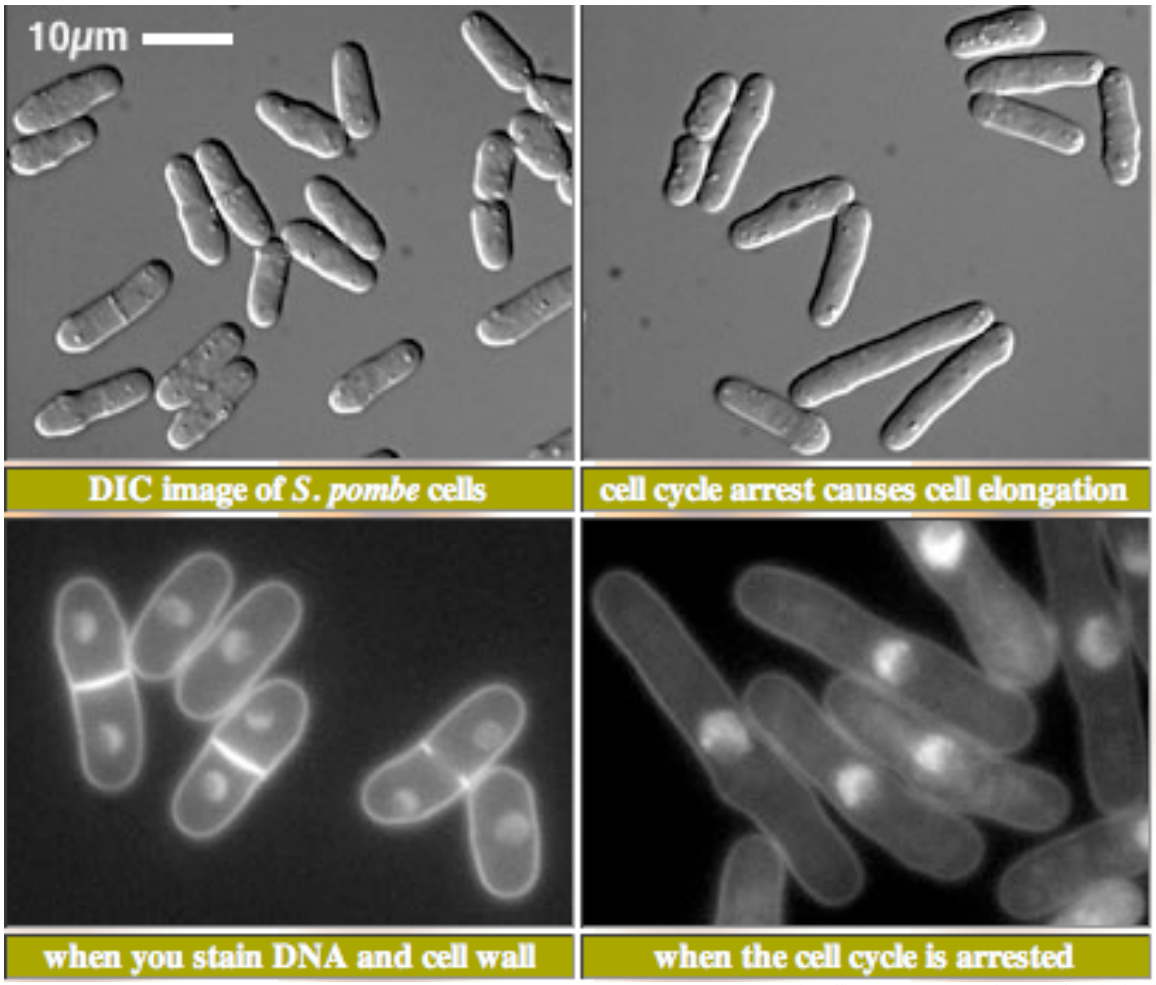
An investigation of DNA damage repair through the comparison of the average percent survival between the wildtype, Rad3 mutant and Rad8 mutant of *Schizosaccharomyces pombe* under varying durations of UV irradiation



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INTRODUCTION

Living organisms are frequently exposed to a variety of mutagens. Many mutagens can cause devastating harm and irreversible damage to cells. Ultraviolet (UV) light is a nonionizing physical mutagen that induces DNA mutations by targeting primarily thymine and causing the formation of thymine dimers and 6-4 photoproducts. (Gauthier and Seroude 2007) The shorter the UV wavelength, the more energy it possesses and the higher the absorbency of target molecules. Higher absorbency of UV light could in return induce more mutations. These mutations may cause cells to die if not removed. Thus, the DNA repair processes are crucial in the survival of all organisms to reverse DNA mutations inflicted by such agents.

