Deciphering the transcriptional regulation of flocculation via CWI pathway in *Saccharomyces cerevisiae*

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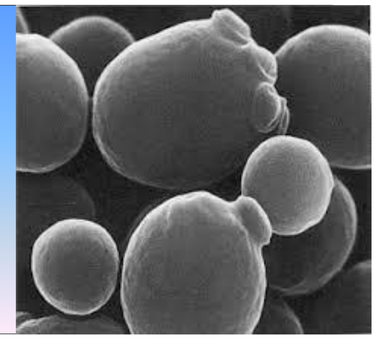
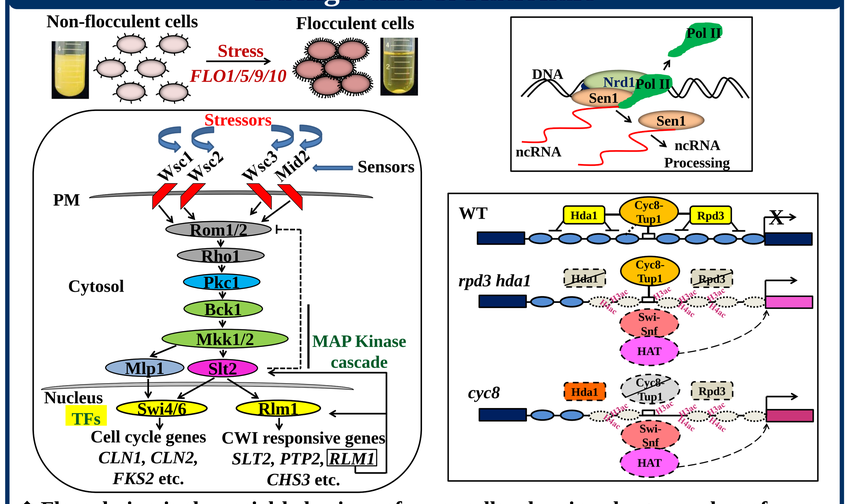


Abstract

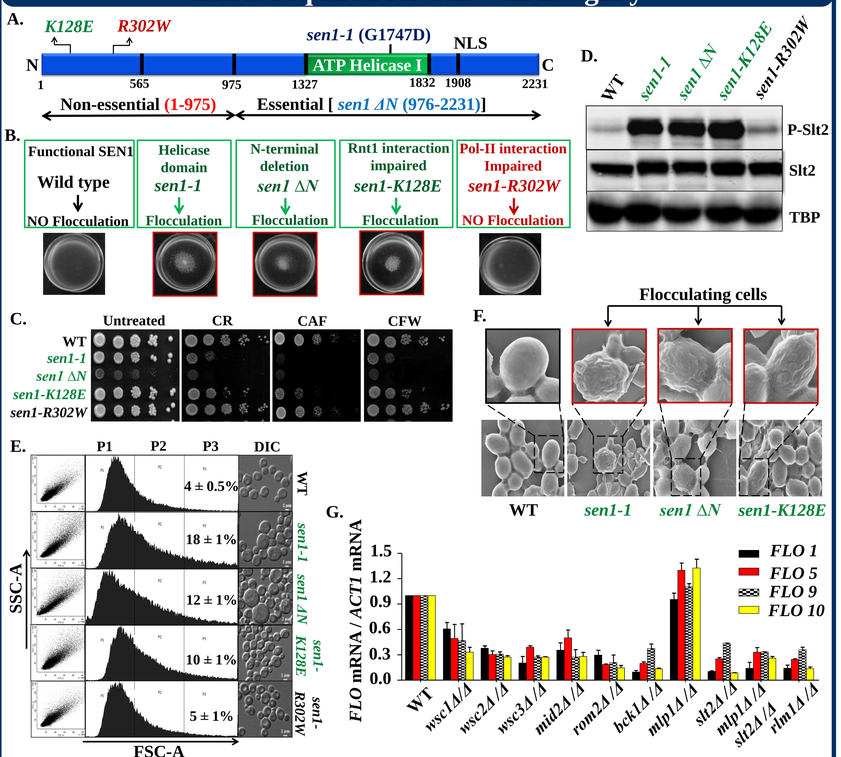
Flocculation is an essential characteristic of yeast cells required for survival under adverse conditions. The multicellular structure (flocs) of yeast provides a suitable microenvironment to enhance the chances of survival during stress conditions. Although the signaling events triggering flocculation have been studied earlier, molecular mechanisms remain elusive. In the present study, we used flocculating *sen1* mutants to identify the mechanism of flocculation. Based on the abnormal cell surface morphology and constitutive phosphorylation of Slt2p in flocculating *sen1* mutant cells, we hypothesized that flocculation was regulated by the cell wall integrity (CWI) pathway. Up-regulation of *FLO* genes in wild-type cells was observed upon the activation of CWI pathway either by chemical treatment or by deleting Slt2 phosphatase (Msg5). Our study with Slt2 mutants reveals that the active state of Slt2 is indispensable for flocculation. Deletion of either *SLT2* or *RLM1* leads to reduced flocculation. Furthermore, we observed overlapping binding sites for Rlm1 and Tup1 at the promoters of almost all the *FLO* genes. Finally, we show higher Rlm1 and lower Tup1 occupancy at the promoters of *FLO1* and *FLO5* in flocculating cells. Altogether we demonstrate that CWI MAPK (Slt2) pathway uses a non-catalytic mechanism to activate the transcription of *FLO* genes.

