Understanding the Role of Histone H2A Serine 122 in DNA Double Strand Break Repair

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Eukaryotes must package a large amount of DNA into the small space of a nucleus, while still being able to access necessary genes. Chromatin is the packaged form of DNA, in which DNA is wrapped around histone proteins to form the nucleosome, which is the smallest part of chromatin. There are four core histones: H2A, H2B, H3, and H4, and two copies of each histone in every nucleosome. Histones have flexible tail regions that include many amino acids that can be covalently modified. These modifications signal numerous cellular processes, including DNA repair. This study focused on histone H2A, in particular the modification on the serine 122 residue (S122). S122 is interesting because when it is mutated to an alanine (S122A mutant) the cells are more sensitive to many types of stress such as DNA damaging agents, like methyl methane sulfonate (MMS). To understand the role of H2A S122 in DNA double strand break repair we performed a suppressor screen, in which we rescued this sensitivity using a genomic library. We set up the suppressor screen by constructing and characterizing a strain of H2A S122A for the genetic screen. After strain construction we then compared the new S122A screen strain with an existing S122A strain to make sure the strain exhibited proper sensitivity to MMS. We then optimized the assay to find the concentration of MMS that gave the biggest difference between wild type S122 and S122A.

Introduction

It is very important for cells to repair DNA because it is constantly getting damaged. There are many causes of DNA damages such as radiation, chemical, and mechanical damage. Double strand breaks are the worst kind of damage for DNA to sustain. It can cause mutations and chromosomal deletions, which will lead to cancer. DNA must be repaired, though, in the context of chromatin. Chromatin is a packaged form of DNA, where it wraps around the histone proteins to form the nucleosome, which is the smallest part of chromatin. Inside the nucleosome, there are four types of histones: H2A, H2B, H3, and H4, with 2 copies of each histone in every nucleosome. Histones have flexible tail regions that include many amino acids that can be covalently modified, and these modifications can include acetylation and phosphorylation. The modifications signal numerous cellular processes, such as DNA repair. This study focused H2A serine 122 mutant (S122A). Previous studies have shown that H2A S122A is sensitive to the repair of chemically induced DNA damage.[1,2] The model organism we used was *Saccharomyces cerevisiae*, also known as baker's yeast. We used *S. cerevisiae* because it has similar chromatin structure and DNA repair as mammalian cells do. To understand the role of H2A S122, we can run the S122A mutant through a genetic screen to try to identify genes that can rescue the methyl methane sulfonate (MMS) growth phenotype. The genetic screen involves transforming the S122A mutant with a genomic library, and growing the cells on MMS. By identifying colonies that grow better we identified cells that received a gene allowing them to repair their DNA more efficiently. Collecting the rescuing plasmids and getting them sequenced will identify the genes responsible for the rescue allowing us to understand the sensitivity of S122A better, and thus the role that H2A S122 plays in DNA repair. The initial step of the screen requires we first make a new S122A strain and test it on different concentrations of MMS. Doing this, we created a strain with appropriate markers for the screen and optimized the concentration of MMS to be used in the screen.

Methods

Strain List

JKY33	existing S122A strain (pRS413 (hta1-S122A-HTB1))
JKY147	wild-type (pRS314(HTA1-HTB1))
JKY148	wild-type (pRS416(HTA1-HTB1))
JKY149	S122A screen strain (pRS416 (hta1-S122A))
JKY154	rad54Δ (negative control)

Strain construction

Preparation of competent cells

JKY148 cells were cultured overnight at 30°C in 5 mL rich media (YPD) in a shaking incubator. 3 mL of the overnight culture were diluted in 50 mL of YPD and cells were incubated in the 30°C shaker for at least 3 hours, until the optical density at 600 nm (OD_{600}) was between 0.500 and 0.600. We then collected the cells by centrifugation @ 3500 rpm for 5 minutes and decanted the media. We washed the pellet with 1 mL of nanopure H_2O, transferred the cells to a 1.5 mL microcentrifuge tube, and spun down the cells at 3500 rpm for 1 minute and decanted the supernatant. The pellet was washed with 1 mL

