Elucidating the function of the essential protein Spn1 in transcription and chromatin organization

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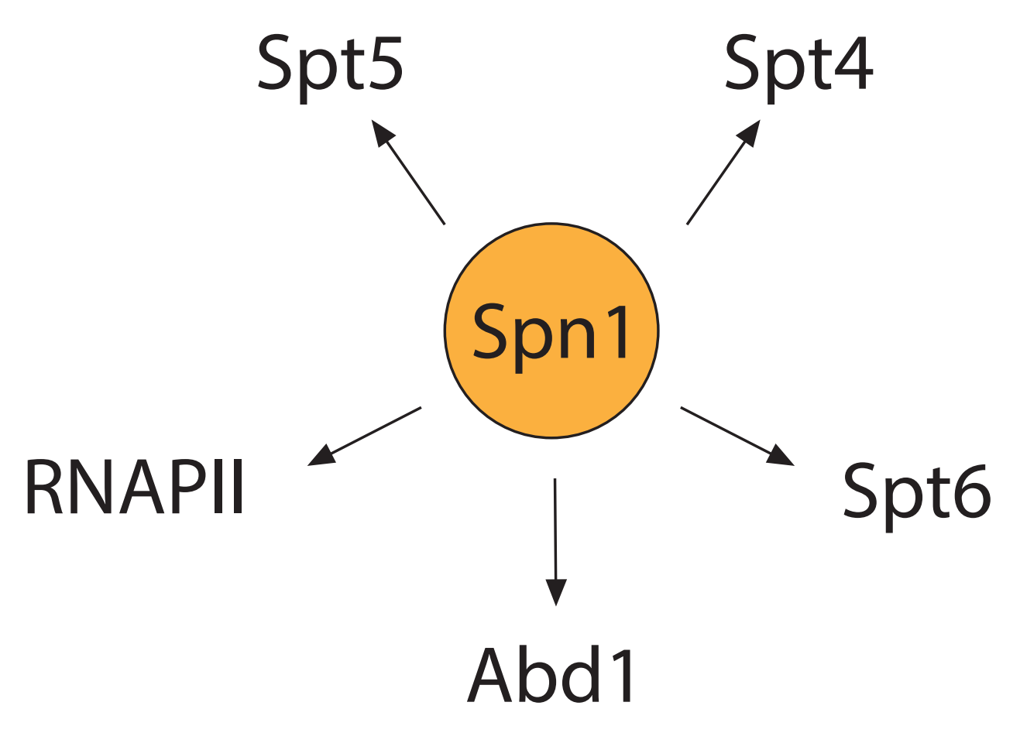
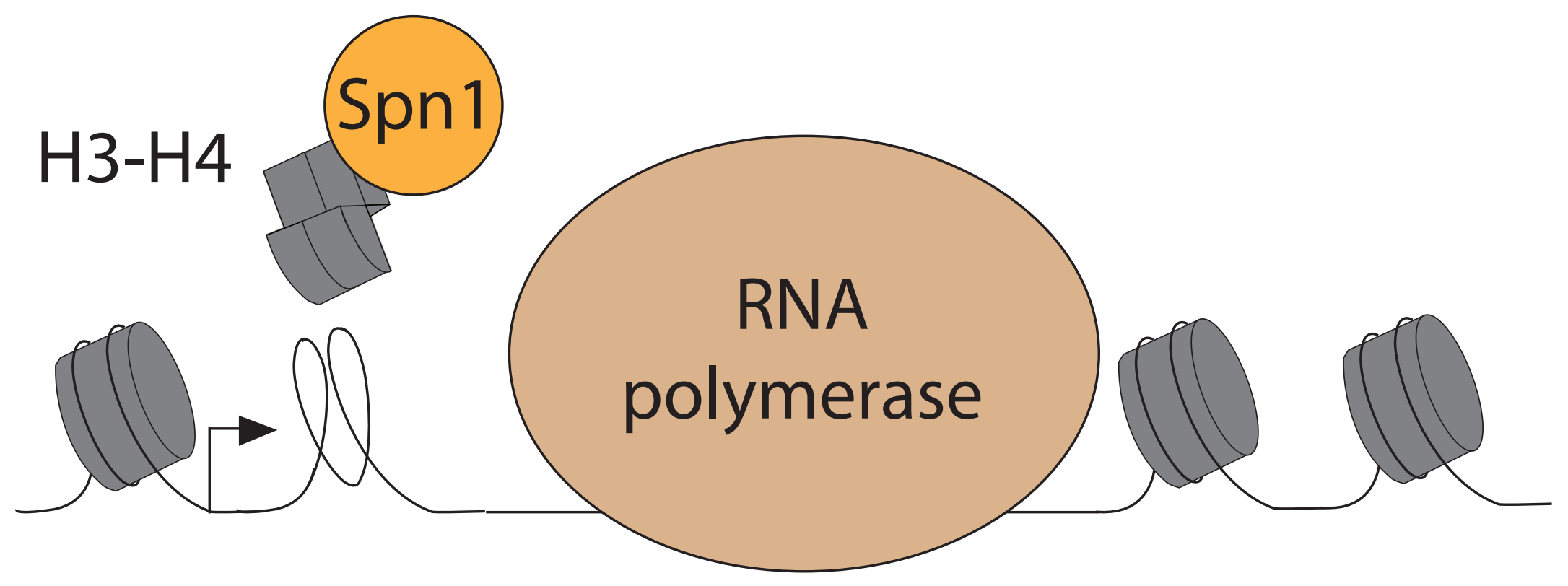
Abstract

