FEBS Letters 589 (2015) 3165–3174

journal homepage: www.FEBSLetters.org

Flocculation in *Saccharomyces cerevisiae* is regulated by RNA/DNA helicase Sen1p

Vikash Singh, Gajendra Kumar Azad [1], Santhosh Kumar Sariki, Raghuvir S. Tomar *

Laboratory of Chromatin Biology, Department of Biological Sciences, Indian Institute of Science Education and Research, Bhopal 462023, India

ARTICLE INFO

Article history:
Received 7 June 2015
Revised 3 September 2015
Accepted 4 September 2015
Available online 10 September 2015

Edited by Francesc Posas

Keywords:
Flocculation
Gene regulation
Sen1
RNA helicase

ABSTRACT

The Nrd1–Nab3–Sen1 (NNS) complex terminates transcription of non-coding RNA genes and mediates degradation of the produced transcript by the nuclear exosome. The NNS complex also represses some stress response genes, by stimulating premature termination. A well-characterized stress response in yeast is flocculation, where cells aggregate to form flocs under expression of lectin-encoding genes designated as FLOs. In this study, we demonstrated the role of the NNS complex and Rrp6p in the expression of flocculation genes: *FLO1*, *FLO5*, *FLO9*, and *FLO10*. Furthermore, a deletion mutant of the RNA processing machinery (*RNT1*), and *SEN1* mutants that are unable to interact with Rnt1p, exhibit a flocculation phenotype. In summary, we have identified a cooperative role of Rnt1p, Rrp6p and the NNS complex in the repression of *FLO* genes.

1. Introduction

Yeast flocculation is a process wherein a large number of yeast cells aggregate to form clumps, or flocs, that accumulate at the bottom of the liquid growth substrate in which they are suspended [1,2]. This aggregation is mediated by specific cell surface lectins, called flocculins. Yeast flocculation is also defined as a social behavior, wherein a cell directs resources to other cells under unfavorable environmental conditions in order to increase the chances of survival of the entire cellular community [3]. The complex phenomenon of flocculation depends on the expression of members of the *FLO* gene family. Nutritional stress and other environmental stresses, such as a high concentration of ethanol, influence the expression of these genes [4]. In addition to flocculation, expression of *FLO* genes also mediates other cell wall-dependent phenotypes, including cell–cell adhesion, biofilm formation, and cell-surface hydrophobicity [5–8]. Aggregates, formed due to flocculation, can be dispersed by adding specific sugars to the culture medium, such as mannose or EDTA (ethylenediaminetetraacetic acid) [9,10]. *Saccharomyces cerevisiae* encodes five distinct functional *FLO* genes: *FLO1*, *FLO5*, *FLO9*, *FLO10*, and *FLO11* [11,12]. Flo8p is a transcription factor and not directly involved in flocculation [13]. Flo11p is involved in flocculation and the formation of fibrous interconnections between cells in a colony of wild-type *S. cerevisiae* [14]. Flo11p is transcriptionally regulated by the mitogen-activated protein kinases (MAPK) (via Ste12p and Tec1p) and cyclic AMP (via Flo8p) pathways [14,15]. Its expression is generally affected by the expression of acetyltransferases, Sir2p and Gcn5p [16]. In contrast, deletion of the acetyltransferase, Sas2p, enhances the expression of *FLO5* [16]. Expression of *FLO1* is regulated at the level of the promoter; its promoter site is characterized by an extensive array of deacetylated nucleosomes that are established or maintained by Cyc8p-Tup1p together with Rpd3p and Hda1p, and that inhibit histone acetylation, block *Swi*/*Snf* binding, and prevent transcription [17]. The *FLO1* gene of *S. cerevisiae* contains many tandem repeats, which significantly affect the expression of *FLO1* in response to changes of pH, temperature, or concentration of mannose in the environment [18]. Reduction in ribosomal levels in fission yeast is linked to flocculation, but the molecular mechanism is not fully understood [19].

Sen1p is a well-studied transcriptional termination factor in *S. cerevisiae*. It is a nuclear superfamily 1 RNA/DNA helicase that is encoded by an essential gene *SEN1*. It is a key component of the NNS complex that terminates transcription of most non-coding transcripts, like small nuclear (sn) and small nucleolar (sno) RNAs, and some coding transcripts at RNA polymerase pause sites [20–23]. The NNS complex interacts with the Trf4/Air2/Mtr4p

Author contributions: Conceived and designed the experiments: RST, VS, GKA and SKS. Performed the experiments: VS and SKS. Analyzed the data: VS, GKA, SKS and RST. Wrote the paper: VS, GKA, SKS and RST.

* Corresponding author. Fax: +91 755 4092392.
E-mail address: rst@iiserb.ac.in (R.S. Tomar).
[1] Current address: Department of Genetics, Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem 91904, Israel.

http://dx.doi.org/10.1016/j.febslet.2015.09.006
0014-5793/

Table 1
List of strains used in this study.

		Relevant genotype	Strain	Reference or source
1	4741	*MAT**a** leu2Δ0 ura3Δ0 his3Δ1 met15Δ0-*	WT (BY 4741)	Yeast deletion collection (O.B.)
2	*sen1-1* (G1747D)	Derived from FWY1 (*MATa ura3-52 leu2-3, _112 pep4-3 trp1 sen1-1*)	*sen1-1*	[31]
3	*sen1-2* (Δ1–975)	Derived from DDY86 (*MATα ade2-101 his3-200 lys2-801 trp1-Δ1 ura3-52 leu2-Δ1T sen1-2*)	*sen1-2*	[47]
4	*sen1-K128E*	Derived from JFY41 (*MATa leu2Δ ura3Δ his3Δ1 trp1Δ* sen1-K128E)	*sen1-K128E*	[33]
5	*sen1-R302W*	Derived from DUY1513 (*MATa leu2Δ ura3Δ his3Δ1 met15Δ* sen1-R302W)	*sen1-R302W*	[33]
6	*RNT1*	MATa lys2Δ0 ura3Δ0 his3Δ200 leu2Δ0	*RNT1*	[51]
7	*rnt1Δ*	MATa lys2Δ0 ura3Δ0 his3Δ200 leu2Δ0 rnt1Δ::HIS3	*rnt1Δ*	[51]
8	YJC610	*MATa ura3Δ0 his3Δ1 leu2Δ0 met15Δ0 LYS2 nrd1Δ::KAN (pJC580 [LEU2 NRD1-HA])*	*Nrd1-HA*	Dr. J. Corden's Lab, Genetics, 154, 557–571 (February 2000)
9	YJC1107	*MATa ura3Δ0 his3Δ1 leu2Δ0 met15Δ0 LYS2 nrd1Δ::KAN (pJC720 [LEU2 nrd1-102)*	*nrd1-102*	Dr. J. Corden's Lab, Genetics, 154, 557–571 (February, 2000)
11	DY30229	*MATα ura3Δ0 his3Δ1 leu2Δ0 nab3Δ0*::kanMX	*nab3-11*	Dr. Daniel Reines, Lab, J. Biol. Chem., 2013, Nov 22, 288(47), 34158–34167
12	ACY1641	MATa leu2Δ ura3Δ his3Δ TRP1 RRP6::KAN *rrp6Δ*	*rrp6Δ*	Dr. Anita H. Corbett's Lab, J. Biol. Chem., 2011 Oct 28, 286(43), 37429–37445

polyadenylation (TRAMP) complex to mediate 3′-end formation of some mRNAs, snRNAs, snoRNAs, and cryptic unstable transcripts (CUTs) [24]. Nrd1p and Sen1p function by interacting with different phosphorylated forms of the C-terminal domain (CTD). Two different mechanisms were reported for the association of Sen1p with Rpb1p: either direct binding to the Ser2-phosphorylated CTD or through indirect interaction with the Ser5-phosphorylated CTD as a component of the NNS complex [25]. Another mechanism was recently proposed, wherein exchange of Nrd1p and Pcf11p on chromatin facilitate RNA Pol II pausing and CTD Ser2 phosphorylation, promoting. Sen1p activity that is required for NNS-dependent transcription termination in vivo [26]. Sen1p interacts with Glc7p, the yeast protein phosphatase 1 and a component of the cleavage and polyadenylation factor (CPF). Glc7p can dephosphorylate Sen1p in vitro, which might affect NNS complex functionality [27]. Sen1p is reported to have different functions, which are separable from its role in RNA processing, including its involvement in resolving R-loops, DNA–RNA hybrids that form due to replication forks clashing with RNA Pol II transcription [28,29]. Sen1p interacts with Srs2p, a DNA helicase protein that prevents DNA recombination and chromosomal rearrangements to maintain genomic integrity [30]. Sen1p also interacts with Rad2p and Rpo21p to assist in transcription, transcription-coupled DNA repair, and RNA processing [31]. The NNS complex also attenuates transcription of some coding genes, such as *FKS2*, which is overexpressed in stress conditions [32]. Sen1p specifically interacts with Rnt1p (RNase III), an endoribonuclease, and with Rpb1p (Rpo21p), a subunit of RNA polymerase II, through its N-terminal domain. Rnt1p is a key component of the RNA processing machinery that interacts with residue K128 on Sen1p [31], and Rpb1p interacts with residue R302 on Sen1p [33]. Mutations to either K128 or R302 of Sen1p in *S. cerevisiae* cause the accumulation of tRNAs, rRNA precursors, and some snRNAs [33]. Mutations in human Senataxin (*SETX*), an ortholog of yeast *SEN1*, causes severe neurological disorders, including ataxia with oculomotor apraxia (AOA) and juvenile amyotrophic lateral sclerosis (ALS) [34–36]. Introduction of the same mutations in yeast *SEN1* perturbed the interaction between Sen1p and Rpb1p [31].