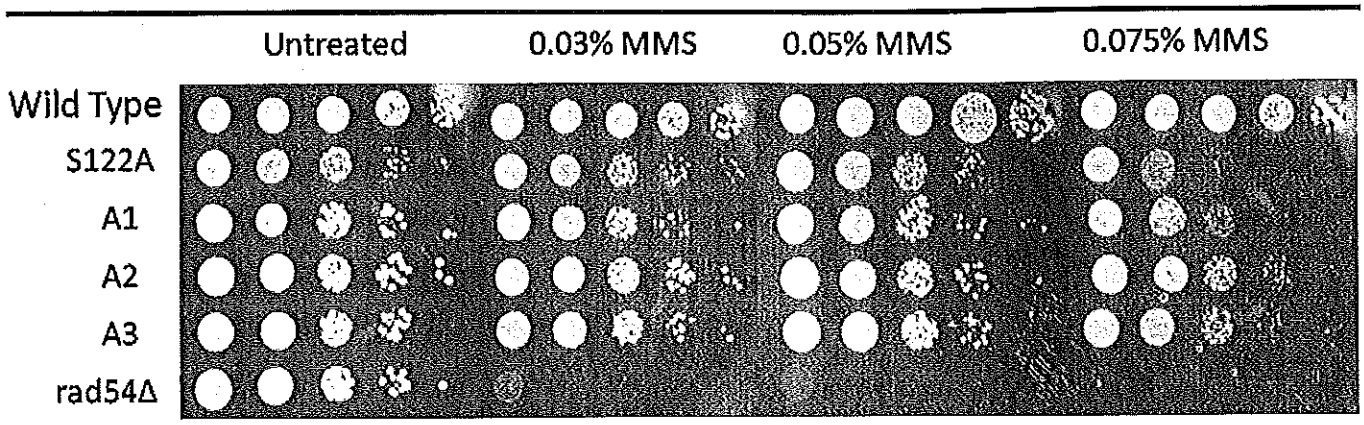

Fig 1. Plating assay to characterize and optimize MMS sensitivity for use in a genetic suppressor screen. Wild-type and rad54Δ are positive and negative controls respectively. S122A indicates an existing S122A mutant strain (JKY33) available, but not suitable for the genetic screen, while A1, A2, and A3 are transformants from the strain construction and designated the S122A screen strain. These results indicate that, while all three transformants are more sensitive to MMS than the WT strain, they are not quite as sensitive as the original S122A mutant. Also seen is that a concentration of 0.075% provides a much stronger phenotype than either of the two lesser concentrations. The robust growth of the WT all the way out to the fifth dilution also means an even higher concentration of MMS could be used

lithium acetate/TE (LiAc/TE; 0.1 M LiAc; 0.1 M Tris-Cl; 1 mM EDTA; pH 7.5), and resuspended in 250 µL of LiAc/TE. Cells were stored for no longer than 48 hours in LiAc/TE at 4°C.

Transformation

For the transformation, we combined as follows: 300 mL PLATE (40 mL 50% PEG-4000; 5 mL 1 M LiOAc; 5 mL 10× TE, pH 7.5), 50 µg carrier (single stranded salmon sperm DNA, 10 mg/mL), 1 µL JKP161 (pRS416(hta1-S122A)), and 50 µL cell suspension and mixing by vortexting. The transformation was incubated at 30°C for 30 minutes, then heat shocked at 42°C for 15 minutes. We then centrifuged cells for 1 minute at 3500 rpm, aspirated the supernatant, and resuspended the cells in 500 mL TE, pH 8.0. Lastly, We plated 150 µL and 50 µL of the transformed cells onto URA- media to select for the plasmid. Colonies from this transformation were named JKY149.

Strain characterization

Plating assay

For each strain (JKY33, JKY147, JKY149, JKY154) 3 mL of YPD was inoculated, and two 10-fold dilutions made. All cultures were grown overnight in a shaking incubator at 30°C. The following day we checked the OD_{600} of the cultures, choosing one culture for each strain that was under 0.9. Using room temperature YPD, we diluted the cultures to an OD_{600} of 0.2, and then made four 10-fold dilutions of each culture. Five µL of each dilution (including the undiluted culture) were plated on YPD plates containing 0%, 0.03%, 0.05%, and 0.075% MMS. Plates were then incubated at 30°C for 48 hours before being photographed.

Results

After the plating assay had been completed, we compared JKY33 with multiple cultures of JKY149 (A1, A2, A3) to make sure our new strain had exhibited proper sensitivity to MMS. We compared different concentrations of MMS (0.03%, 0.05%, and 0.075%) in YPD, and tried to optimize which concentration had the biggest different between wild type and S122A. The new strain (JKY149; A1, A2, A3) was not as sensitive to MMS as the existing S122A strain, but the plating assay indicated that the strongest difference between those strains and the WT was the 0.075%.

Discussion

This project was initially intended to begin the screen and yield candidates from the genomic library. Contamination issues, however, prevented the completion of the full project. We were able to construct a strain for the screen with useful markers still available, and optimized the screen at the same time. This strain of S122A will make it possible to use 5 fluoroorotic acid (5FOA) to select against the H2A plasmid and this, in turn, will make it possible to remove cells that receive wild-type H2A from the library without having to sequence the H2A gene. While 0.075% MMS had the strongest phenotype of the concentrations used, the strong growth of the WT strain indicated that even higher concentrations of MMS could be used to yield increasing differences in the growth patterns between WT and S122A. With the screen available and ready to be

characterized, we are now ready to proceed with the screen.

There are a number of potential outcomes from the genetic screen. It is possible that we will find downstream repair enzymes, repair factors from alternate repair pathways, or an unexpected result from the screen. The screen is unbiased meaning any preconceived notions about the screen will have no affect on the genes we actually identify. The unbiased nature of the screen means that we could identify genes rescuing the phenotype that we would never have normally considered.

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