Loss of F-box motif Encoding Gene *SAF1* and Chromatin Associated factor *CTF8* together contributes to MMS Resistance and HU Sensitive phenotype in *S. cerevisiae*

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ABSTRACT

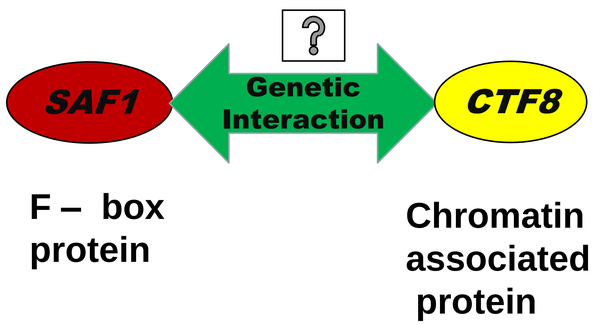
Cohesion related replication factor-C complex constitutes, three subunits called Ctf18, Ctf8 and Dcc1. These three subunit complex assist the loading of PCNA onto the chromosome. None of the replication factor C components are essential for cell viability. The null mutant of *CTF8* in *S.cerevisiae* shows high frequency of chromosome loss. The *SAF1* gene product of *S. cerevisiae* involves in recruitment and degradation of adenine deaminase factor Aah1p through SCF-E3 ligase mediated ubiquitination. Here we have investigated the genetic interaction between *SAF1* and *CTF8* genes. The single and double gene deletions of *SAF1* and *CTF8* were constructed in BY4741 genetic background and evaluated for growth fitness, genome stability and cellular growth response to Genotoxic stress caused by Hydroxyurea (HU) and Methyl methane sulfonate (MMS). The *saf1Δctf8Δ* strain showed the increased growth phenotype in comparison to WT, *saf1Δ*and *ctf8Δ* strains on YPD medium. However *saf1Δctf8Δ* strain when grown in the presence MMS showed resistance and HU sensitive phenotype when compared with *saf1Δ, ctf8Δ.* The frequency of Ty1 retro-transposition was also elevated in *saf1Δctf8Δ* in comparison to either *saf1Δ* or *ctf8Δ.* The number of cells showing the two or multi-nuclei phenotype was also increased in *saf1Δctf8Δ* cells when compared with the either *saf1Δ* or *ctf8Δ.* Based on these observations, we report that the absence of both *SAF1* and *CTF8* genes together contributes to MMS resistance, HU sensitivity, and genome instability phenotype. This report warrants the further investigation into the mechanisms of differential growth phenotype due to loss of *SAF1* and *CTF8* together in presence of Genotoxic stress.

INTRODUCTION

SAF1 **(SCF associated factor 1) of *S.cerevisiae* is involved in degradation of *AAH1* gene during proliferative state to quiescence phase . *SAF1* gene also target with vacuolar / Lysosomal protein such as PRB1, PRC1 and Ybr139w by ubiquitination process.**

CTF8 **(Chromosome transmission fidelity factor 8) gene is functionally conserved from simpler to higher eukaryotes. *CTF8* is the part of Replication factor C (Ctf18p, Ctf8p and Dcc1p) that is mainly involved in Sister Chromatid Cohesion and PCNA loading/unloading.**

The functional relationships between genes could be termed as Genetic interactions. The binary genetic interaction can be explored through double deletion of both the gene which are under investigation and impact on phenotype such as cellular growth fitness.



AIM

To investigate the Genetic Interaction between F - box encoding gene *SAF1* and Chromatid associated gene *CTF8* in the BY4741 and JC2326 genetic background of *S.cerevisiae.*

METHODOLOGY

1. **Primer designing for deletion of an ORF region of *SAF1* and *CTF8 gene* from *S. cerevisiae.***
2. **Amplification of deletion cassettes by PCR from plasmid pFA6a-kanMX6, and pFA6a-His3MX6.**
3. **Yeast transformation of BY4741 and JC2326 strains with deletion marker cassette by lithium acetate protocol.**
4. **Genomic DNA isolation from yeast transformants.**
5. **Confirmation of marker cassette integration at target ORF region by PCR.**
6. **To compare the growth kinetics among the WT and the deletion strains, growth assay based on the OD measurement at 600nm by Spectrophotometer.**
7. **To analyze the frequency of Ty1 retro transposition of deletion Strains in the JC2326 genetic background.**
8. **Comparative analysis among WT and mutants, for nuclear migration status an assay based on fluorescence microscopy.**
9. **To analyze the cellular growth by Semi quantitative spot assay in the presence of Genotoxic drugs.**