In the present study, we investigated the role of Sen1p in the regulation of flocculation. We have shown that all of the individual members of the NNS complex are required for the regulation of flocculation. Our results revealed that mutations in *SEN1* induce upregulation of the *FLO* genes. Interestingly, mutations that interfere with the interaction between Sen1p and Rnt1p or deletion of *RNT1* also induced upregulation of *FLO1*, *FLO5*, *FLO9*, and *FLO10*.

2. Materials and methods

2.1. Strains, chemicals, growth media, and conditions

The *S. cerevisiae* strains used in this study are listed in Table 1. All chemicals used in this study were purchased from Sigma Aldrich. For making synthetic complete (SC) media, all amino acids, YNB (yeast nitrogen base) and ammonium sulfate were mixed by following a standard protocol as described elsewhere [37]. Yeast strains used in this study were grown at 24 °C, unless otherwise stated, in SC media containing 2% dextrose (SCD). For making solid-agar plates, 2% agar was added to SCD [38].

2.2. Complementation of SEN1 mutants with wild-type (WT) SEN1

The *sen1ΔN* and *sen1-1* mutant strains were transformed with the pJF9 plasmid [33] containing a full-length WT *SEN1* gene using the lithium acetate (LioAC) transformation method [39]. Transformants were grown in dropout media plates devoid of uracil, and were confirmed by polymerase chain reaction (PCR).

Table 2
List of primers used for RT-qPCR analysis in this study.

	Oligo name	Sequence
1	ACT1-F	CCTTCTGTTTTGGGTTTGGA
2	ACT1-R	CGGTGATTTCCTTTTGCATT
5	Flo1 – 5′ – F	GGCAGTCTTTACACTTCTGGC
6	Flo1 – 5′ – R	TGTCCTCCGACAGAACCTAGT
7	Flo5 – 5′ – F	TGCATATTTTTGGTAATCTTGGCCT
8	Flo5 – 5′ – R	TGTCCTCCGACAGAACCTAGT
9	Flo9 – 5′ – F	CCATCGTCACATTGCTGGGA
10	Flo9 – 5′ – R	TTTGTCCTCCGACAGAACCC
11	Flo10 – 5′ – F	GTTTGCCAGCTGGAGAGAAG
12	Flo10 – 5′ – R	CCTGGGAAGCGCTATATTCA
13	Flo1 – 3′ F	TACCGGTGAGACAACAACCA
14	Flo1 – 3′ R	TGTTGCCAGTTTCGGATACA
15	Flo5 – 3′ F	TAACAAGTTCCGGGTTGAGC
16	Flo5 – 3′ R	CAGCAATAAGGACGCAATGA
17	Flo9 – 3′ F	TGTATCCGAAACTGGCAACA
18	Flo9 – 3′ R	CAGCAATAAGGACGCAATGA
19	Flo10 – 3′ F	TTGTGACTCCATCCTCCCCT
20	Flo10 – 3′ R	AGTGGTGCGATCACGAGAAA

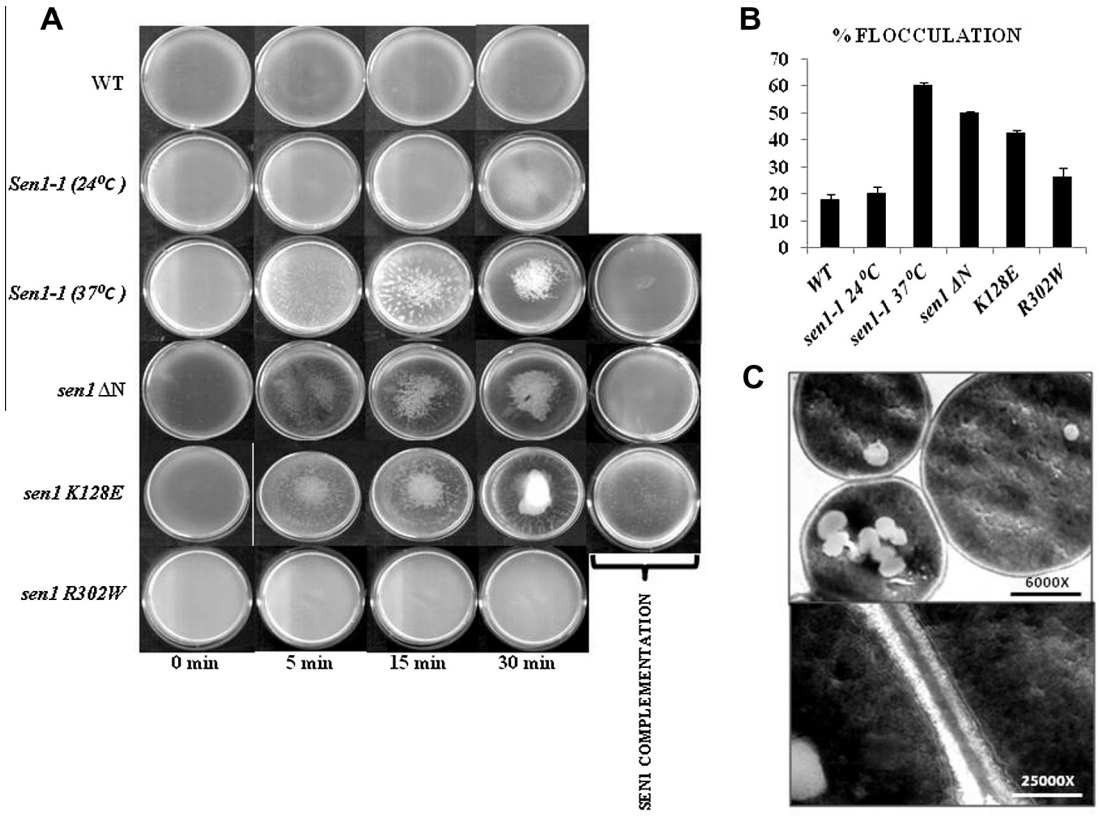


Fig. 1. Mutations in *SEN1* induce non-sexual flocculation in haploid mutant cells. (A) Image showing flocculation of WT, *sen1-1* (24 °C and 37 °C), *sen1ΔN*, *sen1-K128E*, and *sen1-R302W* mutant strains in petri dishes. Complementation of full-length *SEN1* in mutant cells reverts the flocculation phenotype. The *sen1-1* (SEN1) (37 °C) and *sen1ΔN* (SEN1) cells do not show flocculation in petri dishes. Cells were first grown in SC media at 24 °C followed by addition of 3 mL of culture media containing cells into 30 mm petri plates after OD_{600} was adjusted to 2. Images were taken at 0, 5, 10, 15, and 30 min. (B) Flocculation activity of WT, *sen1-1* (24 °C and 37 °C), *sen1ΔN*, *sen1-K128E*, and *sen1-R302W* cells cultured in SC medium. The flocculation activity was determined by measuring the sedimentation rate using the optical density (OD_{600}) of the top layer of the cell suspension after 10 min of settling. Each value represents the mean and standard deviation of three independent experiments. (C) TEM image of *sen1ΔN* mutant cells showing adherent cells as a result of flocculation.

2.3. Observation and analysis of flocculation

Flocculation was observed using the same method as described previously [40] with some modifications. Briefly, 3 mL of cell culture (OD_{600} = 2.0) was placed in a petri dish and images were taken with an HP scanner after 0, 5, 10, 15 and 30 min. Flocculation was measured using the method described by Bony et al. [41] with minor modifications. Briefly, yeast cells were deflocculated by two washes in 50 mM sodium acetate, pH 4.5, 5 mM EDTA (ethylenediaminetetraacetic acid) buffer and twice with distilled water. Cells were resuspended in flocculation buffer (50 mM sodium acetate, 5 mM $CaCl_2$, pH 4.5) while the OD_{600} was adjusted to ~2.0 and incubated at 100×*g* for 30 min (Ca^{2+} ions induce flocculation). Approximately 5 mL of cell suspension was added to 10 mL tubes and kept vertically and undisturbed for 10 min to allow for settling, and 0.3 mL of the samples were taken from just below the meniscus and the OD_{600} was determined using spectrophotometer. Percentage of flocculation (*F*) was determined by the following equation: $F = (1 - B/A) \times 100\%$, where '*A*' is the absorbance at 600 nm immediately before cells were shaken in flocculation buffer and '*B*' is the absorbance at 600 nm after the flocculation settled for 10 min. The percentage of flocculation was represented as a mean of three independent measurements.

