe combined immunodeficiency	No	1	Non-homologous end-joining
LIG4 syndrome	No	1	Non-homologous end-joining
Seckel syndrome	No	1	Damage checkpoints
Li–Fraumeni syndrome	Yes	2	Damage checkpoints

Table 1. DNA Repair Related Diseases. This table illustrates the diseases known to result from DNA repair deficiencies (Lehmann, 2003).

Repair Pathways

One primary DNA repair pathway is Nucleotide Excision Repair (NER).

Nucleotide excision repair is when the DNA on either side of a lesion is cut, and the damaged section is removed and replaced with new DNA by the action of polymerase and ligase. NER is responsible for the repair of many different types of damage, including that resulting from UV exposure (Prakash and Prakash, 2000), and appears to be primarily responsible for the repair of lesions that significantly distort the DNA backbone (de Laat et al, 1999).

Transcription coupled repair (TCR) is a branch of nucleotide excision repair, the other major branch being global genomic repair (GGR) which, as its name implies, repairs DNA regardless of the strand. TCR is a process which causes the transcribed strand of a gene to be repaired quicker than the non-transcribed strand or inactive DNA regions (Svejstrup, 2002). For obvious reasons it is more important that the strand that is being transcribed be repaired quickly, since errors in the template would result in errors in the mRNA produced from it and could ultimately change the protein the gene encodes. Earlier data has shown that UV-induced lesions will block the transcription mechanism. The stalled RNA polymerase then recruits DNA repair factors resulting in much faster repair of the transcribed strand than the non-transcribed strand (Lee et al, 2002; Tijsterman and Brouwer, 1999).

Oxidative Damage and DNA

Oxidative DNA damage has been shown to be a factor in the aging process (Schriner et al, 2005) and numerous cancers. Oxidative damage results from the interaction of reactive oxygen species (ROS), produced in cells during respiration, with the cell's DNA, although ROS can also come from external sources such as hydrogen

peroxide. There are likely over 100 different types of lesions produced by the ROS interactions with DNA, but the most abundant is 8-oxoguanine (8-oxo-G). The mutagenic properties of 8-oxo-G result primarily because it is often mispaired with adenine resulting in GC to TA transversions if it is not repaired. Consequently, every cell has mechanisms in place to prevent or repair the damage created by these ROS.

Role and Function of PC4

The human gene PC4 (positive cofactor 4) was isolated in our lab through a screen of a human cDNA library for genes that would suppress oxidative mutagenesis in an *E. coli* strain hypersensitive to this form of mutagenesis. SUB1 (the yeast homolog of PC4) was shown to be important for cellular resistance to oxidative damage since yeast cells lacking sub1 have higher spontaneous and peroxide induced mutation frequencies (Wang et al, 2004).

Previous experiments have shown that PC4 interacts with XPG, a fact which supports a role for PC4 in DNA repair. XPG (RAD2 in yeast) is an endonuclease responsible for cutting the lesion-containing strand of dsDNA on the 3' side of a lesion, and is critically important for both NER and TCR (Nouspikel et al, 1997). Knocking out rad2 in addition to sub1 actually reduces sensitivity to hydrogen peroxide, suggesting that peroxide sensitivity of sub1 requires the action of Rad2 and suggests sub1 may function in a rad2 dependent pathway. Human PC4 has been shown to directly interact with XPG, and is capable of displacing XPG unpaired regions of double-stranded DNA (Wang et al 2004). This suggests that PC4 may be involved in the removal of XPG from DNA.

The PC4 protein contains two serine-rich acidic domains on its N-terminus, and a single stranded DNA binding domain towards the C-terminal end (Figure 1). PC4 was initially recognized as a transcriptional coactivator, and has the ability to interact with both double stranded and single stranded DNA. However, only the double stranded binding activity is required for its function as a transcriptional coactivator

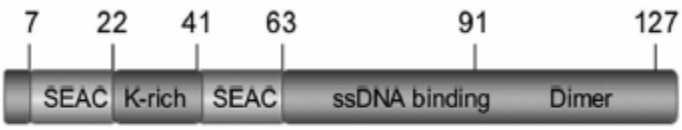


Figure 1. Structure of PC4. This figure shows the structure of the human PC4 gene**.**

(Kaiser et al, 1995). Interestingly, it has been shown that only the ssDNA binding domain is required for PC4s DNA repair function, and this same region was shown to have the ability to repress transcription (Werten et al., 1998; Malik et al., 1998). This is an odd function for something that acts as a transcriptional coactivator, but one that PC4s role in DNA repair might explain. Recently it has been shown that PC4 remains bound to the transcription mechanism throughout the process of transcription (Calvo and Manley, 2005), which is another fact that points to PC4 being involved in transcription coupled repair.

Genes of Interest to This Project

There are a few other genes employed in this project that require some explanation. TRP5 is a tryptophan synthase required for synthesis of tryptophan that is closely related to the *E. coli* tryptophan synthases (Zalkin and Yanofsky, 1982). Like *E. coli*, the *S. cerevisiae* gene contains a single residue that is vitally important to the

function of the gene. In yeast this is the Glu-50 residue, and any mutation in the codon for this residue results in a Trp- cell (a cell that requires tryptophan enriched media in order to grow) (Williams et al, 2005).