RESULTS

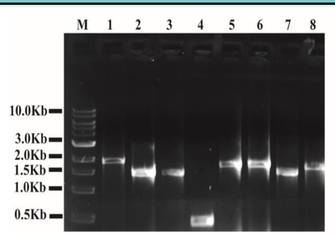


Figure : Confirmation of gene deletions* (*saf1*Δ, *ctf8*Δ, and *saf1*Δ*ctf8*Δ)*in BY4741 genetic background by polymerase chain reaction and agarose gel electrophoresis. Lane M,** *indicates molecular weight marker lane,* ***Lane 1, 4*, indicates SAF1 (1914bp) and CTF8 (402bp) ORF regions,* ***Lane 2*** *indicates HIS3MX6 marker cassette (1403bp),* ***Lane 5*** *indicates KANMX6 marker cassette (1559bp),* ***Lane 3*** *indicates PCR product generated from transformed strain with HIS3MX6 marker cassette having upstream and downstream 40bp homology to SAF1 ORF* (saf1Δ::HIS3MX)*,* ***Lane 6****, indicates PCR product generated from transformed strain with KANMX6 marker cassette having upstream and downstream 40bp homology to CTF8 ORF (*ctf8Δ::KANMX*),* ***Lane 7, 8*** *indicates the PCR product generated from transformed strain (*saf1Δctf8Δ*) having integration ofHIS3MX6 and KANMX6 cassette(*saf1Δ::HIS3MX, ctf8Δ::KANMX*).*

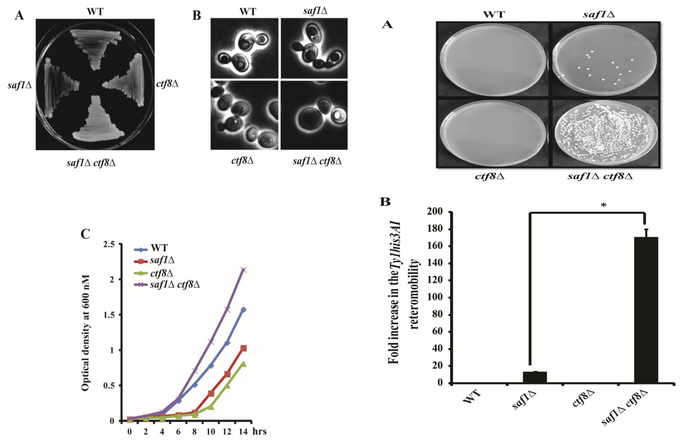
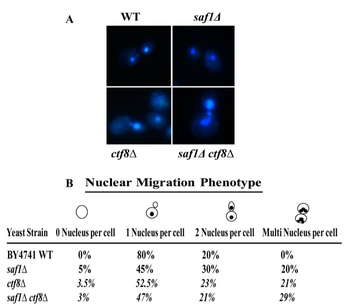


Figure: Comparative analysis of growth and morphology of WT, *saf1*Δ, *ctf8*Δ, *saf1*Δ*ctf8*Δ cells. A growth of streaked strain on YPD plates, streaked culture was incubated for 2 days at 30°C and then photographed**. B.** Phase contrast images of log phase cultures at 100X magnification using Leica DM3000. **C**. Growth Kinetics of strains (WT, *saf1*Δ, *ctf8*Δ, *saf1*Δ*ctf8*Δ). Cells were collected every 2-hour period and cellular growth was measured by optical density (OD) at 600 nm using TOSHVIN UV- 1800 SHIMADZU. The data shown represent the average of three independent experiments. The error bars seen represent the standard deviation for each set of data.