Yeast, a unicellular eukaryotic organism, is an excellent model species to investigate DNA repair systems in eukaryotes. (Forsburg *et al.* 2005). A fission haploid yeast, *Schizosaccharomyces pombe,* was used in this experiment (Gauthier and Seroude 2007). Haploid yeast was employed due to its ability to express all mutations. In addition, *S. pombe* has a relatively short generation time and life cycle (Forsburg *et al.* 2005). The species was also inexpensive to culture and have a complete genome sequence available. (Forsburg *et al.* 2005). *S. pombe* have been extensively studied by researchers and are ideal organisms for examining of DNA repair mechanisms.

Three different strains of *S. pombe*, the wildtype, and two UV sensitive mutant strains, Rad3 and Rad8 mutants, were investigated. The wildtype strain was competent in DNA damage recognition and repair, if provided with sufficient time. The Rad3 and Rad8 mutant strains displayed different defects in the DNA damage repair system (Gauthier and Seroude 2007). The Rad3 mutant strain was known to be impaired in two

checkpoint pathways (Jimenez *et al.* 1992). According to Doe *et al*. (1993), the Rad8 mutant was especially sensitive to UV if irradiation was applied during the G1 and S phases of cell cycle (Doe *et al.* 1993).

In this experiment, varied amounts of UV irradiation were used to induce DNA mutations in the three strains of *S. pombe.* The purpose of this study was to compare the viability of the different strains of *S. pombe* and their DNA repair responses when exposed to varied amounts of UV irradiation.

METHODS AND MATERIALS

This experiment was conducted according to the protocol listed on pages L.2.1.1-L.2.1.6 and pages L 2.2.1-L1.2.5. (Gauthier and Seroude 2007). The concentration of initial *S. pombe* cultures was determined by comparing A_{595} to a standard curve of A_{595} versus concentration (Appendix 1).

RESULTS

Table 1: The relationship between the duration of UV exposure and the number of surviving colonies of wildtype, Rad3 and Rad8 mutant strains of *S. pombe* for three trials.

Duration of UV exposure (seconds)	Wildtype			Rad3 Mutant			Rad8 Mutant		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
0	380	539	200	263	456	283	218	40	948
2	292	398	150	54	155	47	102	29	422
5	240	225	113	19	57	16	73	25	220
10	136	231	50	3	5	6	38	8	143
20	72	142	33	1	3	3	13	3	48
30	30	37	13	0	0	1	2	1	8

Table 2: The relationship between the duration of UV exposure and the percent of surviving colonies of wildtype, Rad3 and Rad8 strains of *S. pombe* for three trials.

Duration of UV	Wildtype (%)			Rad3 Mutant (%)			Rad8 Mutant (%)		
	Trial	Trial	Trial	Trial	Trial	Trial	Trial	Trial	Trial

exposure (seconds)	1	2	3	1	2	3	1	2	3
0	100	100	100	100	100	100	100	100	100
2	76.8	73.8	75.0	20.5	34.0	16.6	46.8	72.5	44.5
5	63.2	41.7	56.5	7.22	12.5	5.65	33.5	62.5	23.2
10	35.8	42.9	25.0	1.14	1.10	2.12	17.4	20.0	15.1
20	18.9	26.3	16.5	0.380	0.658	1.06	5.96	7.50	5.06
30	7.89	6.86	6.50	0	0	0.353	0.917	2.50	0.844

Sample calculation for percent survival:
Using the number of surviving wildtype colonies of *S. pombe* in trial 1 at 5 minutes

% survival = # of colonies at 5 minutes / # of colonies at 0 minutes x 100%
= 240/380 x 100%
= 63.2%

Table 3: The relationship between the duration of UV exposure and the mean percent survival and standard deviation for surviving colonies of wildtype, Rad3 and Rad8 mutant strains of *S. pombe* for three trials.