Spn1 is an essential and conserved protein that has been associated with transcription and chromatin. However, little is known about how Spn1 functions. We performed a genetic screen to discover mutations that suppress the inviability of *Saccharomyces cerevisiae* strains in which the *SPN1* gene has been deleted (*spn1Δ*). We isolated 100 independent *spn1Δ* suppressors, and performed genetic analyses. We found that 99 suppressors are recessive while one is dominant, and that there might be extensive genetic interactions between the suppressor mutations. We also performed whole-genome sequencing of 25 of our mutants, and identified candidate suppressors that may disrupt several proteins with roles in transcription and chromatin organization.

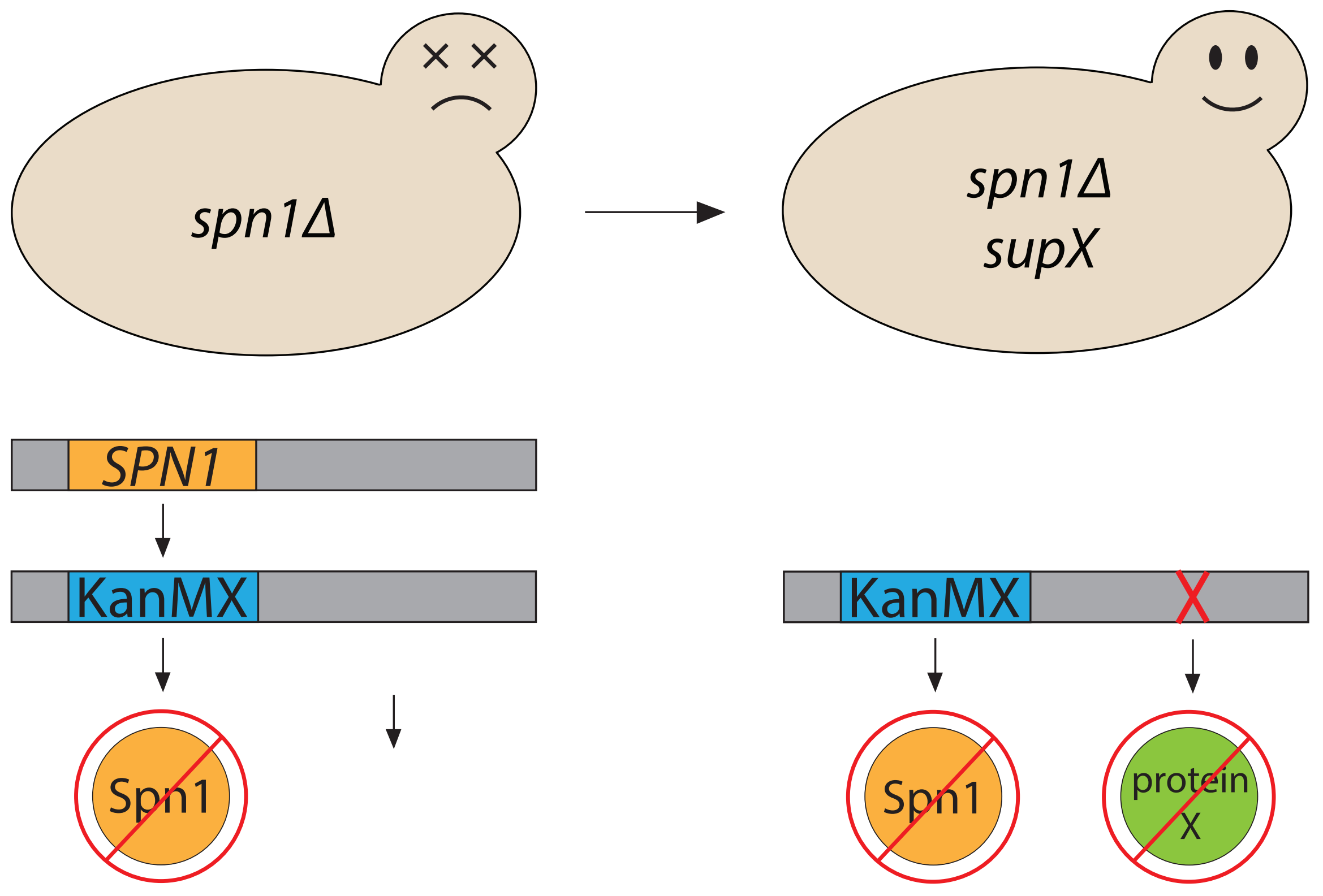
Spn1 is an essential and conserved protein that interacts with transcription factors.



Spn1 has chromatin-associated functions.



