Genetic interaction between RLM1 and F-box motif encoding gene SAF1 contributes to stress response in *Saccharomyces cerevisiae*

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**Abstract**

**Background:** Stress response is mediated by the transcription of stress-responsive genes. The F-box motif protein Saf1p is involved in SCF-E3 ligase mediated degradation of the adenine deaminase, Aah1p upon nutrient stress. The four transcription regulators, \textit{BUR6}, \textit{MED6}, \textit{SPT10}, \textit{SUA7}, are listed for \textit{SAF1} in the genome database of \textit{Saccharomyces cerevisiae}. Here in this study, we carried out an \textit{in-silico} analysis of gene expression and transcription factor databases to understand the regulation of \textit{SAF1} expression during stress for hypothesis and experimental analysis.

**Result:** An analysis of the GEO profile database indicated an increase in \textit{SAF1} expression when cells were treated with stress agents such as Clioquinol, Pterostilbene, Gentamicin, Hypoxia, Genotoxic, desiccation, and heat. The increase in expression of \textit{SAF1} during stress conditions correlated positively with the expression of \textit{RLM1}, encoding the Rlm1p transcription factor. The expression of \textit{AAH1} encoding Aah1p, a Saf1p substrate for ubiquitination, appeared to be negatively correlated with the expression of \textit{RLM1} as revealed by an analysis of the Yeastact expression database. Based on analysis of expression profile and regulatory association of \textit{SAF1} and \textit{RLM1}, we hypothesized that inactivation of both the genes together may contribute to stress tolerance. The experimental analysis of cellular growth response of cells lacking both \textit{SAF1} and \textit{RLM1} to selected stress agents such as cell wall and osmo-stressors, by spot assay indicated stress tolerance phenotype similar to parental strain however sensitivity to genotoxic and microtubule depolymerizing stress agents.

**Conclusions:** Based on \textit{in-silico} and experimental data we suggest that \textit{SAF1} and \textit{RLM1} both interact genetically in differential response to genotoxic and general stressors.

**Keywords:** \textit{S. cerevisiae}, F-box motif, E-3 ligase, Stress

**Introduction**

The natural population of eukaryotic cells, mostly microorganisms remains non-dividing state and proliferate upon nutrients availability [1]. The proliferation of \textit{Saccharomyces cerevisiae} cells from in and out of the quiescence phase due to nutrients availability or stress condition remains an active research area for both basic research and biotechnological purpose. Upon nutrient deprivation, yeast cells stop dividing and enter into a stationary phase. This transition leads to glycogen accumulation and reduced cell wall porosity which makes cells resist stressors which increases survival probability. The mutants with cell cycle transition defects that are not able to enter into the stationary phase showed sensitivity to stress condition and may die due to starvation [2].
The studies on gene expression during entry into stationary phase [3] listed thousands of genes with varied expression profile [4, 5] implying that active transcriptional regulation is necessary for entry or exit from stationary phase. The cell cycle phase transition process is dependent on the ubiquitin-proteasome system [6] and the ubiquitination process is essential for survival during starvation. The SCF^{Saf1}-E3 ligase in *S. cerevisiae* recruits Aah1p (adenine deaminase) for ubiquitin-mediated degradation upon nutrient deprivation condition [7]. The adenine deaminase is an important enzyme of purine biosynthesis and it requires for conversion of adenine to hypoxanthine. The AAH1 expression is down regulated during the transition from proliferative state to quiescence state by Srb10p and Srb11p however regulation at the post-transcriptional level of Aah1p requires SCF^{Saf1}-E3 ligase activity. The SCF^{Saf1}-E3 ligase also requires for degradation of the unprocessed vacuole and lysosomal proteins [8] and three proteins, serine protease B (*PRB1*), protease C (*PRC1*), and Ybr139w reported to be targets of Saf1p for proteasomal degradation [8]. The expression *SAFI* is regulated by regulatory transcription factors (*BHR6, MED6, SLA7*) reported in a study on chromatin immunoprecipitation-chip analysis upon heat treatment [9] and *SPT10* which encodes, a histone H3 acetylase, reported in a microarray RNA expression-based study [10]. The null mutant of *SAFI* exhibits decreased death rate at elevated temperature [11] and resistance to histone deacetylase inhibitor CG-1521 [12].