Background & Rationale



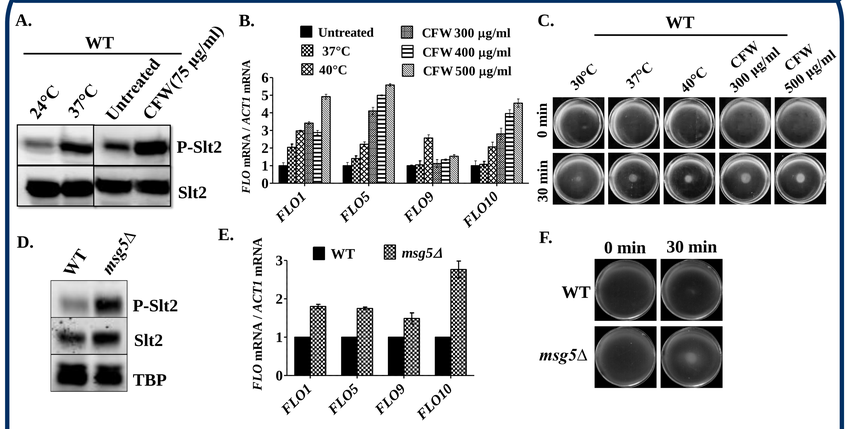
- Flocculation is the social behaviour of yeast cells wherein a large number of yeast cells aggregate to form flocs in unfavourable environmental conditions.
- The CWI pathway is one of MAPK signalling cascades that contributes to cell wall strengthening.
- Sen1p is an ATP-dependent RNA/DNA helicase, required for genomic stability.
- Cyc8-Tup1 global co-repressor complex involved in regulation of many subset of genes including flocculation, osmotic and DNA damage response genes.

Mutations in yeast *SEN1* causes flocculation and is required for cell wall integrity



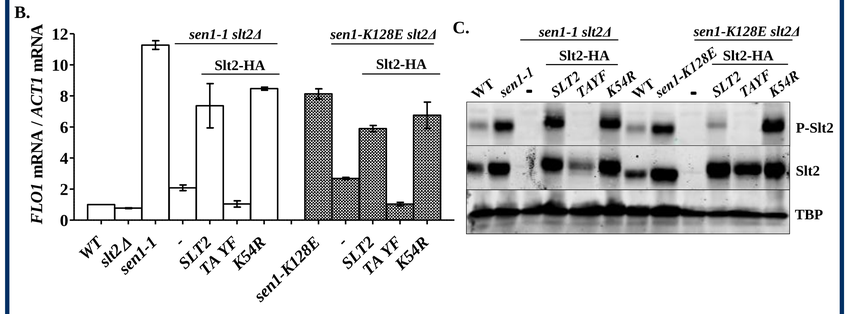
A) Schematic of *sen1* mutants. B) Function impaired and flocculation phenotype in *sen1* mutants. C) Growth phenotype of *sen1* mutants on cell wall perturbing agents CR (Congo Red 30 µg/ml), CAF (Caffeine 6 mM), and CFW (Calcofluor White 75 µg/ml). D) Slt2 phosphorylation by western blotting. E) SEM images of *sen1* mutants. F) Cell size measurement of flocculating *sen1* mutants by FACS & Microscopy. G) Expression of *FLO* genes in the CWI pathway mutants.