OGG1 is a DNA glycolsylase responsible for removing 8-oxoG. Deletion of ogg1 results in an increase in spontaneous and H_2O_2 induced mutation frequency and increased sensitivity to H_2O_2 (Melo et al, 2004; Scott et al, 1999). More specifically, ogg1 deletion increases the frequency of GC to TA transversions but leaves all other base substitutions at wild type levels. These mutations most often result from misincorporation of A opposite 8-oxoG, consequently it is believed that OGG1 is required for the removal of 8-oxoG (Thomas et al, 1997).

PROJECT PURPOSE

The purpose of this project was to use a newly developed TRP5 reversion system to study the effect of sub1 deletion on DNA mutation frequency. Previous data suggested that knocking out sub1 results in increased mutation frequency, but exactly what types of mutations are created was still unknown. The data in this project suggest that deletion of sub1 may not cause an increase in any type of point mutation, however, use of canavanine mutagenesis has confirmed that deletion of sub1 increases overall mutation frequency.

MATERIALS AND METHODS

Yeast Strains and Primers

Strain Number	Mutation	Orientation	sub1Δ Strain Number
MVY400	G148A	Forward	MVY412
MVY401	G148A	Reverse	MVY414
MVY402	G148C	Forward	MVY416
MVY403	G148C	Reverse	MVY418
MVY404	G148T	Forward	MVY420
MVY405	G148T	Reverse	MVY422
MVY406	A149C	Forward	MVY424
MVY407	A149C	Reverse	MVY426
MVY408	A149G	Forward	MVY428
MVY409	A149G	Reverse	MVY430
MVY410	A149T	Forward	MVY432
MVY411	A149T	Reverse	MVY434

Table 2 TRP5 strains: This table lists the strains used in the TRP5 reversion system. Each set of forward and reverse strains represent a single point mutation that results in restoration of wild type TRP5 function. The mutation G148A, for example, represents a strain that will revert to wild type with a mutation from AT to GC. The strain MVY408 and its sub1 mutant MVY428 were used for the UV assay. MVY406 and its sub1 deletion MVY424 were used for the H_2O_2 tests.

Strain Number	Genotype
MVY101	Wild Type
MVY105	sub1Δ

Table 3 Canavanine strains: Strains used in the canavanine mutagenesis assay.

Primer Name	Sequence (5'-3')	Use
sub1f(mv)	ACTGCACACACACGTTTTATC	sub1 knock out primer
sub1KOtf	GCATGGGAATGGCATAAGTAGCCTCG	sub1 knock out primer
sub1+800	TCATGCTCCAACGAACACTCAGCGTA	sub1 confirmation primer
sub1r(mv)	TATCATTCACGCAATTTTCACAG	sub1 confirmation primer
ogg1KO-L	TCTTCCCAATCATCCGAATC	ogg1 knock out primer
ogg1KO-R	AGATTGGGTTACGTCGCATC	ogg1 knock out primer
ogg1-L	TCCTTTTTCAACGCTGGTCT	ogg1 confirmation primer
ogg1-R	TTGCTGGTCGAATTTCCTCT	ogg1 confirmation primer
TRP5-1	CAGGAACGCCTTGGTCACAT	TRP5 PCR primer
TRP5-2	ATGGGTACGGTAACACCTTC	TRP5 PCR primer
CANF1	CTTCAGACTTCTTAACTCC	CAN1 PCR primer
CANR1	GAGGGTGAGAATGCGAAAT	CAN1 PCR primer
CHS84	TGGCCGCACCAAATGC	CAN1 sequencing primer
CHS85	GGTTTGCAGCTTCACCAGC	CAN1 sequencing primer
CHS86	CGTGGAAATGTGATCAAGGT	CAN1 sequencing primer

Table 4 Primers: Primers used for the TRP5 and canavanine assays

Construction of Yeast Knock Out Strains

Amplification of yeast knock out

First a culture of a strain already containing the knock out was started from a frozen glycerol stock and grown in YPD media at 30^{o}C. The genomic DNA was extracted using the Epicentre Masterpure Yeast DNA Purification kit. The knock out gene was amplified via PCR using a set of primers at least 200 base pairs outside of the gene. The primers were created based on sequences obtained from the yeast genome database. The amplified DNA was then purified using the Qiagen PCR purification kit and transformed into the recipient cells.