The *Saccharomyces cerevisiae* RLM1/YPL089C (resistance to lethality of MKK1P386 over expression) is a MADS-box (Mcm1, Agamous, Deficiens, and serum response factor) family transcription factor which is phosphorylated and activated by MAP-kinase Slt2p [13]. The Rlm1p regulates the expression of genes related to cell wall integrity when cells are treated with calcofluor white and zymolyase [14] indicating its role in stress response in *S. cerevisiae*. Deletion of *RLM1* in *S. cerevisiae* results in resistance to cell wall disrupting agents [15]. *S. cerevisiae* *RLM1* homologs have been reported from other filamentous fungi such as *Aspergillus niger* [16], *Aspergillus fumigatus* [17], and *Candida albicans* [18].

The gene expression omnibus (GEO) profile database at the National Centre for Biotechnology Information (NCBI) is a repository of genome-wide gene expression of different model organisms and can be mined for gene expression status using both experiment-centric and gene-centric approach [19, 20]. The gene expression is dependent on the transcriptional network involving the interaction of transcription factors and promoter of genes [21]. The Yeast transcription database YEAST-ARCT (Yeast Search for Transcriptional Regulators And Consensus Tracking) allows the search of association between the transcription factors and gene expression in a variety of experimental conditions (http://www.yeastarct.com). It is a repository of curated associations between transcription factors and genes in *Saccharomyces cerevisiae* [22]. The Yeast stress expression database, yStreX (http://www.ystrexdb.com/) allows analysis of gene-specific expression patterns during stress. The database has a collection of genome-wide expression data on stress response in *S. cerevisiae* [23].

In the present study, we studied the Gene Expression Omnibus profile database (GEO) for the expression status of *SAFI* during stress conditions, and yeast transcription databases were investigated for the association of transcription factors with the *SAFI* expression during stress. The regulatory association analysis was carried out to understand, transcriptional regulation of target genes by transcription factors during stress for hypothesis followed by experimental analysis. We report RLM1 as a transcription factor regulating the expression of *SAFI* during stress through in-silico analysis. Further, we hypothesized that ablation of both genes may contribute to stress tolerance. We report that the loss of *SAFI* and *RLM1* together leads to stress resistance in *S. cerevisiae* selected agents.

### Materials and methods

#### Yeast strains and plasmids

*Saccharomyces cerevisiae* strain, BY4741 (*Mata his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*) and JC2326 (*MAT-ura3, cir0, ura3–167, leu: his, his32 Ty1his3AI-270, Ty1NEO-588, Ty1 (tub: lacs)–146*) and their deletion derivatives used in the study are mentioned in the (Table 1). The list of plasmids used for the generation of deletion marker cassette to replace ORF is mentioned in the (Table 2) and sequences of primers used for PCR are mentioned in (Table 3).

#### Gene expression omnibus profile database (GEO) search for gene expression status

The GEO profile database at (https://www.ncbi.nlm.nih.gov/geo/profiles/) was searched gene-specific keyword (*SAFI*) to study the expression profiles during stress in *S. cerevisiae*.

#### Transcriptional regulation databases (YEASTRACT) search for regulators of gene transcription

For the search of transcriptional regulators of a gene in *Saccharomyces cerevisiae*, the database at (http://www.yeastarct.com) was searched for global transcription factors during normal and stress conditions for *SAFI* and *AAH1*, further analysis for regulatory association of *SAFI* and *AAH1* with common transcription factors (TF) was carried out during stress condition.
Yeast stress expression database (yStreX) analysis

The Yeast stress expression database (http://www.ystrexdb.com/) was analyzed for the expression status of SAF1, AAH1, and RLM1 by using an advanced search tool at default cut-off (log2 Fold Change > 1.5) and (adjusted p-value at < 0.05) under all experimental classes. The results on the expression status of genes included the statistical values and biological features for specific conditions.

Growth assay

To compare the growth rate between WT (BY4741) and its deletion derivatives in YPD broth and solid media standard protocols were adopted. For growth kinetics, a single colony of each strain was inoculated in 25 ml YPD broth and grown overnight at 30 °C. The next day each culture was diluted to 0.05 optical density (OD) at 600 nm and allowed to grow up to 14 h. The OD after every 2 h for each culture was measured using TOSHVIN UV-800 SHIMADZU spectrophotometer. The experiment was carried out in triplicates and the average OD was plotted against each time point to compare the growth rate. To compare the growth on solid media each strain was streaked on YPD + agar plates followed by incubation at 30 °C for 2–3 days.