2.4. Isolation of total RNA and real-time PCR

Exponentially growing wild-type and mutant yeast cells were harvested at OD_{600} = 1.5. Total RNA was extracted by a heat/freeze phenol method as described earlier [42,43]. Approximately 1 μg of total RNA was reverse transcribed to synthesize cDNA using a High Capacity RNA-to-cDNA kit (Bio-Rad) according to the manufacturer's instructions. Real-time PCR experiments were performed as described earlier [44] with slight modifications by using SYBR green mix (Roche Diagnostics) in an ABI real-time PCR instrument. Sequences of all primers used in this study are mentioned in Table 2. Melting curve analysis was performed for each primer pair, and relative changes in mRNA levels between control and mutants were calculated by using the $2_T^{-\Delta\Delta C}$ method [45]. Actin was used to standardize transcript levels. Data represent the means and standard deviation of the results of three independent experiments.

2.5. Transmission Electron Microscopy (TEM)

TEM was performed with WT and *sen1ΔN* mutant following a standard protocol as described previously [46].

3. Results

3.1. Mutations in SEN1 induce non-sexual flocculation of laboratory strains

Sen1 alleles that affect function (*sen1-1*) or protein interactions (*sen1-K128E*, *sen1-R302W*, and *sen1ΔN*) were reported previously [31]. The *sen1-1* mutation (G1747D) is present in a conserved ATP-helicase region of *SEN1*, and this single base change results in a heat-sensitive mutation that alters the cellular abundance of many RNA species [31]. The *sen1ΔN* mutation produces a stable,

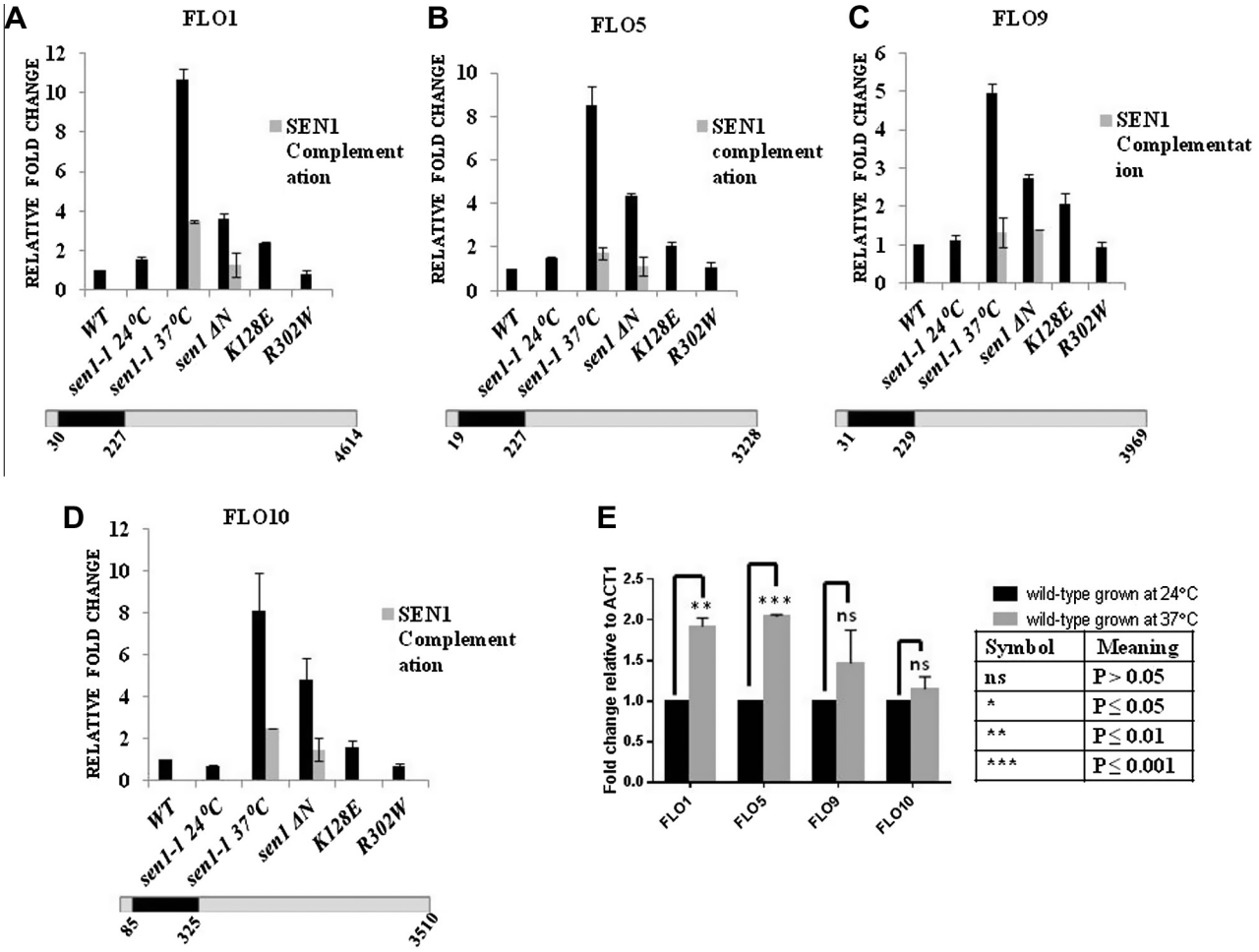


Fig. 2. Upregulation of *FLO1*, *5*, *9*, and *10* transcripts in Sen1p mutants. (A–D) The RT-qPCR quantification of total *FLO1*, *5*, *9*, and *10* transcripts was performed with WT, *sen1-1* (24 °C and 37 °C), *sen1ΔN*, *sen1-K128E*, and *sen1-R302W* cells cultured in SC medium at 24 °C unless mentioned otherwise. Complementation of full-length *SEN1* in mutant cells reverts the increased expression of *FLO* genes. The RT-qPCR quantification of total *FLO1*, *5*, *9*, and *10* transcripts was performed before and after complementation with full-length wild-type *SEN1* (pJF9) in sen1-1 (24 °C and 37 °C) and sen1ΔN mutant (shown as grey bars) strains grown in SC media at 24 °C. Actin was used to standardize transcript levels. The schematic diagram below the graph (A, B, C, and D) shows the position of the amplicon (black shaded region) that was used for RT-qPCR. (E) The RT-qPCR quantification of total *FLO1*, *5*, *9*, and *10* transcripts was done with WT grown at 24 °C and 37 °C. Asterisk represents significant difference at *P*-value <0.05. Error bars represent standard deviation. Each value represents the mean and standard deviation from three independent experiments. *ACT1* was used for normalization.

truncated protein that does not contain the first 975 amino acids of Sen1p [47]. This deletion removes the binding domains required for interactions with the RNase III cleavage enzyme, Rnt1p, and the RNA polymerase II subunit, Rpb1p. Selected point mutations in the *SEN1* N-terminal region reduce the binding affinity of Sen1p with its interaction partners. For example, previous reports indicate that *sen1-K128E* and *sen1-R302W* impair the Sen1p–Rnt1p and Sen1p–Rpb1p interactions, respectively [33]. A number of phenotypes result from impairing Sen1p's activity or interactions. Upon N-terminal truncation of Sen1p (*sen1ΔN*), yeast cells grow 50% slower than WT cells [48]. Surprisingly, when *sen1ΔN* cells were cultured in SC medium, they aggregated to form floccules (Fig. 1A); however, flocculation was not observed for WT cells grown in the same medium. We tested whether other mutants of *SEN1* can also form floccules. We cultured *sen1-1* (at permissive [24 °C] or restrictive [37 °C] temperatures), *sen1-K128E*, and *sen1-R302W* in SC medium. We found that *sen1-1* grown at 37 °C and *sen1-K128E* formed floccules robustly compared to WT and *sen1-R302W* (Fig. 1A). We used the flocculation assay to quantify the percentage of flocculating cells in culture medium (Fig. 1B). The *sen1-1* (grown at 37 °C), *sen1ΔN*, and *sen1-K128E* mutants showed 60.2%, 50.0%, and 42.7% flocculation, respectively, which were higher than the 18.0% and 26.3% flocculation shown by WT and *sen1-R302W* cells, respectively.

To rule out the possibility that flocculation occurred due to a cell separation defect, we examined flocculating *sen1ΔN* mutants by transmission electron microscopy (TEM). We were able to observe clear and distinct cell walls between adherent cells (Fig. 1C). These results suggested that Sen1 is responsible for the flocculation phenotype in mutant cells.