Duration of UV exposure (seconds)	Wildtype		Rad3 Mutant		Rad8 Mutant	
	Mean (%)	SD (%)	Mean (%)	SD (%)	Mean (%)	SD (%)
0	100	0	100	0	100	0
2	75.2	1.51	23.7	9.13	54.6	15.5
5	53.8	11.0	8.46	3.59	39.7	20.4
10	34.6	9.01	1.45	0.578	17.5	2.45
20	20.6	5.11	0.699	0.342	6.17	1.23
30	7.08	0.72	0.118	0.204	1.42	0.936

The mean percent survivals for each of the wildtype, Rad3 mutant and Rad8 mutant strains of *S. pombe,* at a specific duration of UV exposure, were calculated through the addition of percent survivals from each trial and dividing by the number of trials performed for each *S. pombe* strain at that particular duration of UV exposure.

The correlation between the average percent survival and duration of UV irradiation in the wildtype, Rad3 and Rad8 strains of *S. pombe* is shown in Figure 1. It was found that the average percent survival decreased for all three strains when the amount of UV exposure increased. The wildtype strain of *S. pombe* exhibited the least decrease in

average percent survival. The Rad8 mutant curve showed a greater decrease in average percent survival than wildtype, but less than the Rad3 mutant. In addition, the Rad8 mutant also showed a UV threshold at 25 seconds of UV exposure. The Rad3 mutant strain displayed a dramatic decrease in average percent survival at low doses of UV irradiation and had the greatest decrease among all three strains.

The morphologies of colonies of each *S. pombe* strain differed considerably from each other after UV irradiation treatments. The colonies of all three strains were consistently observed to be round in shape. The Rad8 mutant colonies were extremely tiny in size. The Rad3 mutant strains were large-sized and formed distinct colonies. The wildtype displayed medium-sized colonies compared to the other two UV-sensitive mutant strains. However, the wildtype strain exhibited a lawn of colonies at low doses of UV irradiation.

DISCUSSION

In the present study, the average percent survivals of all three strains of *S. pombe* decreased as the duration of UV irradiation increased. However, minor variations existed in different strains. The wildtype strains of *S. pombe* exhibited the least decrease in average percent survival. The Rad8 mutant showed a greater decrease than wildtype, but less than the Rad3 mutant. In addition, the Rad8 mutant reached a UV threshold where the curve decreased rapidly towards 0 % survival at 25 seconds of UV exposure. The Rad3 mutant strain expressed a dramatic decline at low doses of UV irradiation and had the greatest decrease in survival among all three strains. These observations could be explained by the differences in UV sensitivity on DNA damage and DNA repair mechanisms between the three strains of *S. pombe.*

The wildtype strain of *S. pombe* showed the smallest decrease in average percent survival as UV exposure treatment increased. The wildtype strain was more UV-resistant than the other two mutant strains (Lieberman *et al*. 1989). The wildtype was also capable of detecting and repairing DNA damage, if supplied with enough time (Gauthier and Seroude 2007). Usually, when a DNA mutation was induced by mutagens, DNA repair mechanisms responded to the damage by delaying the progression of mitosis and temporarily retaining the cell in the G2 phase (Jimenez *et al.*1992). This delay provides time for DNA repair. DNA repair can occur through the common nucleotide excision repair (NER) pathway and the extra UV photoproducts excision pathway, with the latter only present in *S. pombe* (McCready *et al.* 2000). Typically, upon detection of DNA damage, the NER responds to lesions by removing them and restoring the proper DNA sequences. Therefore, DNA repair system ensures the accuracy of DNA sequences and hence, the correct gene expression. The UV resistance of the wildtype strain and its ability to perform DNA repair, with sufficient time provided, might explain the small survival decrease in comparison to other mutant strains.