Microscopy

To compare the morphological features between WT and deletion derivative yeast strains, phase contrast microscopy was carried out on log-phase grown cultures in YPD medium at 30 °C using Leica DM3000 microscope at ×100 magnification. To study the chitin distribution in the WT and mutant strains Calcofluor white staining method was adopted. Briefly, WT and mutant strains were grown overnight at 30 °C and the next day a small aliquot was inoculated in fresh YPD medium in 1:10 ratio for growth till log phase. The cells were suspended in 100 μl of the solution containing Calcofluor white (50 μg/ml solution) fluorescent dye, after staining cells were observed under ×100 magnification of fluorescence microscope using ultraviolet filters as per instructions mentioned in the manual of Leica DM3000 fluorescence microscope.

To study the nuclear migration defects in WT and its deletion derivatives, assay described [24, 25] was adopted. The number of nuclei per cell in yeast strains was determined with nuclear binding dye (DAPI). Briefly, strains were grown to early log phase (OD600 ~ 0.8) at 30 °C. Yeast cells were washed with distilled water and suspended in 1X PBS (Phosphate Buffer Saline). Further, fixation was done by the addition of 70% ethanol before DAPI staining. Cells were washed with 1X PBS buffer then again centrifuged for 1 min at 2500 rpm. DAPI stain (1 mg/ml stock) to a final concentration of 2.5 μg/ml was added and incubated for 5 min at room temperature and visualized under a fluorescent microscope with 100 X magnifications. A total of 200 cells were counted and grouped according to 0, 1, 2, and multi nuclei per cell, more than two nuclei per cell indicated the nuclear migration defect.

Spot assay for comparative cellular growth response

For comparative assessment of WT and its deletion derivatives for cellular growth response to stress agents, standard semi-quantitative spot assays were performed. Briefly, wild type (BY4741) and its deletion derivatives strains were grown in the 25 ml YPD (Yeast Extract 1% w/v, Peptone 2% w/v, dextrose 2% w/v) medium overnight at 30 °C. The next day the cultures were diluted and grown in fresh YPD medium for 3–4 h to reach the log phase (OD600 0.8–1.0). The cultures concentration was adjusted by dilution (equal OD at 600 nm) serially diluted, from each dilution, an aliquot of 3 μl volume was spotted onto YPD agar plates, without stress agents

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**Table 1** List of Saccharomyces cerevisiae strains used in the study

| Strains | Genotype | Source |
|---------|----------|--------|
| BY4741  | MATα his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 | This study |
| MRS1    | saf1Δ::His3 | This study |
| MRS2    | rlm1Δ::KanMX | This study |
| MRS3    | saf1Δ::His3, rlm1Δ::KanMX | This study |
| JCS2526 | MAT::ura3, c10Δ, ura3–167, leu::hisG, his332 Ty1his3Al-270, Ty1NEO-588, Ty1 (tyb::lacZ)-146 | Prof. M. Joan Curcio USA |
| MRS4    | saf1Δ::KanMX | This study |
| MRS5    | rlm1Δ::KanMX | This study |
| MRS6    | saf1Δ::KanMX, rlm1Δ::LEU2 | This study |

**Table 2** List of plasmids used for generating deletion cassette

| Plasmids | Deletion module | Selection media |
|----------|-----------------|-----------------|
| pFA6a- KanMX6 | KanMX6 | YPD + G418 |
| pFA6a- His3MX6 | HIS3MX6 | SD/ His – |
and plated containing Hydroxyurea (HU), Methyl methanesulfonate (MMS), Nocodazole, Benomyl, Calcofluor White, SDS, NaCl, Glycerol, DMSO, H₂O₂ as each separate agents. The plates were incubated at 30 °C for 2–3 days and cellular growth of the WT and mutants were recorded. The plates with spot assays were analyzed as per the method mentioned [26] for quantification of the relative yeast growth. The cellular growth of yeast colonies at 10⁻¹, 10⁻² and 10⁻³ dilutions were analyzed for quantification.