3.2. Sen1p mutation provokes upregulation of FLO genes

Because we observed flocculation in *SEN1* mutants, we examined the expression of flocculins using RT-qPCR. Flocculins are specific cell-surface proteins that mediate flocculation in *S. cerevisiae* by binding directly to mannose residues that are present on adjacent yeast cell walls [49]. Major genes that encode flocculins are *FLO1*, *FLO5*, *FLO9*, *FLO10*, and *FLO11*. We analyzed the expression of *FLO1*, *FLO5*, *FLO9*, and *FLO10* in *sen1-1* (grown at 24 °C), *sen1-1* (grown at 37 °C), *sen1ΔN*, *sen1-K128E*, and *sen1-R302W* mutants (Fig. 2A–D). *FLO1*, *FLO5*, *FLO9*, and *FLO10* expression was upregulated in *sen1-1* (grown at 37 °C), *sen1ΔN*, and *sen1-K128E* cells, which correlates with their flocculation

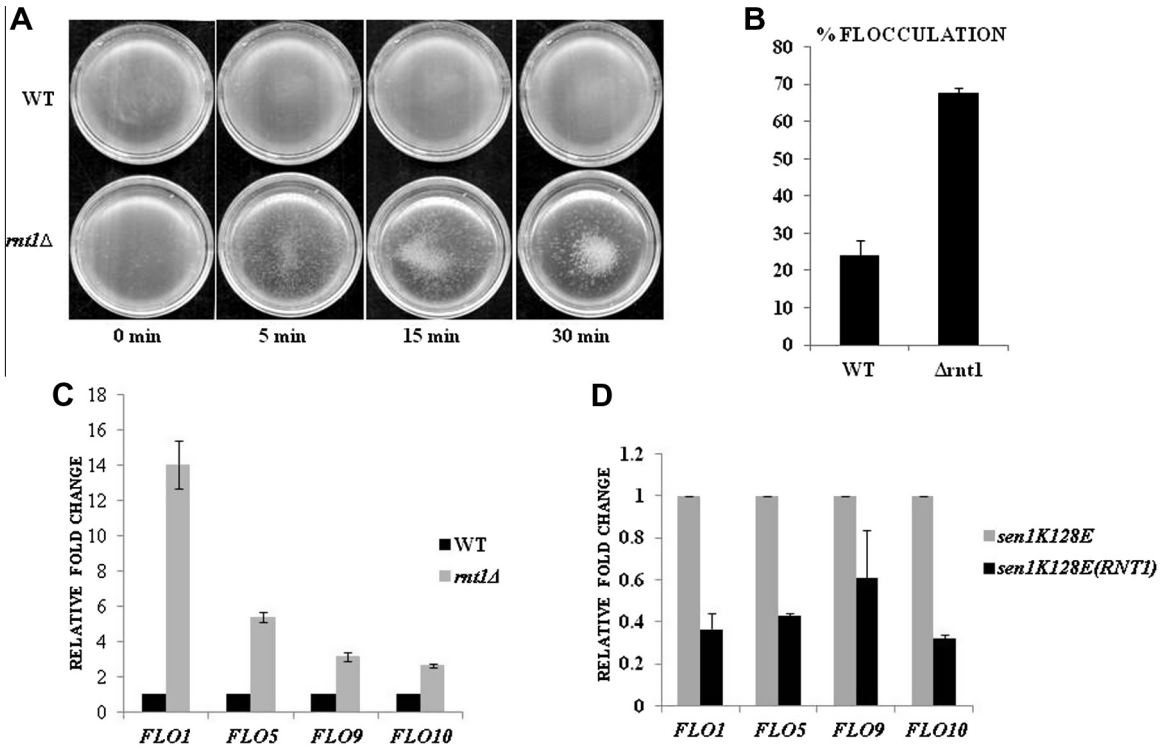


Fig. 3. Rnt1p is required for the regulated expression of flocculation. (A) Flocculation of WT and *rnt1Δ* mutant strains in petri dishes, cells were first grown in SC media at 24 °C and then 3 mL of culture media containing cells were poured in a 30 mm petri plate after OD_{600} was adjusted to 2. Images were taken at 0, 5, 15, and 30 min. (B) Flocculation activity of WT and *rnt1Δ* cells cultured in SC medium at 24 °C. The flocculation activity was determined by measuring the sedimentation rate using the optical density (OD_{600}) of the top layer of the cell suspension after 10 min of settling. (C) The RT-qPCR quantification of total *FLO1*, *5*, *9*, and *10* transcripts in WT and *rnt1Δ* cells cultured in SC medium at 24 °C, (D) The RT-qPCR quantification of total *FLO1*, *5*, *9*, and *10* transcripts in *sen1-K128E* and *sen1-K128E* transformed with pJF12 (*RNT1*). *ACT1* was used to standardize transcript levels. Each value represents the mean and standard deviation from three independent experiments.

phenotype (Fig. 1). Expression of these genes was significantly higher in *sen1-1* cells (grown at 37 °C) because the mutation located in the helicase domain causes loss of Sen1p function at an elevated temperature [50]. To rule out that *FLO* genes are non-specifically induced in *sen1-1* due to a temperature increase (37 °C), we cultured WT cells at 24 °C and 37 °C and analyzed the expression of all *FLO* genes. We have observed change in the expression *FLO1* and *5* at elevated temperature but the change was not that robust as it was in case of *sen1-1* (Fig. 2E).

To rescue the flocculation phenotype we transformed highly flocculating *sen1ΔN* and *sen1-1* mutants with pJF9, which carries the WT *SEN1* gene [25,31]. We did not observe flocculation with *sen1ΔN* (*SEN1*), *sen1-1* (*SEN1*), or WT cells. These results confirmed that non-sexual flocculation in *sen1ΔN* or *sen1-1* cells were due to the loss of Sen1p function. We used RT-qPCR to measure *FLO1*, *FLO5*, *FLO9*, and *FLO10* transcript levels in *sen1ΔN* (*SEN1*) and *sen1-1* (*SEN1*) cells. As expected, the expression of these genes returned to levels similar to those in WT cells (Fig. 2A–D). Therefore, our findings suggest that Sen1p represses expression of *FLO* genes.

3.3. Nrd1–Nab3–Sen1 complex regulates FLO gene expression

We have observed flocculation in *sen1-K128E* cells, which were unable to process transcripts because of an impaired interaction with Rnt1p [33]. This result agreed with other studies which reported that Rnt1p regulates gene expression by degrading various coding RNAs [51]. Thus, we predicted that Rnt1p might be involved in the processing of *FLO1*, *FLO5*, *FLO9*, and *FLO10* mRNAs. To test this hypothesis, we first examined WT *RNT1* and *rnt1Δ* cells for flocculation, and observed extensive flocculation in *rnt1Δ* cells (Fig. 3A). Furthermore, *rnt1Δ* and WT cells showed 67.6% and 24.1% flocculation, respectively, in flocculation assay (Fig. 3B). We also detected a significant increase in mRNA levels of *FLO1*, *FLO5*, *FLO9*, and *FLO10* in *rnt1Δ* cells, indicating a role for Rnt1p in the regulation of *FLO* genes (Fig. 3C).

As Sen1p and Rnt1p are known to interact with each other [33], we examined whether this interaction is required for *FLO* gene expression. We hypothesized that the overexpression of Rnt1p in the *sen1-K128E* mutant, which is unable to interact with Rnt1p, should rescue the overexpression of *FLO* genes. To test this prediction we transformed pJF12 plasmid carrying WT *RNT1* into *sen1-K128E* mutant cells. We performed RT-qPCR to detect the expression of *FLO* genes. We observed a decrease in the expression of *FLO* genes after overexpression of Rnt1p (Fig. 3D), suggesting that a defect in Rnt1p recruitment is responsible for the flocculation phenotype of the *sen1*-K128E mutant.

Since the NNS complex is required for premature transcription termination, and therefore repression, of several protein-coding genes, we studied its role in regulation of flocculation genes. We cultured *nrd1-102* and *nab3-11*, temperature-sensitive mutants of *NRD1* and *NAB3* [52,53], at 37 °C, which formed floccules robustly (Fig. 4A). The *nrd1-102* and *nab3-11* mutants showed 51.3% and 72.2% flocculation, respectively (Fig. 4B). Furthermore, we analyzed the expression of *FLO* genes in these mutants. We cultured WT and mutants at 37 °C and performed RT-qPCR to analyze the expression of *FLO* genes. We observed an upregulation of *FLO* genes, suggesting a role of the NNS complex in the regulation of *FLO* gene expression (Fig. 4C and D). Altogether, our results support a role for the NNS complex in the molecular mechanism of flocculation.