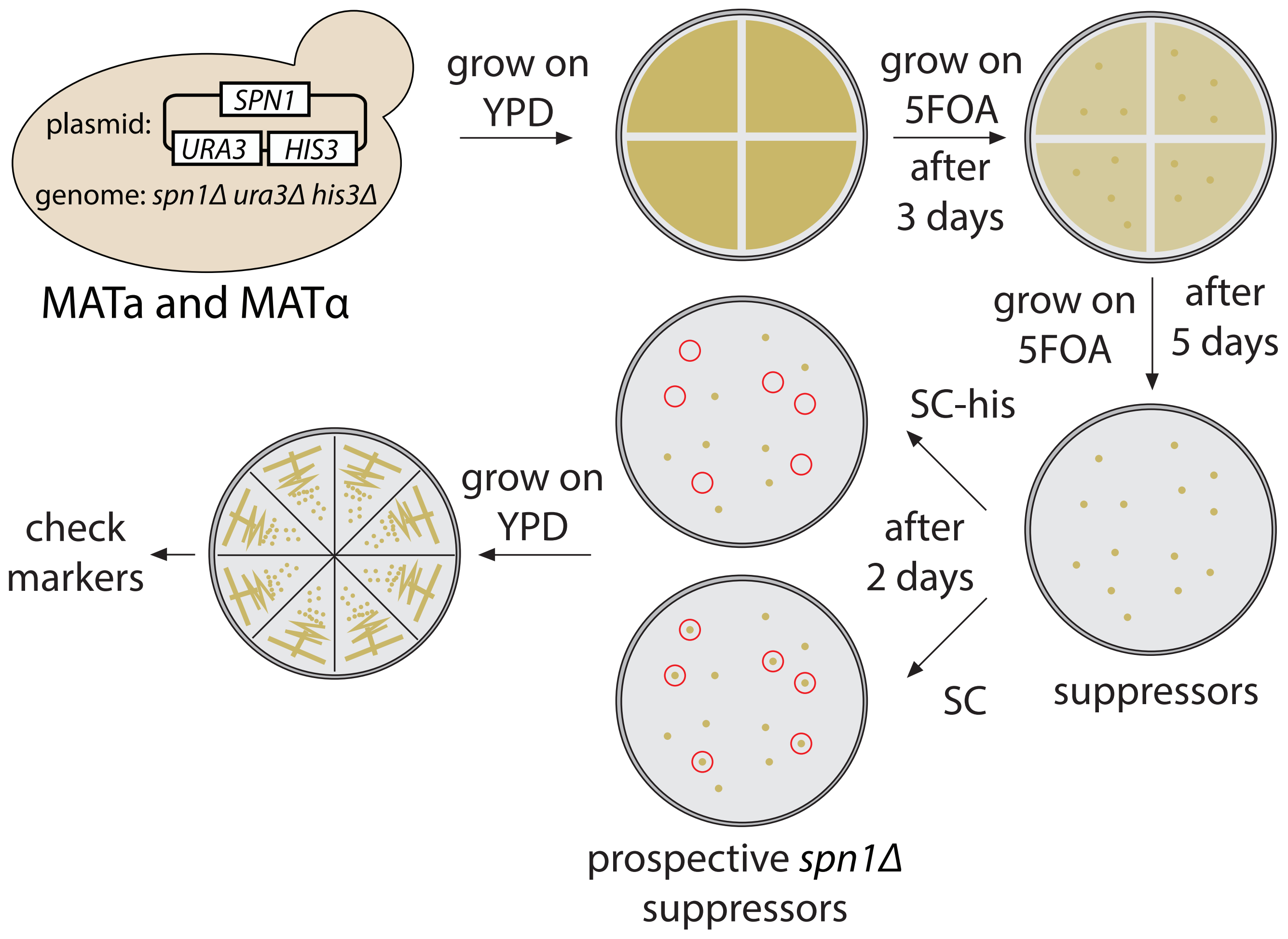
Yeast cells that lack Spn1 (*spn1Δ*) are inviable.