**Assay for Ty1 retromobility**

To assess the Ty1 retro-mobility in WT and its deletion derivatives assay was adopted mentioned in [27, 28]. The reporter strain JC2326 was used for the construction of deletion derivatives. The reporter and its deletion derivatives contain a single Ty1 element marked with indicator gene HIS3AI. The indicator gene HIS3 is interrupted by an artificial intron (AI) in an orientation opposite to the HIS3 gene transcription. During transcription of marked Ty1 with this arrangement followed by splicing and reverse transcription, generates cDNA with the functional HIS3 ORF which upon integration into the genome, generates HIS3 positive phototrophs. The quantitative measurement of the HIS3 positive colonies was considered as the frequency of retromobility. To measure the Ty1 retrotransposition frequency in the WT (JC2326; reporter strain) and the deletion derivatives, a single colony of each strain was inoculated into 10 ml YPD broth and grown overnight at 30 °C. The overnight grown cultures were again inoculated in 5 ml YPD at 1:1000 dilutions. The cultures were grown for 144 h at 20 °C. The culture was serially diluted and plated on minimal media (SD/His⁻ plates) followed by incubation at 30 °C for 3–7 days. The three independent replicates of each strain were used for serial dilution and plated on SD/His⁻ plates. The numbers of HIS+ colonies were counted from each plate and plotted for comparative assessment of Ty1 retrotransposition.

**Statistical analysis**

Statistical significance of observations was determined using the paired student t-test. *P*-value is less than 0.05 indicated significance. To compare the statistical significance between groups and within groups one-way ANOVA and posthoc Bonferroni analysis was performed.

**Results**

**GEO profile database exhibits increased expression of SAF1 during stress condition**

The analysis of the GEO profile database showed elevated expression of SAF1 when cells of S.cerevisiae were treated with Clioquinol, Pterostilbene, Gentamicin, hypoxia, genotoxic, desiccation, and heat stress agents. The summary of the results is mentioned and Table 4 and in the supplementary data file (Figure S1-S7; source GEO profile database NCBI).

### Table 4 Status of SAF1 expression during stress conditions (source: GEO)

| Stress Factor | Up-regulation of SAF1 during Stress | GEO Profile ID |
|--------------|------------------------------------|---------------|
| Clioquinol   | ↑                                  | ID: 66726869  |
| Pterostilbene| ↑                                  | ID: 52693374  |
| Gentamicin   | ↑                                  | ID: 46082374  |
| Hypoxia      | ↑                                  | ID: 57031974  |
| Genotoxic    | ↑                                  | ID: 11783774  |
| Desiccation  | ↑                                  | ID: 38619274  |
| Heat Shock   | ↑                                  | ID: 89011     |

**Table 3 List of primers sequences used for amplification of deletion cassette**

| Gene Name | Primer code | Sequence |
|-----------|-------------|----------|
| SAF1      | SAF1F       | 5’CCAAGGATATACCTCAATTTAAAAATGA  |
|           | SAF1R       | 5’ACGGATCCAAATTCAGGAAATGTA  |
|           | SAF1FL      | 5’TAAATTTAAAGTGTCGCAAAACTATAC  |
|           | SAF1RL      | 5’TAGATACAACGCGATCAGGTTAATTAA-3’ |
| RLM1      | RLM1F       | 5’TCTTTTGGAATATTTTCAGGAAATC-3’ |
|           | RLM1R       | 5’TCTTTTGGAATATTTTCAGGAAATC-3’ |
|           | RLM1FL      | 5’TCTTTTGGAATATTTTCAGGAAATC-3’ |
|           | RLM1RL      | 5’TCTTTTGGAATATTTTCAGGAAATC-3’ |
**SAF1** expression during stress correlates with expression of **RLM1**

An *in-silico* analysis using the YEASTRACT database (http://www.yeastract.com) for the association of global transcription factors with **SAF1** during normal and stress conditions revealed a total of three transcription factors i.e. Rlm1p, Spt23p, and Cin5p (Table 5), among three Rlm1p and Cin5p found to be the positive regulator. However six other transcription factors i.e. Rpn4p, Msn2p, Msn4p, Aft1p, Rap1p, and Rlm1p showed an association with **AAH1** during stress (Table 5). The Rlm1p was found to be a common transcription regulator of both **SAF1** and **AAH1** (Fig. 1). Regulatory association analysis showed **RLM1** as a positive regulator of the **SAF1** and negative regulator of the **AAH1**. Furthermore, an analysis of the yeast stress expression database (yStreX) showed a 2-fold elevated expression of **SAF1** and **RLM1** upon stress condition (Table 6). Based on *in-silico* analysis we hypothesized that upon exposure to stress, upregulation of **RLM1** leads to increase expression of **SAF1** and downregulation of **AAH1** resulting in transitioning of cells into the quiescence phase. The Rlm1p was found to be the common regulator of both **SAF1** and **AAH1**. It is reported that Saf1p during nutrient stress regulates Aah1p activity post-transcriptionally [7]. Further, based on *in-silico* analysis we hypothesized that deletion of **SAF1** and **RLM1** together may contribute to stress tolerance.