Phosphorylation of Slt2 correlates with the expression of *FLO* genes



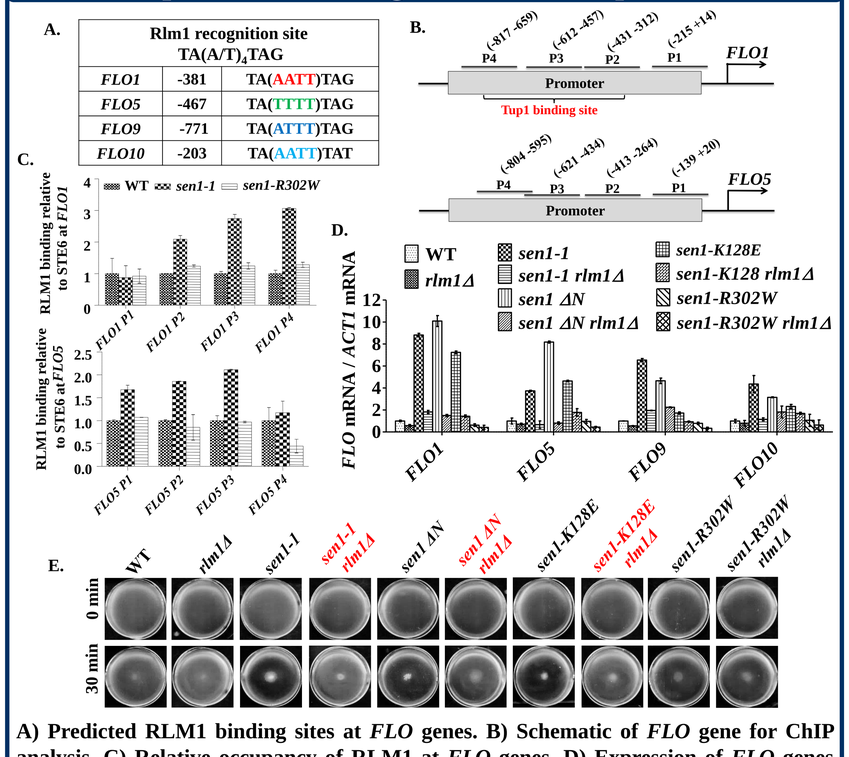
A & D) Slt2 phosphorylation in WT cells upon cell wall stress & *msg5*Δ. B & E) Expression of *FLO* genes in WT cells upon cell wall stress & *msg5*Δ. C & F) Visualization of flocculation in WT cells upon cell wall stress & *msg5*Δ.

Phosphorylation of Slt2 is crucial in regulation of flocculation



A) Flocculation, B) *FLO* gene expression, & C) Western blotting, upon *SLT2* deletion and complementation with *SLT2* plasmids [full length *SLT2*, phosphorylated mutant of *SLT2* (*T190A Y192F*), and catalytic dead *SLT2* mutant (*K54R*)] in *sen1-1*, and *sen1-K128E* mutants.

Expression of *FLO* genes is Rlm1 dependent



A) Predicted RLM1 binding sites at *FLO* genes. B) Schematic of *FLO* gene for ChIP analysis. C) Relative occupancy of RLM1 at *FLO* genes. D) Expression of *FLO* genes upon deletion of *RLM1* in *sen1* mutants. E) Flocculation in *sen1* mutants after deletion of *RLM1*.