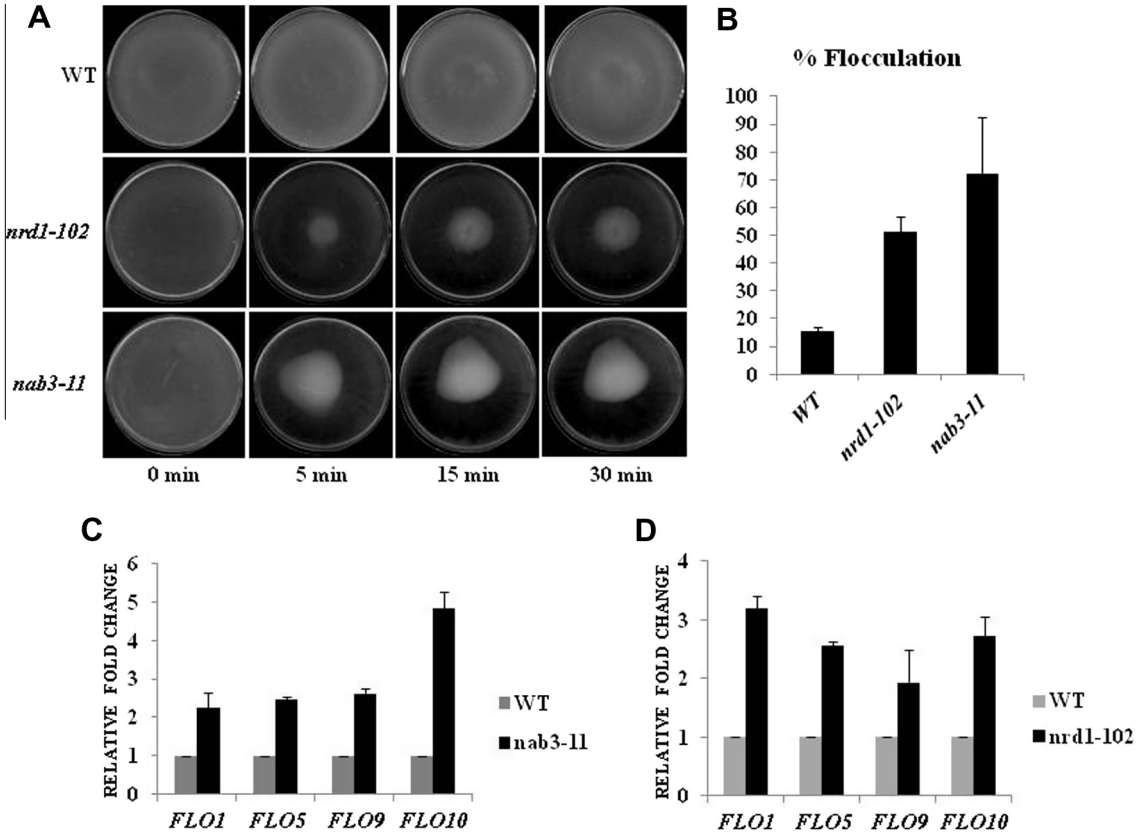


Fig. 4. Nrd1p and Nab3p, the components of the NNS complex are also required for flocculation. (A) Flocculation of WT, *nrd1-102*, and *nab3-11* mutant strains in petri dishes; cells were first grown in SC media at 37 °C and then 3 mL of culture media containing cells were poured in a 30 mm petri plate after OD_{600} was adjusted to 2. Images were taken at 0, 5, 15, and 30 min. (B) Flocculation activity of WT, *nrd1-102*, and *nab3-11* cells cultured in SC medium at 37 °C. The flocculation activity was determined by measuring the sedimentation rate using the optical density (OD_{600}) of the top layer of the cell suspension after 10 min of settling. (C and D) The RT-qPCR quantification of total *FLO1*, *5*, *9*, and *10* transcripts was done with WT, *nrd1-102*, and *nab3-11* grown initially at 24 °C and transferred to 37 °C and incubated for 3 h, after OD_{600} reached 0.8.

3.4. Rrp6 deletion causes upregulation of FLO genes without exhibiting flocculation phenotype

As a component of nuclear exosome machinery Rrp6p mutants are defective in degradation of prematurely terminated RNA during transcription [24,54]. We cultured *rrp6Δ* mutant cells to test whether *FLOs* mRNA are degraded by Rrp6p or not. We have detected the upregulation of *FLO1*, *5*, *9* and *10* mRNA in *rrp6Δ* (exosomal mutant) cells (Fig. 5A). Moreover, we have performed the flocculation assay and plate assay, and our results revealed that there is no flocculation in *rrp6Δ* (Fig. 5B and C). We reasoned the non-appearance of flocculation is due to the formation of cryptic unstable transcripts (CUTs) therefore, we tested the presence of CUTs for *FLO* gene in WT, *rrp6Δ*, *rnt1Δ* and NNS complex mutants. To measure CUTs for *FLO* genes we designed primers for extreme 5′ (designated as Head) and extreme 3′ (designated as Tail) regions of *FLO1*, *5*, *9* and *10* ORFs. We cultured WT, *sen1-1*, *sen1-2*, *sen1K128E*, *nrd1-102*, *nab3-11 rnt1Δ* and *rrp6Δ cells* in SC medium and analyzed *FLO* gene expression by RT-qPCR using above mentioned primer sets. We found enrichment for extreme 5′ region of *FLO1*, *FLO5*, and *FLO9* in *rrp6Δ* cells, while both the 5′ and 3′ regions for *FLO1*, *5*, *9* and *10* were enriched in *sen1-1*, *sen1-2*, *sen1K128E*, *nrd1-102*, *nab3-11* and *rnt1Δ* cells (Fig. 6A–D). Interestingly, we found enrichment of both the 5′- and 3′-regions of *FLO10* transcripts in *rrp6Δ* cells (Fig. 6D), suggesting transcription of *FLO 10* gene is not prematurely terminated by NNS-complex. Altogether, our result revealed the collaborative role of NNS complex, Rnt1p and Rrp6p to maintain the levels of FLOs gene.

4. Discussion

Yeast cells have a remarkable ability to form complex structures (flocs) that enable them to adhere to different surfaces, cells, and tissues. Flocculation is governed by *FLO* genes and quite a few flocculation activators and suppressor genes have been described in literature [11]. FLO genes appears to be regulated by upstream genetic elements where different activator and repressor proteins are known to bind including Set1 (COMPASS) methylation complex [55] and Histone deacetylases (HDACs) such as Gcn5p, Had1 [56]; however, the mechanisms that control this complex process is not fully understood. Our current study sheds light on the process of flocculation control by identifying Sen1p as a novel regulator.

Yeast Sen1p is a well-studied protein known to function in different transcriptional and/or co-transcriptional events [33]. Previously, Sen1p mutants were generated to characterize it's in vivo functions: *sen1-1* mutation (*G1747D*) leads to a temperature-sensitive phenotype that alters the cellular abundance of many RNA species [57], and *sen1ΔN* (truncation of the first 975 amino acids of Sen1p) abolishes its interactions with Rnt1p and Rpb1p [31]. Our results revealed that both of these Sen1p mutants (*sen1-1* and *sen1ΔN*) exhibited a flocculation phenotype (Fig. 1). The flocculation phenotype exhibited by *sen1ΔN* cells indicates the requirement of protein–protein interactions, since the deletion of the N-terminal domain of Sen1p leads to the loss of interactions with its interacting partners. To identify the specific interactions that are responsible for flocculation, we tested *sen1-K128E* and