**Loss of SAF1 and RLM1 together impacts growth rate**

Comparative growth assessment of WT, saf1Δ, rlm1Δ, and saf1Δrlm1Δ yeast strains in liquid and solid rich medium showed a slight reduction in the growth rate of saf1Δ, rlm1Δ, and saf1Δrlm1Δ strains compared to WT (Fig. 2A, C). The analysis of comparative morphological features of cells of WT and mutants strains showed no distinguishable differences (Fig. 2B).

**Loss of SAF1 and RLM1 together results in tolerance to CFW and SDS stress**

Calcofluor white (CFW) is a fluorochrome stain, which binds to chitin, and perturbs the cell wall whereas SDS is a membrane disrupting agent. The cells lacking both **SAF1** and **RLM1** showed altered chitin distribution in **RLM1** when compared to WT and saf1Δ (Fig. 3A) when stained with CFW. The quantification of relative fluorescence intensity of CFW fluorescence in the strains after staining indicated the elevated level of fluorescence in the cells lacking both **SAF1** and **RLM1** (Fig. 3B). The spot assay on comparative cellular growth response to CFW (30 μg/ml) and SDS (0.0075%) also showed stress tolerance by cells lacking both **SAF1** and **RLM1** when compared with WT and rlm1Δ (Fig. 3C, D). Further, the quantification of the relative growth of yeast strains on plates with stress agents showed a lack of sensitivity by the cells lacking both **SAF1** and **RLM1**, when compared with the WT; however cells lacking only, **RLM1** showed reduced growth when spotted on plates containing SDS as stress agent.

**Loss of SAF1 and RLM1 together results in tolerance to oxidative and osmotic stress**

The hydrogen peroxide (H$_2$O$_2$) and Dimethyl sulfoxide (DMSO) both are oxidative stress agents [29–31]. The cells lacking both **SAF1** and **RLM1** showed tolerance to 2 mM H$_2$O$_2$, 8% DMSO (Fig. 4A, B), and 4% of glycerol (Fig. 4C) in the spot assay when compared to WT and saf1Δ strains. The high concentration of external glycerol and NaCl leads to osmotic stress. However, mutants strains grew similar to WT when 1 M NaCl was used as a stress agent (Fig. 4D). This suggests that cells of saf1Δrlm1Δ strain tolerate osmotic stress mediated by glycerol and are unaffected by salt stress.

### Table 5 List of global transcription factors regulating **SAF1** and **AAH1** during normal and stress condition (Source: YEASTRACT database)

| Name of Gene | Name of TFs | Reference |
|--------------|-------------|-----------|
| SAF1         | Rlm1p, Spt23p and Cin5p | [http://www.yeastract.com](http://www.yeastract.com) |
| AAH1         | Rpn4, Msn2, Msn4, Aft1, Rap1 and Rlm1p | [http://www.yeastract.com](http://www.yeastract.com) |

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![Image](https://via.placeholder.com/150)
Table 6 Expression status of SAF1 and RLM1 during stress condition (source: yStreX (http://www.ystrexdb.com

| Gene  | Class          | Sub – class      | Control       | Case     | Strain     | Log –FC | P-value |
|-------|----------------|------------------|---------------|----------|------------|---------|---------|
| RLM1  | Cultivation    | Fermentation     | 2 day         | 14 days  | 285        | 2.282   | 0       |
| SAF1  | Extra stress   | Desiccation      | Control       | 100%     | BY4743     | 2.142   | 6.8E-13 |
| SAF1  | Limiting       | Amino acid (AA- Lim) | YPD | 15 min and SD media | W303a | 2.224 | 5.4E-11 |
| SAF1  | Media          | SD               | YPD           | 15 min and SD media | BY4741 | 2.224 | 5.4E-11 |
| SAF1  | Inhibitor      | Rapamycin        | Control       | 200 ng/ml | BY4741 | 2.057 | 9.91154747E-6 |
| SAF1  | Temperature    | 37 °C            | 25 °C         | 5 min    | BY4741 | 2.006 | 4.88851412E-6 |
| SAF1  | Inorganic      | Hydrogen peroxide| Control       | 0.4 mM   | BY4741 | 2.185 | 8.132709E-8 |