Yeast Transformation

The cells were grown to an OD_{600} of approximately 1.6 overnight in 5ml of liquid YPD at 30^{o}C. Next, the cells were centrifuged at 5,000rpm for 5min and then resuspended in 2.5mL of sterile water. The cells were centrifuged again for 5min at 5,000rpm and resuspended in 100uL of 100mM Lithium Acetate and transferred to a microfuge tube. The suspensions were spun in a microcentrifuge at top speed for 10 seconds to pellet the cells which were then resuspended in 50uL of 100mM Lithium acetate. Carrier DNA (Herring Sperm DNA 2ug/mL) was boiled for 5 minutes and then put on ice. The LiAc suspension was spun down to pellet the cells and then the following were added in order: 240uL 50% PEG, 36uL 1M LiAc, 25uL 2ug/mL Carrier DNA, 50ul DNA in TE with one control sample not receiving any DNA. The samples were incubated at 30^{o}C for 30 minutes then heat shocked at 42^{o}C for 15 minutes. After

incubation, the samples were spun down to pellet the cells and then resuspended in 200uL of water. 100uL of sample were plated on a selection plate and allowed to grow for 3-5 days. The knock out is created by the yeast cells recombining the knock out segment with the wild type gene, consequently replacing the wild type with the cassette containing the selectable marker. In yeast the selectable markers that are generally used are URA3, TRP1, HIS3, LEU2 which confer a wild type phenotype on transformed cells, and KanMX4 which makes transformed cells resistant to Geneticin (G418). The first four would be selected on synthetic complete media with the appropriate amino acid or nucleotide dropped out, which is written as SC-Ura, etc. For transformation with KanMX4 Geneticin is added to YPD media in varying amounts depending on the strain being transformed.

Confirmation of knock out

Several colonies were picked from the selection plates and grown overnight in 5mL of YPD. Genomic DNA was isolated and then amplified with confirmation primers. Confirmation primers are a set of primers designed to amplify a region slightly larger than the original knock out. This is done to confirm that the knock out recombined into the appropriate location in the yeast genome. Discrimination between wild type and replacement can be made either on the basis of size of the PCR product, restriction digest, or both.

Reversion Assay with UV Treatment

The culture was grown overnight in 5mL of YPD at 30^{o}C, and an OD_{600} reading was taken. The sample was centrifuged for 5 minutes at 5,000rpm and then resuspended

in enough 1xPBS to make the sample have a concentration of approximately $5x10^8$ cells per mL. A sample was diluted to 10^{-6}, and 100uL of each of the 10^{-5} and 10^{-6} dilutions were plated on two YPD plates. 100uL of the undiluted sample was plated on at least 3 SC-Trp plates. One set of dilution plates and all but one of the plates containing undiluted sample were then exposed to UV at $1J/m^2/s$ for 20 seconds. All of the plates were incubated at 30^oC, the plates that had been exposed to UV were kept in the dark. The YPD plates were counted after 3 days to determine cell viability, and the SC-Trp plates were counted after 4-5 days.

Assay using 50mL cultures and Trp starvation

A 5mL culture was grown up overnight in liquid SC media at 30^oC. The following day the 5mL culture was used to inoculate a 50mL culture of SC media which was again grown overnight. After centrifugation for 5min at 5000rpm the sample was resuspended in 50mL liquid SC-Trp and incubated at 30^oC for 2 hours. An OD_{600} reading was taken and the cultures were centrifuged for 5min at 5000rpm.The sample was resuspended in enough 1xPBS to give the sample a concentration of approximately $5x10^8$ cells per mL. A sample was diluted to 10^{-6} and 100uL of each of the 10^{-5} and 10^{-6} dilutions were plated on two YPD plates. 100uL of the undiluted sample was plated on at least 3 SC-Trp plates. One set of dilution plates and all but one of the plates containing undiluted sample were then exposed to UV at $1J/m^2/s$ for 20 seconds. All of the plates were incubated at 30^oC, the plates that had been exposed to UV were kept in the dark to prevent photoreactivation. The YPD plates were counted after 3 days to determine cell viability, and the SC-Trp plates were counted after 4-5 days.

Reversion Assay with H_2O_2 Treatment

The culture was grown overnight in 5mL of YPD at 30°C, and an OD_{600} reading was taken. The sample was centrifuged for 5 minutes at 5,000rpm and then resuspended in 5mL of 1xPBS. It was split into 5 1mL aliquots each receiving a different amount of H_2O_2 with one receiving none. The samples were incubated at 30°C for 1 hour, and then the reactions were stopped with catalase. The samples were spun down to pellet the cells and then resuspended in 1mL 1xPBS. Appropriate dilutions of each sample were plated on YPD and used to determine cell viability and 100uL of undiluted sample was plated on SC-Trp plates. All plates were incubated at 30°C, the YPD plates were counted after 3 days, and the SC-Trp plates were counted after 4-5 days.

Spontaneous Canavanine Mutagenesis

Samples were grown to mid-log phase (OD_{600}~2.5) overnight in YPD media at 30°C. The cultures were centrifuged and resuspended in 5mL 1xPBS. Appropriate dilutions were plated on YPD and undiluted samples were plated on SC plates lacking arginine and containing 60ug/mL canavanine. The YPD plates were counted after 3 days and the mutagenesis plates were counted after 4-5 days.