Figure: Assay for genome instability by measuring of *his3AI* marked Ty1 transposition frequency in WT, *saf1*Δ, *ctf8*Δ, *saf1*Δ*ctf8*Δ. A. Images of plates showing the Ty1 transposition induced colonies on SC plate lacking His media. **B.** Bar diagram showing the frequency of *Ty1his3AI* transposition in each strain. The data shown represent the average of three independent experiments. The significance of transposition was determined by using two tailed t- test. P - value (p) less than 0.05 indicate significant difference and the symbol * represent to p<0.05



Yeast Strain	0 Nucleus per cell	1 Nucleus per cell	2 Nucleus per cell	Multi Nucleus per cell
BY4741 WT	0%	80%	20%	0%
*saf1*Δ	5%	45%	30%	20%
*ctf8*Δ	3.5%	52.5%	23%	21%
*saf1*Δ *ctf8*Δ	3%	47%	21%	29%

Figure : Assessment of nuclear migration phenotype using DAPI (4′,6-diamidino-2-phenylindole) staining of WT, *saf1*Δ, *ctf8*Δ, *saf1*Δ*ctf8*Δ. A Representative fluorescent image of WT, *saf1*Δ, *ctf8*Δ, *saf1*Δ*ctf8*Δ cells showing the status of Nuclear DNA migration. The images were acquired at the 100X magnification using Leica DM3000 fluorescent microscope. **B** Table showing the percentage from the count of 200 cell as, 0, 1, 2 and multi nucleus in each strain, more than two nuclei indicate the nuclear migration defect.

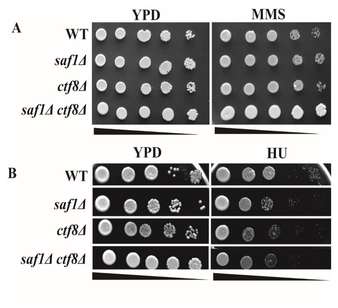


Figure: Comparative assessment of growth phenotype of WT *saf1*Δ, *ctf8*Δ, *saf1*Δ*ctf8*Δ strains in presence of Methyl methane sulfonate and Hydroxyurea by spot analysis. A. Each strain was grown to log phase was equalized by O.D 600nm and tenfold serially dilution was made. From each dilution 3µl spotted on YPD, YPD + HU (200mM). **B.** Ten-fold serial diluted culture of WT *saf1*Δ, *ctf8*Δ, *saf1*Δ*ctf8*Δwere spotted on YPD, YPD + MMS (0.035%) containing agar plates. The *saf1*Δ*ctf8*Δ showed the résistance to MMS and sensitivity to HU

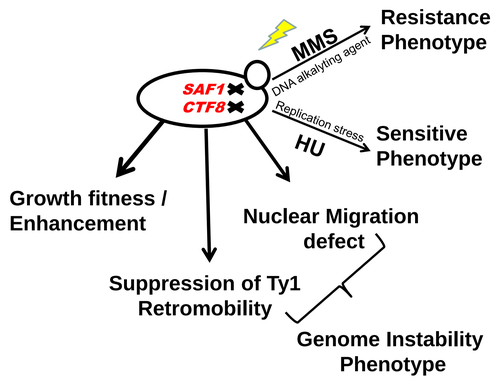


Figure: A model showing the role of double deletion of *SAF1* and *CTF8* genes in the maintenance of genome stability and stress response.

CONCLUSION

- **The binary genetic interaction between *SAF1* and *CTF8* genes showed growth fitness or phenotypic enhancement feature.**
- **Double deletion of *saf1*Δ*ctf8*Δ showed resistant against DNA alkalyting agent Methyl methane sulphonate (MMS) and sensitive in the presence of replication stress drug Hydroxyurea (HU).**
- **The Genome stability assay such as nuclear migration and Ty1 retromobility indicate that double deletion *saf1*Δ and *ctf8*Δ involved in genome stability.**

FUTURE DIRECTIONS

- **Investigation into the Mechanism of Genome stability conferred by the double deletion of *SAF1* and *CTF8* genes.**
- **Find out the mechanism of differential growth phenotype due to loss of *SAF1* and *CTF8* together in presence of Genotoxic stress.**

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