Fig. 2 Genetic interaction between SAF1 and RLM1 impacts growth rate: Comparative analysis of growth and morphology of cells of WT, saf1Δ rlm1Δ saf1Δ rlm1Δ strain. A Comparative growth of streaked strains on YPD agar plates. B Representative Phase-contrast images of cells of indicated strains, no major difference concerning morphology observed between WT and mutants C Growth kinetics of indicated strains. The saf1Δ rlm1Δ saf1Δ rlm1Δ strains show a slightly reduced growth rate when compared to the WT strain. The data shown represent the average of three independent experiments. The error bars seen represent the standard deviation for each set of data.
Loss of SAF1 and RLM1 together results in sensitivity to genotoxic stressors

DNA alkylating agent, methyl methanesulfonate (MMS) elicits DNA damage in cells, which results in activation of DNA damage repair pathways [32]. The replication checkpoint inhibitor Hydroxyurea causes DNA replication defects by inhibiting the activity of ribonucleotide reductase (RNR) activity [33]. The cells lacking both SAF1 and RLM1 showed growth similar to WT in presence of 0.035% MMS, however, showed sensitivity to 200 mM HU in the spot assay when compared with WT (Fig. 5B, C). The cells lacking both SAF1 and RLM1

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**Fig. 3** Genetic interaction between SAF1 and RLM1 impacts chitin distribution and cellular growth response to cell wall stressors.  
A Representative Fluorescence images of Calcofluor white, specific to chitin in the cell wall, stained cells of WT, saf1Δ, rlm1Δ, saf1Δrlm1Δ strains. The cells of rlm1Δ and saf1Δrlm1Δ strains showed the altered chitin distribution in the cell wall when compared to cells of WT and saf1Δ strain.  
B Relative fluorescence intensity of the Calcofluor white stained cells of WT and mutant strains. The saf1Δrlm1Δ strain showed 10-fold increases in the intensity of fluorescence signal in comparison to WT cells.  
C Images of semi-quantitative spot assay for comparative analysis of cellular growth response of cells in presence of 30 μg/ml Calcofluor white and 0.0075% SDS of WT, saf1Δ, rlm1Δ and saf1Δrlm1Δ strains. The cells lacking SAF1 and RLM1 together show tolerance to CFW compared to WT, however slight sensitivity to SDS in comparison to WT. The cells lacking RLM1 show sensitivity to SDS compared to WT cells.
also showed the sensitive phenotype to microtubule depolymerizing agents, benomyl (100 μg/ml) and Nocodazole (50 μg/ml) in the spot assay (Fig. 5C, D) including WT. All the strains showed the characteristics of elongated buds and altered morphological features when cells were exposed to Hydroxyurea and Nocodazole (Fig. 5E) when compared to the morphology of cells grown on YPD+ agar media alone.

Fig. 4 Genetic interaction between SAF1 and RLM1 contributes to the cellular growth response to oxidative (DMSO and H2O2) and Osmotic (Glycerol and NaCl) stressors. A, B, C, D. Comparative cellular growth response of cells of WT, saf1Δ, rlm1Δ, and saf1Δrlm1Δ strains in presence of 8% DMSO, 2 mM H2O2, 4% glycerol, and 1 M NaCl by spot assay. The cells lacking both SAF1 and RLM1 together show tolerance to DMSO, hydrogen peroxide, and glycerol compared to WT cells however the response to NaCl appeared similar to WT cells.
Fig. 5 (See legend on next page.)
Loss of *SAF1* and *RLM1* together results in increased multi-nuclei phenotype and Ty1 retro-transposition