RESULTS

Confirmation of the TRP5 Reversion Assay

The TRP5 reversion assay used in this project was only recently developed, as shown in the Materials and Methods section the system makes use of a set of twelve strains. In each strain there is a single point mutation at one of two bases in the TRP5 active site glu codon that results in disruption of the gene (Trp-). For each of the six possible mutations there is a forward and reverse strain, with forward and reverse referring to the orientation of the gene relative to the nearest active replication start site. Reversion back to the original base results in a return of wild type function of the TRP5 gene (Trp+). This allows for easy selection of cells in which the reversion has occurred by growing them on SC plates lacking tryptophan. Another mechanism for confirmation of reversion built into this system is a TaqI restriction site. The wild type TRP5 gene contains a TaqI site, but mutation of either of the bases results in elimination of the restriction site. Reversion, therefore, restores the restriction site, so restriction digestion of DNA from revertants can conclusively show if the reversion did in fact result in restoration of the glu codon to wild type rather than a suppressor mutation elsewhere. While suppressor mutations are expected to be rare because the glu codon is required for function, exceptions may occur. The segment of the gene amplified by the TRP5 PCR primers is shown below in Figure 2A with the TaqI restriction site highlighted. Figure 2B shows the specific base pairs used in this system.

A.

CAGGAACGCCTTGGTCACATTTATGACCGCAGGTTACC
CAACAGTCAAAGACACTGTCCCTATTCTCAAGGGTTTC
CAGGATGGTGGTGTAGATATCATCGAATTGGGTATGCC
CTTCTCTGATCCAATTGCAGATGGTCCTACAATTCAATT
ATCTAATACTGTGGCTTTGCAAAACGGTGTTACCTTGC
CTCAAACTCTAGAAATGGTCTCCCAAGCTAGAAATGAA
GGTGTTACCGTACCCAT

B.

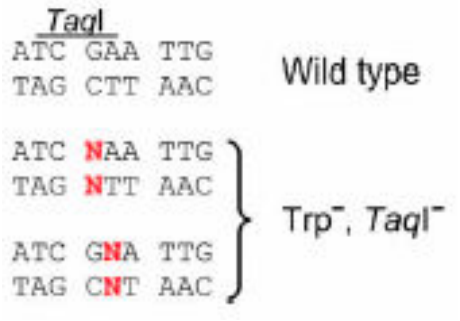


Figure 2 TRP5 Reversion System: A. This shows the segment of the TRP5 gene amplified by the PCR primers, the 4 highlighted bases are the TaqI restriction site. B. This figure shows the two base pairs used in the TRP5 system. Changes in either of these results in a Trp- strain, and also interrupts a Taq1 restriction site. For each strain reversion to the wild type base pair through mutation results in a Trp+ cell and restoration of the Taq1 site. Plates lacking tryptophan can be used to select for revertants, and Taq1 restriction can be used to confirm reversion. (Williams et al, 2005)

Since this is a newly developed system, it was first necessary for me to determine if it would be possible for me to reproduce the data presented by the group who developed the system. The spontaneous mutation frequency in this system is too low to count, so my initial focus was on UV induced mutagenesis. A problem that I encountered in my preliminary study was the appearance of some larger colonies surrounded by many smaller ones on the Trp- plates (Figure 3). Testing for restoration of the TaqI restriction site indicated that only the larger colonies were actually revertants, the small colonies appear to result from weak suppressor mutations. In subsequent trials only the large, true revertants were counted.

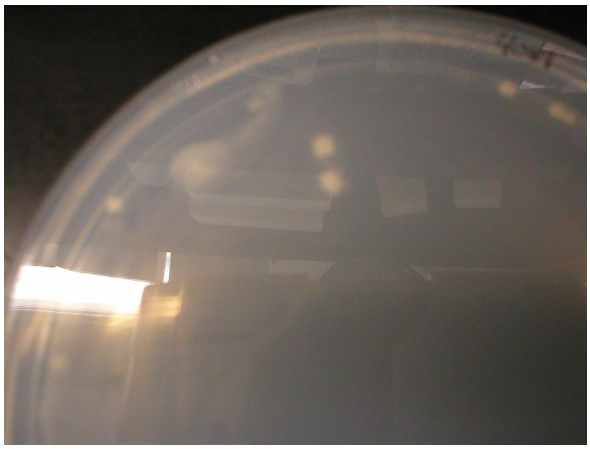
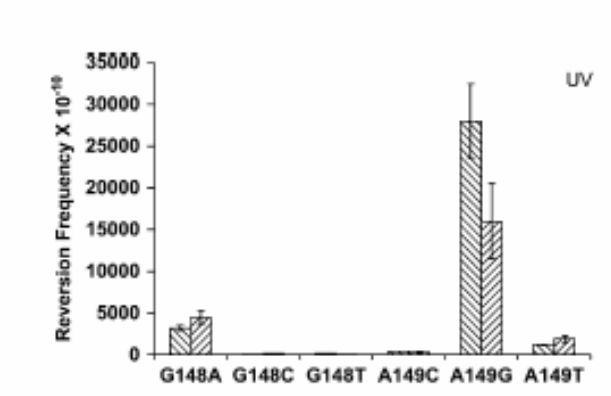


Figure 3 TRP5 Reversion Plate.

Figure 4A shows the data for UV reversion frequency given by Williams et al, and Figure 4B shows the data I obtained from my trials with this system. As expected, the highest frequency for UV induced mutations is seen in the strain that reverts only by a GC to TA transition which is the most common mutation resulting from UV exposure. As this figure shows, my mutation frequencies were equivalent to those demonstrated in the Williams et al paper, so it was then possible to continue on to the study of SUB1 in this system.