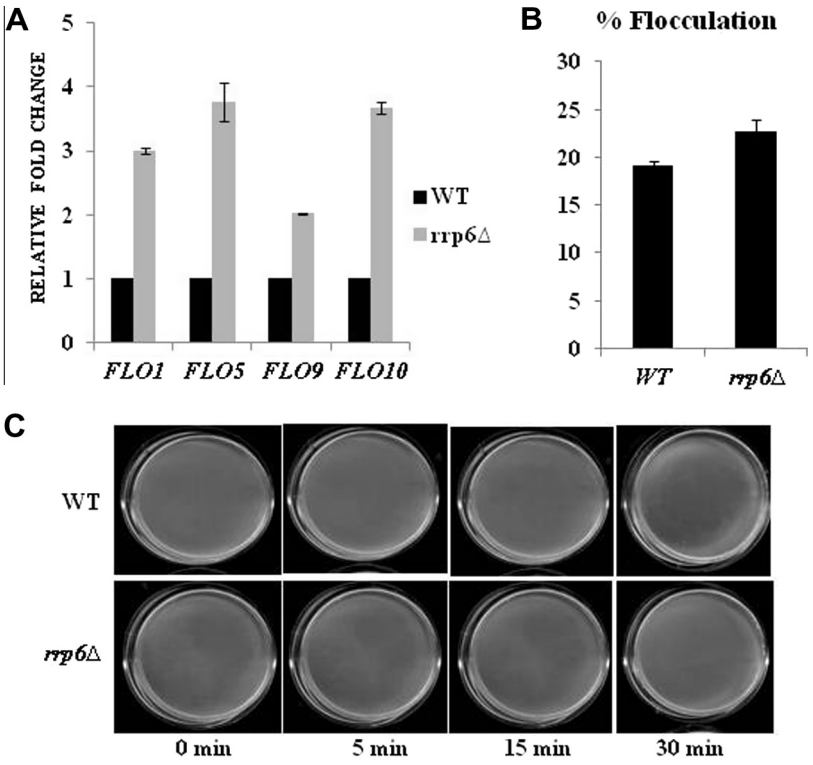


Fig. 5. Role of Rrp6p in flocculation (A) The RT-qPCR quantification of total *FLO1*, *5*, *9* and *10* transcripts in WT and *rrp6Δ* cells cultured in SC medium at 24 °C, *ACT1* was used to normalize transcript levels. Each value represents the mean and standard deviation from three independent experiments. (B) Percentage flocculation activity of WT and *rrp6Δ* cells cultured in SC medium at 24 °C. The flocculation activity was determined by measuring the sedimentation rate as the optical density (OD_{600}) of the top layer of the cell suspension after 10 min of settling. (C) Flocculation Plate Assay: WT and *rrp6Δ* cells cultured in SC medium at 24 °C and then 3 mL of culture media containing cells were poured in 30 mm petri plate after OD_{600} was adjusted to 2. Images were taken at 0, 5, 10, 15 and 30 min.

sen1-R302W (*sen1-K128E* and *sen1-R302W* disrupt the Sen1–Rnt1 and Sen1–Rpb1 interactions [33], respectively) mutants using a flocculation assay. Only the *sen1-K128E* mutant formed floccules robustly, suggesting that the disruption of interaction between Sen1p and Rnt1p promotes flocculation (Fig. 1A). Previous studies have indicated that Rnt1p, which possesses endonuclease activity, regulates a number of protein-coding genes by limiting the number of active transcripts [51]. Furthermore, overexpression of Rnt1p in *sen1-K128E* rescues the flocculation phenotype, suggesting the importance of the interaction between Sen1p and Rnt1p in flocculation. This finding supports our result that Rnt1p is involved in the processing and degradation of *FLO* transcripts, as demonstrated by flocculation in *rnt1Δ* cells (Fig. 3C). We also explored the possibility of an impairment in transcriptional termination (Fig. 2), using the *sen1-R302W* mutant which disrupts the Rpb1–Sen1 interaction [25,33]. Our results revealed that the expression of *FLO* genes is not upregulated in the *sen1-R302W* mutant, which agrees with a previous report wherein no major defects of termination for coding genes were reported with this mutant [25]. Furthermore, the molecular mechanism of flocculation control, which we have described here, is distinct from a previously described mechanism for *FLO1* and *FLO11* genes. Normally, the promoter of *FLO1* is repressed by Tup1p and Ssn6p, and association of the *Swi*/*Snf* complex with the *FLO1* promoter de-represses transcription of the *FLO1* gene [58]. Tec1–Ste12–Msa1/2 complex is a known transcriptional activator that binds to the promoter of *FLO11* gene and regulates its expression in a cell cycle dependent manner. We propose that these mechanisms are also operating in flocculating Sen1p mutants and future studies are warranted. Flocculation exhibited by Sen1p mutants suggests that under normal environmental conditions Sen1p negatively regulates the transcription of *FLO1*, *FLO5*, *FLO9*, and *FLO10*, which prevents flocculation during favorable growth conditions.

We have also determined the involvement of the other two members of the NNS complex (Nrd1p and Nab3p) in the regulation of flocculation (Fig. 4C and D). We also tested the likelihood of the regulation of *FLO* gene expression through the formation CUTs, using the exosomal mutant, *rrp6Δ* that genetically interact with *Sen1-K128E* (Fig. 6A–D) [33]. We found that the 5′-regions of *FLO1*, *5* and *9* transcripts were enriched in *rrp6Δ* cells, while both the 5′ and 3′ regions for *FLO1*, *5*, *9* and *10* were enriched in NNS complex mutant as well as *rnt1Δ* cells (Fig. 6). These results indicate that at least a part of transcription of *FLO* genes are prematurely terminated by the NNS complex, leading to the production of truncated versions of the mRNAs that are degraded by the exosome and Rrp6p. The proper transcriptional termination of most primary transcripts that code for snoRNAs and snRNAs requires the NNS complex and the CTD of RNA Pol II [27,33]. Our results indicate that the whole NNS complex is involved in the regulation of flocculation and the transcription of *FLO* genes. Previous studies suggest that interactions with Glc7p, a protein phosphatase subunit of the cleavage/polyadenylation factor, and Nab3p, require Sen1p [27,52]. Sen1p physically interacts with Nab3p and Nab3p directly binds to Nrd1p, but Sen1p and Nrd1p do not physically interact [23]. Apart from the NNS complex, Nrd1p, Nab3p, and Sen1p are also components of a larger complex that includes RNA Pol II, the nuclear cap binding proteins Cbp20p and Cbp80p, Spt5p, the TRAMP (Trf4/Air2/Mtr4p Polyadenylation complex) and exosome complexes [59]. Based on our results, we propose a model in which the NNS complex along with Rnt1p and Rrp6p are required for the repression of *FLO* genes (Fig. 7). The mutation in any of these components (NNS-complex/Rrp6p/Rnt1p) causes

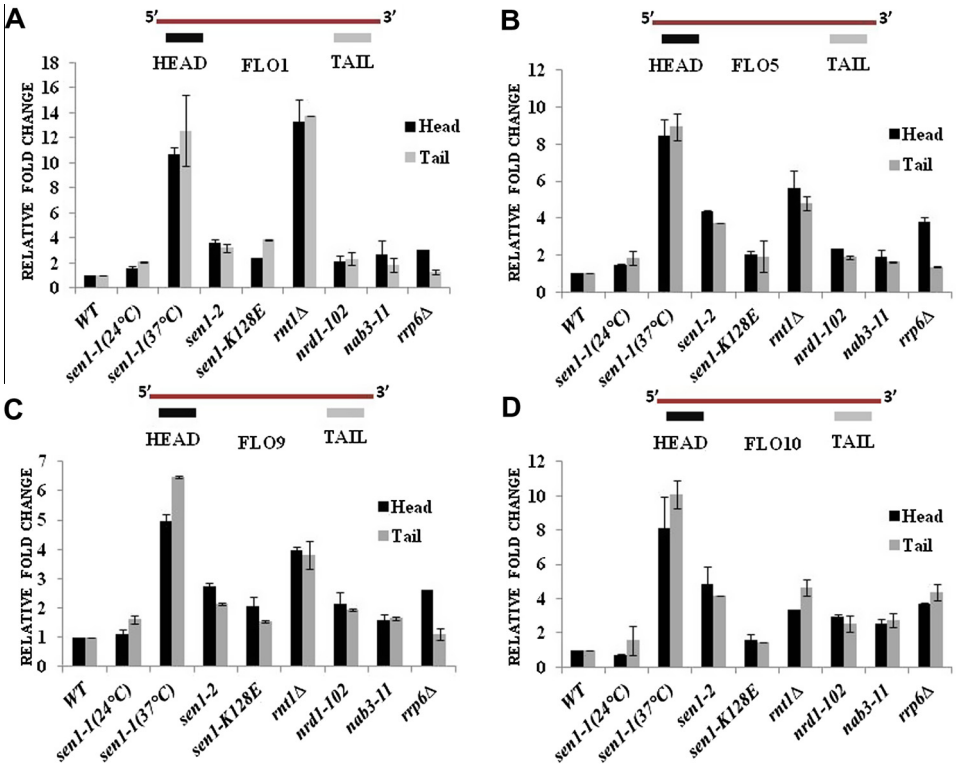
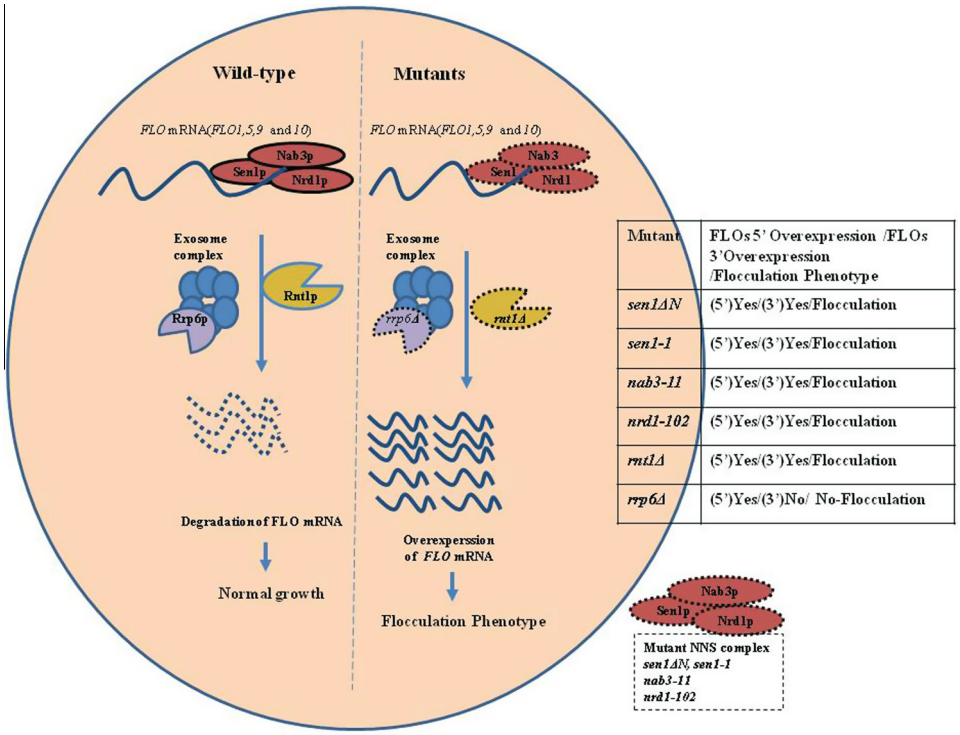