The sensitivity to genotoxic stress of the cells with loss of both *SAF1* and *RLM1* prompted us to study the nuclear status in the cells using DAPI staining followed by fluorescence microscopy. The DAPI stained cells showed compact nuclei as single dot and multi-nuclei feature (Fig. 6A). The quantification of the single and multi-nuclei features in cells of WT, *saf1Δ*, *rlm1Δ*, and *saf1Δrlm1Δ* (Fig. 6B) showed 28% of cells of *saf1Δrlm1Δ* strain with multi nuclei phenotype compared to WT cells which mostly showed compact nuclei. It is well established that DNA replication stress and DNA damage both lead to an increase in Ty1 retromobility in *S. cerevisiae* [34]. The cells lacking both *SAF1* and *RLM1* in JC2326, Ty1 reporter strain background showed ~120-fold increase in Ty1retromobility in comparison to WT (Fig. 7A, B) whereas *rlm1Δ* strain showed nearly ~40-fold increase in the marked Ty1 retro-mobility. The results on multi-nuclei features and Ty1 retro-mobility suggest the role of both the *SAF1* and *RLM1* in genome maintenance through an unknown mechanism that needs to be investigated in future studies.

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**Fig. 5** SAF1 and RLM1 both require cellular growth response to genotoxic and microtubule depolymerizing agents. A, B, C, D Comparative cellular growth response of cells of WT, *saf1Δ rlm1Δ saf1Δrlm1Δ* strains in presence of 0.035% MMS, 200 mM HU, 50 μg/ml Nocodazole and 100 μg/ml benomyl by spot assay. The cells lacking SAF1 and RLM1 together show sensitivity to HU, Nocodazole and Benomyl compared to WT however, similar growth profile to WT in case of growth on plates with MMS. E Comparative morphology of cells of WT, *saf1Δ rlm1Δ* and *saf1Δrlm1Δ* strains grown on YPD + agar media alone and media with HU and Nocodazole stress agents. Cells grown on genotoxic agents show altered cell division.

**Fig. 6** Loss of *SAF1* and *RLM1* together impacts nuclear migration in *S. cerevisiae*. A Representative DAPI (4',6-diamidino-2-phenylindole) stained fluorescent images of log-phase grown cells of WT, *saf1Δ*, *rlm1Δ*, *saf1Δrlm1Δ* strains showing the status of Nuclear DNA. B Table showing the percent cells with 0, 1, 2, and multi-nuclei feature from the count of 200 cells for each strain, more than two nuclei indicate the nuclear migration defect. The 28% of cells of *saf1Δrlm1Δ* strain showed multi-nuclei phenotype compared to WT strains.
Fig. 7 Loss of SAF1 and RLM1 together results in the elevated level of retrotransposition of the Ty1 element. 

A Images of plates showing the Ty1 transposition induced HIS+ prototrophs formation on SD + agar plate lacking Histidine. 

B Bar diagram showing the relative numbers of HIS+ prototrophs generated through the retro-transposition activity of the marked Ty1his3AI element in each strain. The data shown represent the average of three independent experiments. The significance of retro-transposition event was determined using a two-tailed t- test and P-value less than 0.05 was considered as significant. To compare the differences between the groups the one-way ANOVA and posthoc Bonferroni analysis was performed. The one-way ANOVA analysis showed the p-value (4.1E-08) between the groups whereas posthoc Bonferroni analysis results showed the p-value as (0.0042) when saf1Δ versus rlm1Δ and p-value as (0.0031) when saf1Δ versus rlm1Δ saf1Δ was compared.
Discussion
In this study, we report the genetic interaction between transcription factor encoding RLM1 and F-box motif encoding SAF1 which impacts the growth rate, response to stress agents, nuclear migration, and retrotransposition of Ty1 element in S. cerevisiae. The increased expression of SAF1, supported by results of in-silico analysis, during a variety of stress conditions suggests the crucial role of SAF1 in response to stress by S. cerevisiae. It is reported earlier that Saf1p is needed for induction of quiescence.