A.



B.

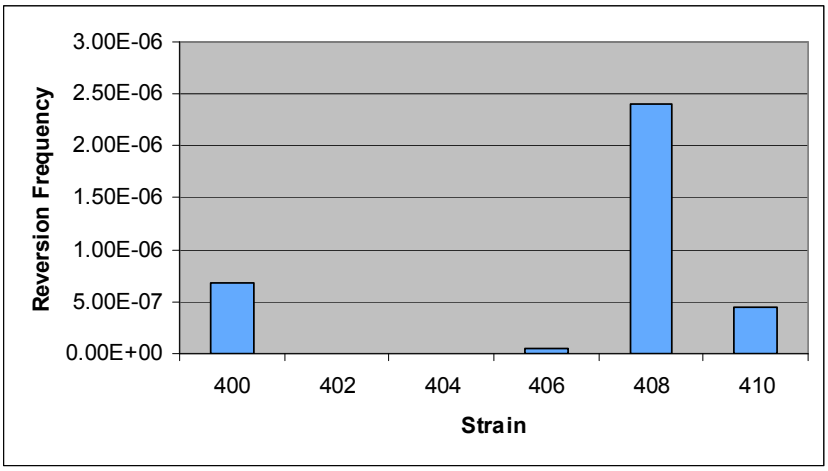


Figure 4 Test of TRP5 Reversion System: A. This figure contains the original data obtained from the Williams et al paper. B. This is my data using only the forward strains as a means of comparison.

UV Reversion of sub1Δ Strains

First, it was necessary to transform a sub1 deletion into the set of twelve TRP5 strains. Figure 5 shows how a deletion strain is created. While completing the transformations I began exposing the sub1 deletion strains to UV to see if there would be any increase in mutation frequency. Figure 6 shows the results of the initial test containing all but the 406 deletion mutant. From this data it appeared that in each case there was some small increase in reversion frequency, however, it was necessary to determine if it was enough to be statistically significant. For this I focused on the strain showing the highest frequency, MVY408, and the sub1 deletion of that strain, MVY428. By completing the experiment with several cultures of each strain I was able to check the consistency of the mutation frequency. As can be seen in Figure 7 the increase in mutation frequency is not high enough to be considered significant when the variation in mutation frequency between cultures is measured.