Fig. 6. Analysis of CUT (Criptic Unstable Transcripts) of *FLO1*, *5*, *8*, *9* and *10* transcripts. (A–D) qPCR quantification of total 5′ region(in black bar) and 3′ region(in gray bar) enrichment of *FLO1*, *5*, *9* and *10* transcripts in WT, *sen1-1*, *sen1-2*, *rnt1Δ*, *nrd1-102*, *nab3-11* and *rrp6Δ* mutant cells cultured in SC media. *ACT1* was used to standardize transcript levels; data represent the means ± standard errors of the results of three independent experiments. Specific primers were made for quantification of 5′ and 3′ region of *FLO1*, *5*, *8*, *9* and *10* transcripts.



Mutant	FLOs 5' Overexpression /FLOs 3'Overexpression /Flocculation Phenotype
sen1ΔN	(5')Yes/(3')Yes/Flocculation
sen1-1	(5')Yes/(3')Yes/Flocculation
nab3-11	(5')Yes/(3')Yes/Flocculation
nrd1-102	(5')Yes/(3')Yes/Flocculation
rnt1Δ	(5')Yes/(3')Yes/Flocculation
rrp6Δ	(5')Yes/(3')No/ No-Flocculation

Fig. 7. Proposed model showing the regulation of flocculation by the NNS complex; wild-type cells during normal growth conditions target processing and degradation of *FLO* mRNA through Rnt1p/Rrp6p via the NNS complex. This leads to reduced expression of flocculins making them non-flocculent, while mutation in either RNA processing/ degradation machinery or the NNS complex components cause overexpression of flocculins leading to flocculation.

the overexpression of *FLO* genes (Fig. 7) leading to flocculation phenotype. Altogether, our findings suggest a regulatory function of Rnt1p, Rrp6p and the NNS complex in *FLOs* gene expression.

Acknowledgments

We thank Michael Culbertson (University of Wisconsin), Jeffry Corden (Johns Hopkins University School of Medicine), Daniel Reines (Emory University), Anita H. Corbet (Emory University) and Sherif Abou Elela (University of Sherbrooke) for providing us the Sen1p, Nrd1p, Nab3p, Rrp6p and Rnt1p mutant yeast strains, respectively. We again thank Michael Culbertson (University of Wisconsin) for providing pJF9 and pJF12 plasmids. Council of Scientific and Industrial Research (CSIR), India is acknowledged for fellowship support to VS. Members of the chromatin biology laboratory were acknowledged for helpful discussions throughout this work. This work was supported by funds from the Department of Biotechnology, Govt. of India to RST.