Fig. 8 Evidence of sustained expression of AAH1 during heat stress when expression of SAF1 and RLM1 is minimal. Data generated using Serial Pattern of Expression Levels Locator (SPELL Version 2.0.3) database

Fig. 9 Model indicating the regulation of stress response, mediated by genetic interaction between SAF1 and RLM1. A. Stress mediated increased expression of RLM1 results in decreased transcription of AAH1 whereas increased transcription of SAF1 that results in post-transcriptional degradation of Aah1p in S. cerevisiae, that may contribute to sensitivity to stress agents and transition from actively dividing state to quiescence phase. B. The absence of both RLM1 and SAF1 leads to resistance phenotype in presence of a general stressor, that may result due to a steady level of Aah1p in the cells.
state in actively dividing cells upon nutrients deprivation stress [35]. The Saf1p as SCF SAF1 E-3 ligase complex recruits Aah1p for ubiquitination and subsequent degradation by 26S proteasome during nutrients stress [7, 35]. This implies that the steady level of Aah1p is needed for the active dividing state of S. cerevisiae cells. These reports suggest the role of Rlm1p in response to osmo-stressors (Glycerol and NaCl) compared to parental strain however, cells showed sensitivity to genotoxic stress (HU) and microtubule inhibitors (Nocodazole and Benomyl) suggesting, the role of other alternate pathways constituted SAF1 and RLM1 as part in response to genotoxic stress. This is supported by the fact that cells lacking both SAF1 and RLM1 together exhibit an elevated level of Ty1 retrotransposition and multi-nuclei phenotype. It must be noted that the lack of RLM1 and SAF1 may contribute to the chronic presence of adenine deaminase, Aah1p encoded by AAH1. The adenine deaminase enzyme (Aah1p) is crucial for purine metabolism and is involved in the conversion of adenine to hypoxanthine [40] and imbalance in the purine metabolites may have phenotypic consequences [41].

The analysis of Serial Pattern of Expression Levels Locator (SPELL Version 2.0.3) database [42] showed the sustained increased expression of AAH1 during heat stress when the expression of RLM1 and SAF1 was minimal [4] (Fig. 8). We propose that the stress tolerance phenotype of cells lacking SAF1 and RLM1 could be due to sustained expression of AAH1. Understand the mechanism of general stress tolerance by cells lacking SAF1 and RLM1 requires future studies, however, the present study suggests a model (Fig. 9) where the continuous presence of Aah1p could be one of the reasons for the stress tolerance phenotype of cells lacking RLM1 transcription factor and F-box motif protein-encoding SAF1, as both require for regulation of Aah1p. Based on the in-silico data analysis and experimental validation we suggest that RLM1 is a transcriptional regulator of SAF1 and AAH1 during stress.

Conclusions

Based on in-silico and experimental data we suggest that SAF1 and RLM1 both interact genetically in differential response to genotoxic and general stressors.

Abbreviations

DAPI: 4′,6-diamidino-2-phenylindole; PBS: Phosphate buffered saline; HU: Hydroxyurea; MMS: Methyl methanesulfonate; DMSO: Dimethyl sulfoxide; SDS: Sodium dodecyl sulfate; NaCl: Sodium chloride; H2O2: Hydrogen peroxide; His: Histidine; YEASTRACT database: Yeast search for Transcriptional Regulators And Consensus Tracking; GEO: Gene expression omnibus; yStreX: Yeast stress expression database; SAF1: SCF associated factor 1; AAH1: Adenine Amino Hydrolase; RLM1: Resistance to lethality of MKK1P386 overexpression

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s41021-021-00218-x.

Additional file 1. Supplementary Figures from S1 to S7.

Acknowledgments

We thank Prof. M. Joan Curio, Dr. Deepak Sharma, IMTECH for strains and plasmids. We also thank Dr. Jitendra Thakur, NIPGR, New Delhi for lab support. The authors also thank Dr. Samer Singh, BHU, India, and the anonymous reviewers for their careful reads and constructive suggestions in improving the manuscript.
Authors’ contributions
NKB conceived and directed the study and wrote the paper with MS and VV. MS and NKB studied and analysed the in-silico data. MS performed the experiments and analysed the data with NKB. VV provided the bioinformatics facility and analysed the data. All the authors reviewed the results and approved the final version of the manuscript.

Funding
The research work in the laboratory of N.K.B is supported by Ramalingaswami fellowship grant (BT/RLF/Re-entry/40/2012) from the Department of Biotechnology and SERB-DST, GOI grant number (EEQ/2017/0000087) and support from SMVDU, Jammu & Kashmir, India.

Availability of data and materials
All data generated or analysed during this study are included in this article.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no conflicts of interest with the content of this article.

Received: 24 March 2021 Accepted: 19 September 2021

Published online: 09 October 2021

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