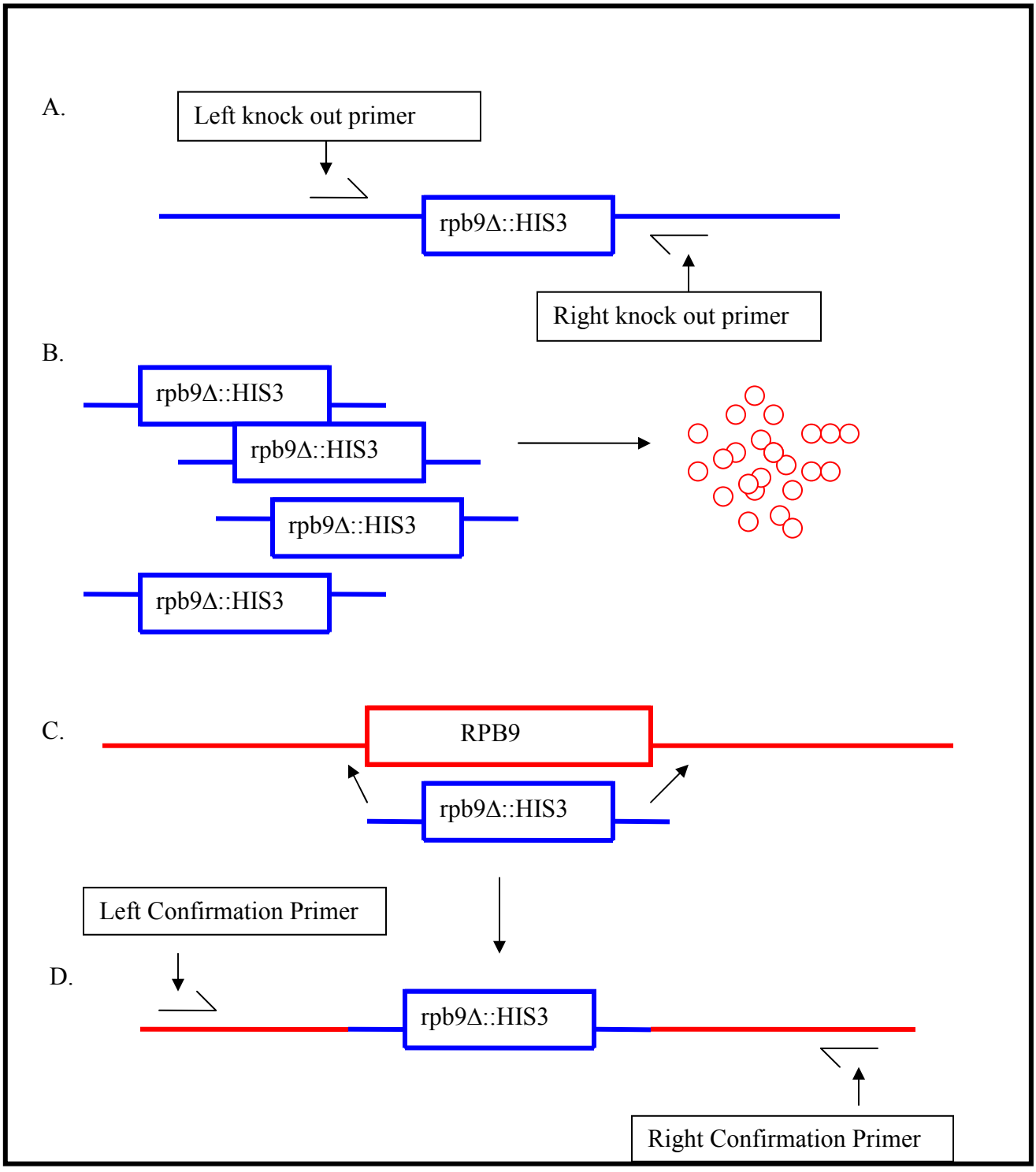


Figure 5 Creating a Yeast Knock Out A. The knock out is amplified using PCR from a strain already containing it. The knock out primers should be at least 200bp from the end of the gene. B. The amplified knock out segments are transformed into the receiving yeast cells. C. Through recombination the yeast incorporates the knockout into its genome replacing the wild type gene. D. Confirmation primers which are further out than the knock out primers are used to amplify the region to confirm that colonies picked from selection plates contain the knock out in the appropriate location.

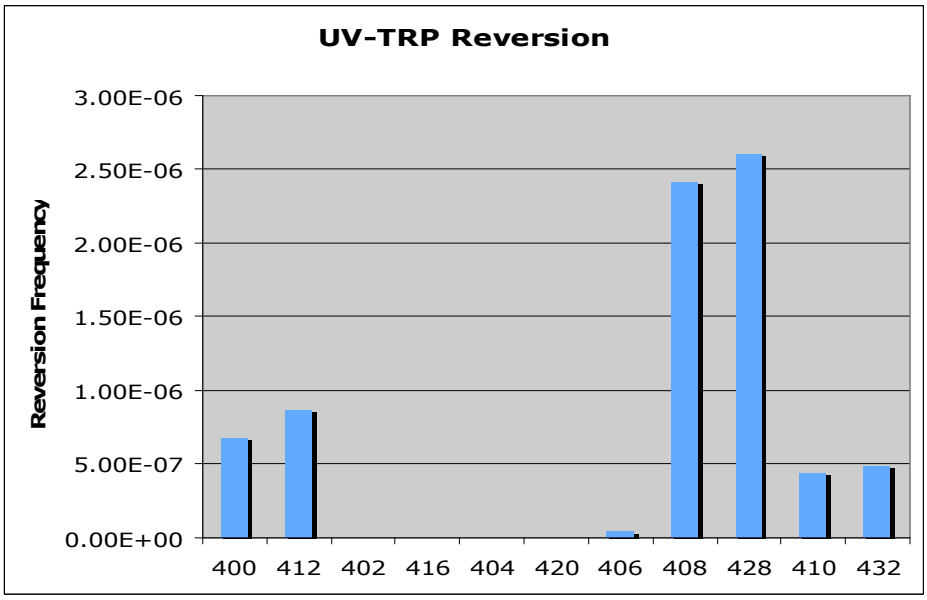


Figure 6 Reversion Frequency Comparison: This figure compares the reversion frequency of the wild type strains with the sub1 deletion of that strain. The data for the sub1 deletion of 406 is unavailable because that strain had not been successfully created by the time of this experiment.