Acknowledgement

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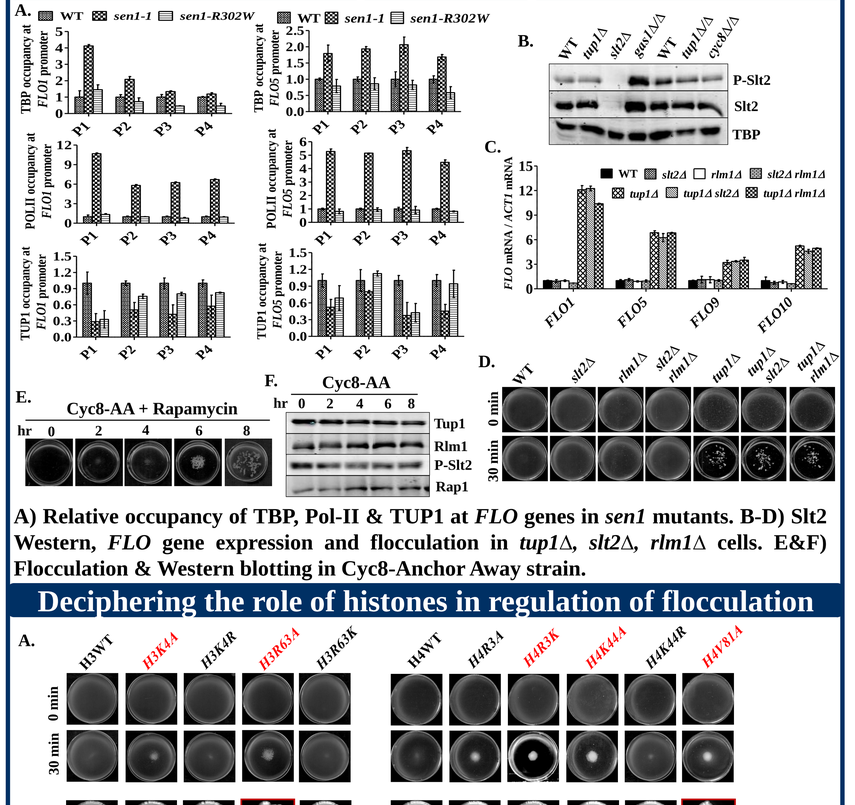
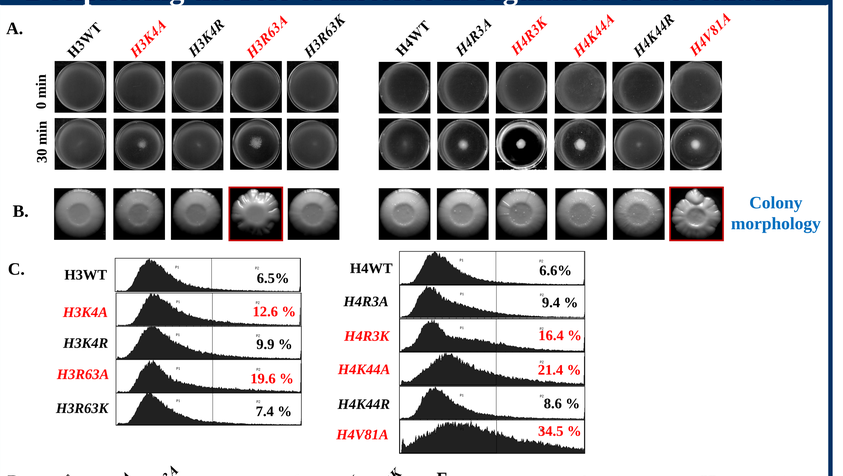
Antagonism between Rlm1 and Tup1 in regulation of transcription of *FLO* genes



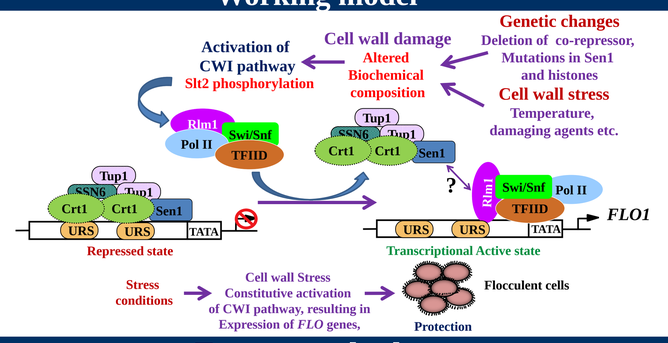
A) Relative occupancy of TBP, Pol-II & TUP1 at *FLO* genes in *sen1* mutants. B-D) Slt2 Western, *FLO* gene expression and flocculation in *tup1*Δ, *slt2*Δ, *rlm1*Δ cells. E&F) Flocculation & Western blotting in Cyc8-Anchor Away strain.

Deciphering the role of histones in regulation of flocculation



A) Flocculation in histone H3 & H4 point mutants. B) Colony morphology. C) cell size measurement using FACS. D) Flocculation after deletion of *RLM1*. E) Expression of *FLO* genes upon deletion of *RLM1*. F) Growth phenotype of histone mutants on cell wall perturbing agents CFW, CR, and CAF. G) Slt2 phosphorylation by western blotting.

Working model



Future work plan

- To study the interaction between Sen1 and Rlm1 by CoIP.
- To study the antagonism between Rlm1 & Tup1 by checking their occupancy in Cyc8-AA strain.
- To study the role of various Histone acetylases and deacetylases in regulation of flocculation.
- Studying the various MAPK cascades in sensing the stress that leads to derepression of *FLO* genes.

Conclusions

- Sen1p is required for maintenance of cell wall via CWI pathway.
- CWI pathway is required for the expression of *FLO* genes.
- Phosphorylation of Slt2 is crucial than the catalytic activity in regulation of *FLO* genes.
- Rlm1 and Tup1 plays antagonistic role to in regulating flocculation.
- Histones involved in regulation of *FLO* gene expression and colony morphology.