The Rad8 mutant strain revealed a greater decrease in average percent survival than the wildtype strain and less than that of the Rad3 mutant strain. In addition, a UV threshold was obtained. This might be due to the sensitivity of the Rad8 mutant to UV irradiation. A study done by Doe *et al.* (1993) indicated that the maximal sensitivity of the Rad8 mutant strain occurred during G1/S phase. This might suggest that if DNA mutations were implemented during these early stages of cell cycle, there would be sufficient time for DNA repair to occur before mitosis. Thus, this delay in the entry into mitosis might have enhanced the survival of the Rad8 mutant strain compared to the

Rad3 mutant. In addition, according to Doe *et al.* (1993), an isolated plasmid pRAE2, found at the Rad8 locus, can re-establish UV sensitivity to near wildtype conditions. This could also possibly explain the mild decrease in the average percent survival of the Rad8 mutant at low doses of UV exposure similar to that of the wildtype strain. However, if the dosage of UV irradiation increased, the Rad8 mutant strain eventually approached a UV threshold where no more surviving colonies were observed. This could be explained by a critical threshold of DNA mutations that the Rad8 mutant can repair. Once past a certain point, the Rad8 mutant strain could be unable to remove the large amount of DNA damage. The Rad8 mutant showed a UV threshold, a larger decrease in survival than the wildtype and a smaller decrease than the Rad3 mutant; this might be due to its greater sensitivity to UV exposure than wildtype and less than the Rad3 mutant.

In the Rad3 mutant strain, a dramatic decrease in the average percent survival at low doses of UV exposure was observed, compared to the other strains of *S. pombe*. The Rad3 mutant was found to be defective in two checkpoint functions in the DNA repair system (Jimenez *et al.* 1992). This could explain the significant decrease in survival of Rad3 at low doses of UV irradiation. According to a study conducted by Jimenez *et al.* (1992), the Rad3 mutant strain was tested to be incapable of delaying mitosis and arresting cells in the G2 phase following DNA damage. A similar study done by Bentley *et al*. (1996) indicated that the Rad3 gene was fundamental in all DNA structure checkpoints for damage repair. These studies suggested that without the functional Rad3 gene, DNA damage would not be repaired in *S. pombe* and cell death would be extremely common.

In addition, a study performed by Martinbo *et al.* (1998) indicated that the Rad3 gene of *S. pombe* was necessary to activate Chk1 response for mitotic arrest following DNA damage and also to activate Cds1 response for DNA replication blocks. Defects in these two responses of the DNA repair process could result in the failure to excise lesions in DNA following UV exposure and the entry of incomplete DNA into mitosis. As a result, mitosis could advance when DNA was unreplicated, thereby producing lethal missegregations from partially replicated or unreplicated DNA (Bentley *et al.* 1996). Moreover, hypersensitivity to DNA mutagens could occur when mutant strains with malfunctioning DNA checkpoints attempted mitosis without any repair (Bentley *et al.* 1996). This hypersensitivity to mutagens could decrease the survival of the Rad3 mutant strain. Therefore, DNA repair aids in the successful completion of DNA replication and helps to maintain the accuracy of DNA sequences during replication, and thereby increasing the survival of organisms. The Rad3 mutant strain of *S. pombe* was unable to arrest the progression into mitosis upon detection of DNA damage and replication blocks, and could explain the significant decrease in survival after the application of the first dosage of UV treatment.

In this experiment, the wildtype, Rad3 and Rad8 mutant strains of *S. pombe* all showed decreases in average percent survival with increasing UV exposure. The wildtype was observed to be the most UV resistant. The Rad8 mutant displayed a decrease in average percent survival that was similar to the wildtype up to a UV threshold. The Rad3 mutant had the most dramatic decrease in survival at low doses of UV irradiation possibly due to its defects in two checkpoint functions for DNA repair. This experiment indicated the important roles of the DNA repair and checkpoint pathways in the

maintenance of accurate DNA sequences and gene expression. Defects in DNA repair could result in genetic disorders such as Xeroderma pigmentosum (Doe *et al.* 1993). Additional studies could be done to provide further insight into the complex mechanisms of the various genetic disorders caused by defects in DNA repair systems.

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