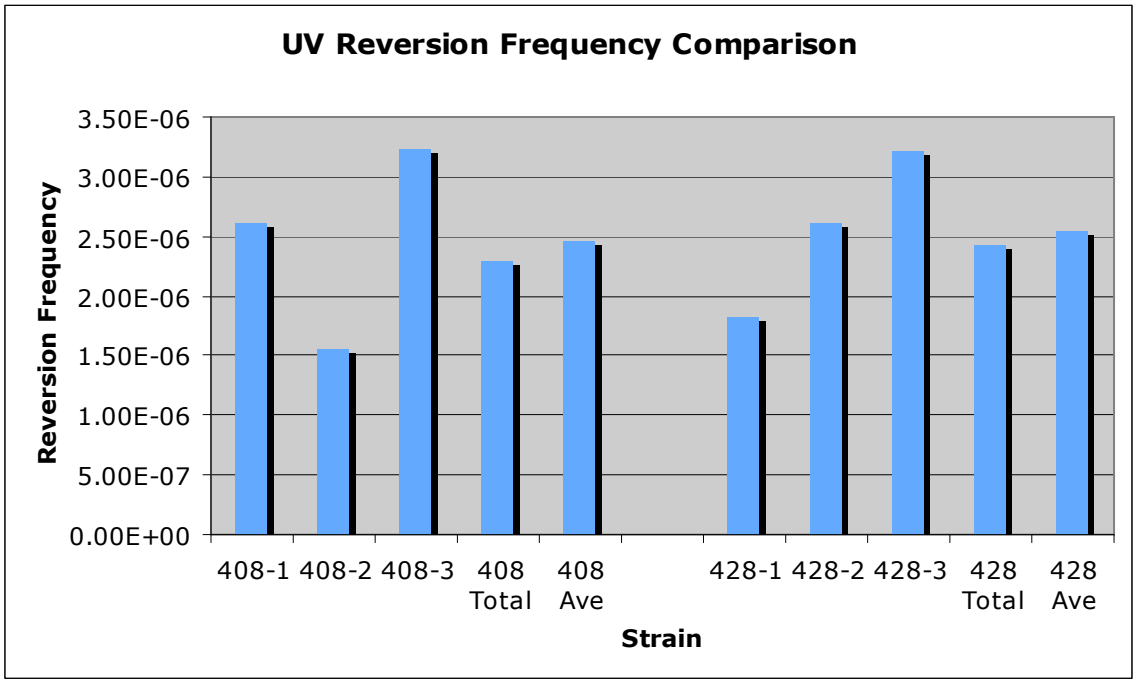


Figure 7 Reversion Frequency of MVY408 and MVY428: This figure shows the reversion data for the UV treatment of 408 and its sub1 deletion 428. The total column is the total number of colonies from all three cultures divided by the overall number of viable cells. The average was also taken for the two sets of data.

Since the initial data indicated no observable change resulted from a sub1 deletion, I duplicated the same procedure but added a 2 hour incubation in media lacking tryptophan prior to UV treatment. If SUB1 is involved in a transcription related pathway it may be necessary to have the TRP5 gene be actively transcribed before any difference could be seen. Contrary to what was expected the sub1 deletion strain showed a significant decrease in mutation frequency (Figure 8).

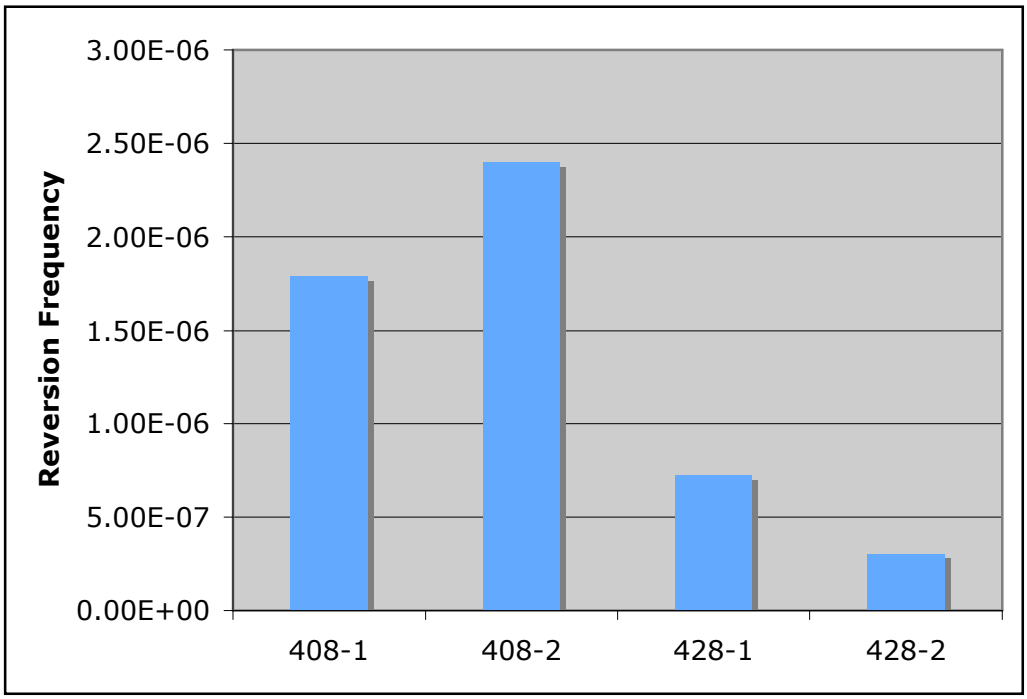


Figure 8 Trp Starvation: This figure shows the significant decrease in UV induced reversion frequency seen in the sub1 deletion strain (428) after Trp starvation.

H_2O_2 Reversion

Along with UV exposure I also began testing this system with H_2O_2 treatment to determine if directly increasing oxidative stress would alter the mutation frequency in the TRP5/sub1 strains. Unfortunately, at the time of writing this the use of H_2O_2 has not produced any detectable change in mutation frequency in any of the strains.

Canavanine Mutagenesis

Since the TRP5 reversion system was not producing any observable changes in mutation frequencies it was necessary to consider returning to an older mutational assay. Canavanine resistance is conferred on any cells that have a mutation that inactivates the CAN1 gene, preventing canavanine from entering the cells. In this system mutants can be selected by growing cultures on plates containing canavanine. This system differs from the TRP5 reversion assay because it is sensitive not only to base pair changes, but also to insertions, deletions, and frame shift mutations. Previous data had suggested that a sub1 deletion increases both spontaneous and H_2O_2 induced mutations. I repeated the spontaneous mutation test, and the results are shown in Figure 9 below. I found a 3-10 fold increase in spontaneous CanR mutations when the sub1 deletion mutants are compared with their SUB1+ parent strains.