References

[1] Stratford, M. (1989) Evidence for two mechanisms of flocculation in *Saccharomyces cerevisiae*. Yeast 5. Spec No, S441-5.
[2] Bony, M., Thines-Sempoux, D., Barre, P. and Blondin, B. (1997) Localization and cell surface anchoring of the *Saccharomyces cerevisiae* flocculation protein Flo1p. J. Bacteriol. 179, 4929–4936.
[3] Herker, E. et al. (2004) Chronological aging leads to apoptosis in yeast. J. Cell Biol. 164, 501–507.
[4] Verstrepen, K.J., Derdelinckx, G., Verachtert, H. and Delvaux, F.R. (2003) Yeast flocculation: what brewers should know. Appl. Microbiol. Biotechnol. 61, 197–205.
[5] Cunha, A.F., Missawa, S.K., Gomes, L.H., Reis, S.F. and Pereira, G.A.G. (2006) Control by sugar of *Saccharomyces cerevisiae* flocculation for industrial ethanol production. FEMS Yeast Res. 6, 280–287.
[6] Govender, P., Domingo, J.L., Bester, M.C., Pretorius, I.S. and Bauer, F.F. (2008) Controlled expression of the dominant flocculation genes FLO1, FLO5, and FLO11 in *Saccharomyces cerevisiae*. Appl. Environ. Microbiol. 74, 6041–6052.
[7] Reynolds, T.B. and Fink, G.R. (2001) Bakers' yeast, a model for fungal biofilm formation. Science 291, 878–881.
[8] Guo, B., Styles, C.A., Feng, Q. and Fink, G.R. (2000) A Saccharomyces gene family involved in invasive growth, cell-cell adhesion, and mating. Proc. Natl. Acad. Sci. U.S.A. 97, 12158–12163.
[9] Miki, B.L., Poon, N.H. and Seligy, V.L. (1982) Repression and induction of flocculation interactions in *Saccharomyces cerevisiae*. J. Bacteriol. 150, 890–899.
[10] Stratford, M. (1992) Lectin-mediated aggregation of yeasts–yeast flocculation. Biotechnol. Genet. Eng. Rev. 10, 283–341.
[11] Teunissen, A.W. and Steensma, H.Y. (1995) Review: the dominant flocculation genes of *Saccharomyces cerevisiae* constitute a new subtelomeric gene family. Yeast 11, 1001–1013.
[12] Verstrepen, K.J., Reynolds, T.B. and Fink, G.R. (2004) Origins of variation in the fungal cell surface. Nat. Rev. Microbiol. 2, 533–540.
[13] Kobayashi, O., Suda, H., Ohtani, T. and Sone, H. (1996) Molecular cloning and analysis of the dominant flocculation gene FLO8 from *Saccharomyces cerevisiae*. Mol. Gen. Genet. 251, 707–715.
[14] Rupp, S., Summers, E., Lo, H.J., Madhani, H. and Fink, G. (1999) MAP kinase and cAMP filamentation signaling pathways converge on the unusually large promoter of the yeast FLO11 gene. EMBO J. 18, 1257–1269.
[15] Octavio, L.M., Gedeon, K. and Maheshri, N. (2009) Epigenetic and conventional regulation is distributed among activators of FLO11 allowing tuning of population-level heterogeneity in its expression. PLoS Genet., 5.
[16] Rodriguez, M.E., Orozco, H., Cantoral, J.M., Matallana, E. and Aranda, A. (2014) Acetyltransferase SAS2 and sirtuin SIR2, respectively, control flocculation and biofilm formation in wine yeast. FEMS Yeast Res. 14, 845–857.
[17] Fleming, A.B., Beggs, S., Church, M., Tsukihashi, Y. and Pennings, S. (2014) The yeast Cyc8-Tup1 complex cooperates with Hda1p and Rpd3p histone deacetylases to robustly repress transcription of the subtelomeric FLO1 gene. Biochim. Biophys. Acta 1839, 1242–1255.
[18] Yue, F., Du, Z., Guo, X., He, X. and Zhang, B. (2013) Effect of tandem repeats adjacent to 3′-terminal of FLO1 on the flocculation function of *Saccharomyces cerevisiae*. Wei Sheng Wu Xue Bao 53, 1276–1284.
[19] Li, R. et al. (2013) Reduction of ribosome level triggers flocculation of fission yeast cells. Eukaryot. Cell 12, 450–459.
[20] Winey, M. and Culbertson, M.R. (1988) Mutations affecting the tRNA-splicing endonuclease activity of *Saccharomyces cerevisiae*. Genetics 118, 609–617.
[21] Steinmetz, E.J. and Brow, D.A. (1996) Repression of gene expression by an exogenous sequence element acting in concert with a heterogeneous nuclear ribonucleoprotein-like protein, Nrd1, and the putative helicase Sen1. Mol. Cell. Biol. 16, 6993–7003.
[22] Rasmussen, T.P. and Culbertson, M.R. (1998) The putative nucleic acid helicase Sen1p is required for formation and stability of termini and for maximal rates of synthesis and levels of accumulation of small nucleolar RNAs in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 18, 6885–6896.
[23] Steinmetz, E.J., Conrad, N.K., Brow, D.A. and Corden, J.L. (2001) RNA-binding protein Nrd1 directs poly(A)-independent 3′-end formation of RNA polymerase II transcripts. Nature 413, 327–331.
[24] LaCava, J., Houseley, J., Saveanu, C., Petfalski, E., Thompson, E., Jacquier, A. and Tollervey, D. (2005) RNA degradation by the exosome is promoted by a nuclear polyadenylation complex. Cell 121, 713–724.
[25] Chinchilla, K., Rodriguez-Molina, J.B., Ursic, D., Finkel, J.S., Ansari, A.Z. and Culbertson, M.R. (2012) Interactions of Sen1, Nrd1, and Nab3 with multiple phosphorylated forms of the Rpb1 C-terminal domain in *Saccharomyces cerevisiae*. Eukaryot. Cell 11, 417–429.
[26] Grzechnik, P., Gdula, M.R. and Proudfoot, N.J. (2015) Pcf11 orchestrates transcription termination pathways in yeast. Genes Dev. 29, 849–861.
[27] Nedea, E. et al. (2008) The Glc7 phosphatase subunit of the cleavage and polyadenylation factor is essential for transcription termination on snoRNA genes. Mol. Cell 29, 577–587.
[28] Alzu, A. et al. (2012) Senataxin associates with replication forks to protect fork integrity across RNA-polymerase-II-transcribed genes. Cell 151, 835–846.
[29] Yuce, O. and West, S.C. (2013) Senataxin, defective in the neurodegenerative disorder ataxia with oculomotor apraxia 2, lies at the interface of transcription and the DNA damage response. Mol. Cell. Biol. 33, 406–417.
[30] Chiolo, I., Carotenuto, W., Maffioletti, G., Petrini, J.H., Foiani, M. and Liberi, G. (2005) Srs2 and Sgs1 DNA helicases associate with Mre11 in different subcomplexes following checkpoint activation and CDK1-mediated Srs2 phosphorylation. Mol. Cell. Biol. 25, 5738–5751.
[31] Ursic, D., Chinchilla, K., Finkel, J.S. and Culbertson, M.R. (2004) Multiple protein/protein and protein/RNA interactions suggest roles for yeast DNA/RNA helicase Sen1p in transcription, transcription-coupled DNA repair and RNA processing. Nucleic Acids Res. 32, 2441–2452.
[32] Kim, K.Y. and Levin, D.E. (2011) Mpk1 MAPK association with the Paf1 complex blocks Sen1-mediated premature transcription termination. Cell 144, 745–756.
[33] Finkel, J.S., Chinchilla, K., Ursic, D. and Culbertson, M.R. (2010) Sen1p performs two genetically separable functions in transcription and processing of U5 small nuclear RNA in *Saccharomyces cerevisiae*. Genetics 184, 107–118.
[34] Chen, Y.Z. et al. (2004) DNA/RNA helicase gene mutations in a form of juvenile amyotrophic lateral sclerosis (ALS4). Am. J. Hum. Genet. 74, 1128–1135.
[35] Chen, Y.Z. et al. (2006) Senataxin, the yeast Sen1p orthologue: characterization of a unique protein in which recessive mutations cause ataxia and dominant mutations cause motor neuron disease. Neurobiol. Dis. 23, 97–108.
[36] Moreira, M.C. et al. (2004) Senataxin, the ortholog of a yeast RNA helicase, is mutant in ataxia-ocular apraxia 2. Nat. Genet. 36, 225–227.
[37] Adams, A., Kaiser, C. and Laboratory, Cold Spring Harbor. (1998) Methods in Yeast Genetics: a Cold Spring Harbor Laboratory Course Manual, Cold Spring Harbor Laboratory Press, Plainview, N.Y..
[38] Azad, G.K., Singh, V. and Tomar, R.S. (2014) Assessment of the biological pathways targeted by isocyanate using N-succinimidyl N-methylcarbamate in budding yeast *Saccharomyces cerevisiae*. PLoS ONE 9, e92993.
[39] Brachmann, C.B., Davies, A., Cost, G.J., Caputo, E., Li, J., Hieter, P. and Boeke, J.D. (1998) Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast 14, 115–132.
[40] Li, R. et al. (2013) Reduction of ribosome level triggers flocculation of fission yeast cells. Eukaryot. Cell 12, 450–459.
[41] Bony, M., Barre, P. and Blondin, B. (1998) Distribution of the flocculation protein, flop, at the cell surface during yeast growth: the availability of flop determines the flocculation level. Yeast 14, 25–35.
[42] Singh, V., Azad, G.K., Reddy, M.A., Baranwal, S. and Tomar, R.S. (2014) Anticancer drug KP1019 induces Hog1 phosphorylation and protein ubiquitylation in *Saccharomyces cerevisiae*. Eur. J. Pharmacol. 736, 77–85.
[43] Azad, G.K., Singh, V., Golla, U. and Tomar, R.S. (2013) Depletion of cellular iron by curcumin leads to alteration in histone acetylation and degradation of Sml1p in *Saccharomyces cerevisiae*. PLoS ONE 8, e59003.
[44] Baranwal, S., Azad, G.K., Singh, V. and Tomar, R.S. (2014) Signaling of chloroquine-induced stress in the yeast *Saccharomyces cerevisiae* requires the Hog1 and Slt2 mitogen-activated protein kinase pathways. Antimicrob. Agents Chemother. 58, 5552–5566.
[45] Azad, G., Singh, V., Thakare, M., Baranwal, S. and Tomar, R. (2014) Mitogen-activated protein kinase Hog1 is activated in response to curcumin exposure in the budding yeast *Saccharomyces cerevisiae*. BMC Microbiol. 14, 317.
[46] Azad, G.K., Singh, V., Baranwal, S., Thakare, M.J. and Tomar, R.S. (2015) The transcription factor Rap1p is required for tolerance to cell-wall perturbing agents and for cell-wall maintenance in *Saccharomyces cerevisiae*. FEBS Lett. 589, 59–67.
[47] DeMarini, D.J., Winey, M., Ursic, D., Webb, F. and Culbertson, M.R. (1992) SEN1, a positive effector of tRNA-splicing endonuclease in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 12, 2154–2164.
[48] Golla, U., Singh, V., Azad, G.K., Singh, P., Verma, N., Mandal, P., Chauhan, S. and Tomar, R.S. (2013) Sen1p contributes to genomic integrity by regulating expression of ribonucleotide reductase 1 (RNR1) in *Saccharomyces cerevisiae*. PLoS ONE 8, e64798.

[49] Miki, B.L., Poon, N.H., James, A.P. and Seligy, V.L. (1982) Possible mechanism for flocculation interactions governed by gene FLO1 in *Saccharomyces cerevisiae*. J. Bacteriol. 150, 878–889.
[50] DeMarini, D.J., Winey, M., Ursic, D., Webb, F. and Culbertson, M.R. (1992) SEN1, a positive effector of tRNA-splicing endonuclease in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 12, 2154–2164.
[51] Catala, M., Aksouh, L. and Abou Elela, S. (2012) RNA-dependent regulation of the cell wall stress response. Nucleic Acids Res. 40, 7507–7517.
[52] Conrad, N.K., Wilson, S.M., Steinmetz, E.J., Patturajan, M., Brow, D.A., Swanson, M.S. and Corden, J.L. (2000) A yeast heterogeneous nuclear ribonucleoprotein complex associated with RNA polymerase II. Genetics 154, 557–571.
[53] Kim, M., Vasiljeva, L., Rando, O.J., Zhelkovsky, A., Moore, C. and Buratowski, S. (2006) Distinct pathways for snoRNA and mRNA termination. Mol. Cell 24, 723–734.
[54] Vanacova, S. et al. (2005) A new yeast poly(A) polymerase complex involved in RNA quality control. PLoS Biol. 3, e189.
[55] Dietvorst, J. and Brandt, A. (2008) Flocculation in *Saccharomyces cerevisiae* is repressed by the COMPASS methylation complex during high-gravity fermentation. Yeast 25, 891–901.
[56] Dietvorst, J. and Brandt, A. (2010) Histone modifying proteins Gcn5 and Hda1 affect flocculation in *Saccharomyces cerevisiae* during high-gravity fermentation. Curr. Genet. 56, 75–85.
[57] Ursic, D., Himmel, K.L., Gurley, K.A., Webb, F. and Culbertson, M.R. (1997) The yeast SEN1 gene is required for the processing of diverse RNA classes. Nucleic Acids Res. 25, 4778–4785.
[58] Fleming, A.B. and Pennings, S. (2001) Antagonistic remodelling by Swi-Snf and Tup1-Ssn6 of an extensive chromatin region forms the background for FLO1 gene regulation. EMBO J. 20, 5219–5231.
[59] Vasiljeva, L. and Buratowski, S. (2006) Nrd1 interacts with the nuclear exosome for 3′ processing of RNA polymerase II transcripts. Mol. Cell 21, 239–248.