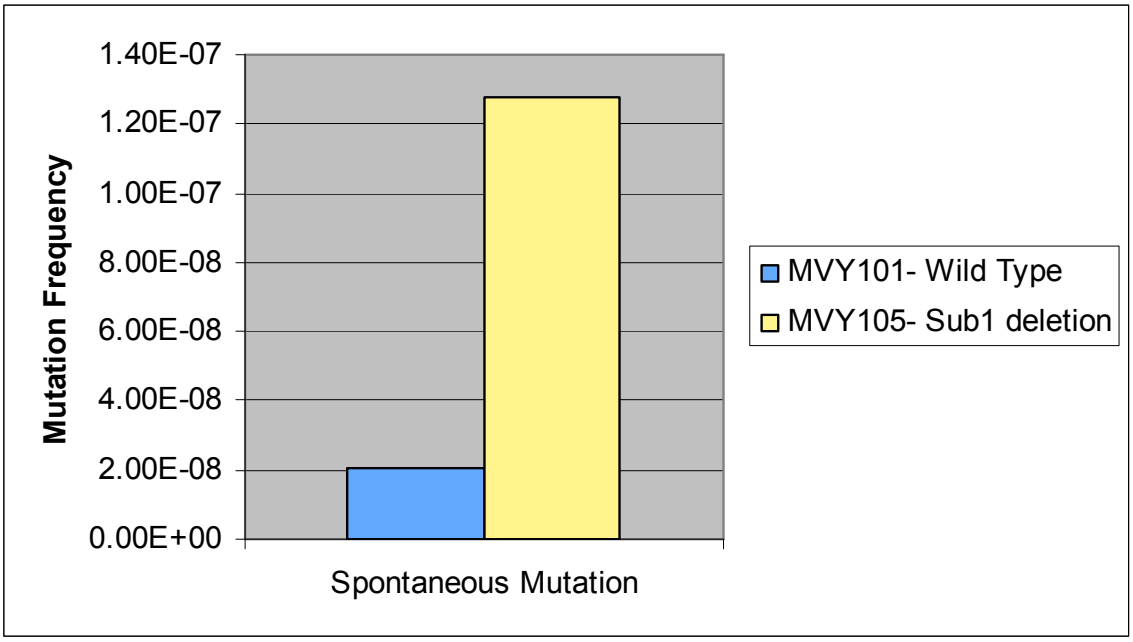


Figure 9 Spontaneous Canavanine Mutagenesis: This figure shows the data obtained from an assay of spontaneous mutation in the wild type (blue) and sub1 (yellow) deletion strains.

Currently I am working to sequence a group of the wild type and sub1 deletion mutants to determine what types of mutations are seen in each case, and if there is a difference in the mutational spectrum resulting from the sub1 mutation.

DISCUSSION

The goal of this project was to determine if deletion of sub1 increases yeast DNA mutation frequency and if it increases any one type of mutation or produces an overall increase of all mutation types. Since UV treatment generally results in GC to AT transitions, the strain representing that mutation was the focus of UV induced mutagenesis. The TRP5 reversion results, however, remain inconclusive. There was no measurable increase in mutation frequency resulting from the deletion of the sub1 gene, and at this time it is difficult to determine why this could be. It is possible that the system simply is not sensitive enough to allow detection of any difference between wild type and sub1Δ, or that sub1Δ forces the creation of something besides point mutations. Another possibility is that previous data suggesting that knocking out sub1 increases mutation frequency was inaccurate. The canavanine test, however, confirms that such an increase is seen. Since the canavanine system detects all types of mutation, not just point mutation, this points toward the possibility that the sub1 deletion creates some other type of mutation.

The results of the trp starvation experiment produce more questions than answers. If SUB1 simply played a role in TCR you would expect to see a significant increase in mutation frequency when the TRP5 gene is being actively transcribed. The data, however, shows that the exact opposite occurs. This could tie into the idea of SUB1 being in someway involved in the reestablishment of transcription after repair. It is possible that deletion of sub1 could result in fatal strand breaks instead of just base substitutions causing a majority of the cells to die therefore decreasing the observed

frequency of mutation. The possible connection between sub1 and transcription/replication requires further experiments before any real conclusions may be drawn.

Oxidative DNA damage causes GC to TA transversions. In wild type and sub1 strains such transversions are not seen. Therefore, it is not clear if sub1 has no effect, or if sub1 increases their frequency, but even the higher frequency is below the threshold of detection. Since OGG1 repairs 8-oxoG, the lesion that is largely responsible for GC to TA transversions, I am currently working on knocking out the gene ogg1 in the TRP5 strains to see if this increases mutation frequency enough to make the H_2O_2 induced mutations visible. Further study of both the TRP5 and canavanine system is necessary to determine what exactly is going on. The sequencing of the canavanine mutants could possibly conclusively show the type of mutations resulting from sub1 deletion. This data, along with the UV survival curve data I present in my MQP submitted to the Biology and Biotechnology departments could help determine more solidly the role SUB1/PC4 plays in DNA mutation and repair.

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