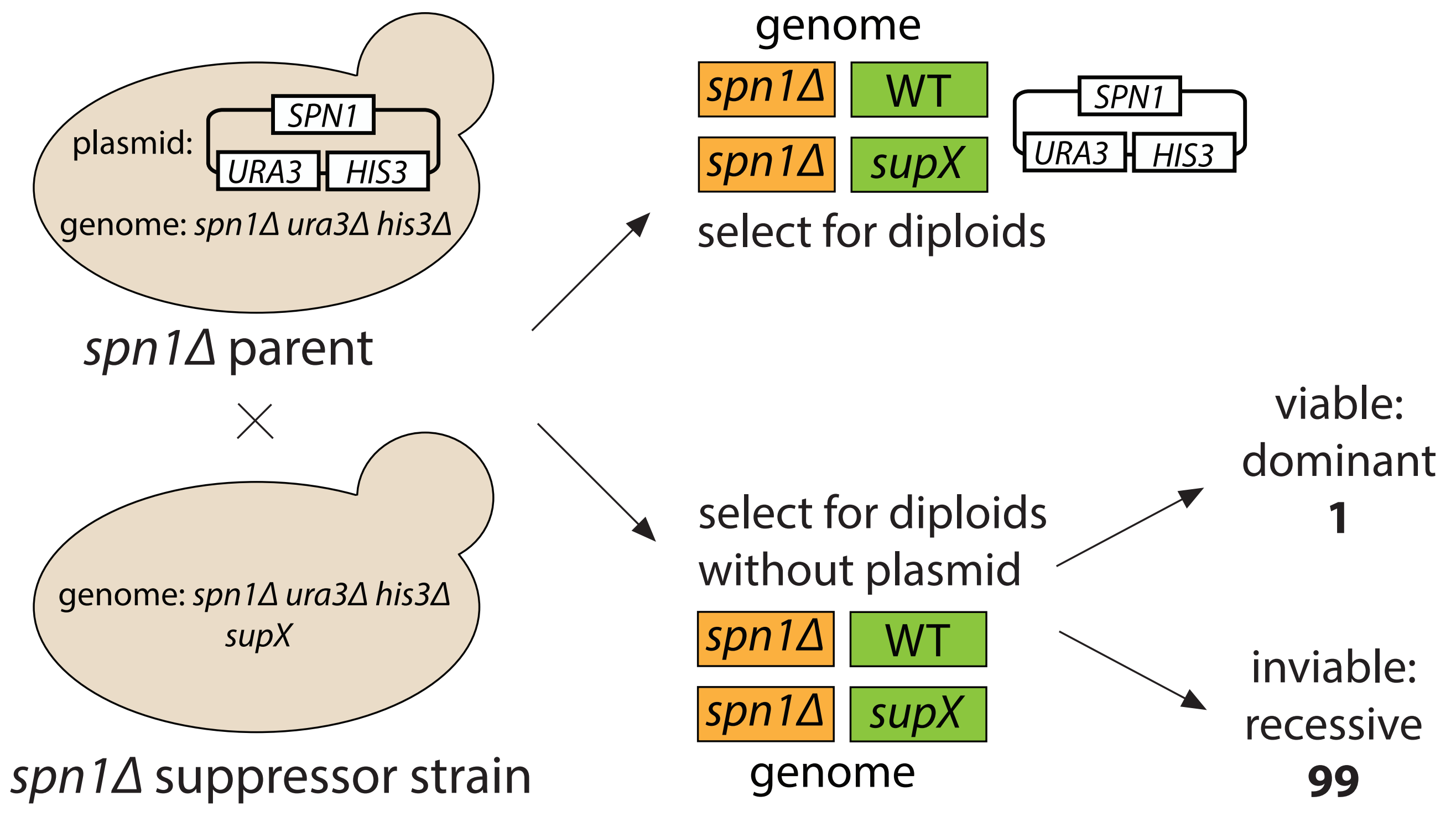
Motivation for this study: a pilot screen isolated five mutations disrupting chromatin-associated genes that suppress *spn1Δ* inviability.

Set2	Rpd3S		FACT	
set2-E45X	*rco1-H448Y*	*eaf3-E65X*	*spt16-A644P*	*pob3-E171K*

We isolated 100 additional *spn1Δ* suppressors using a large-scale screen.



We found that 99 *spn1Δ* suppressors are recessive and one is dominant.

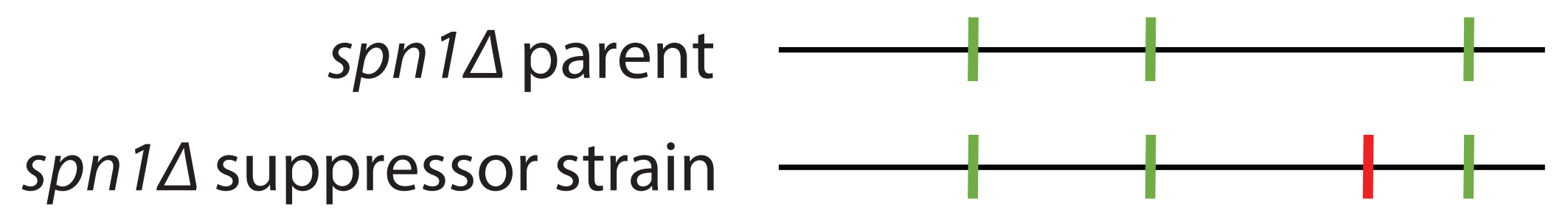


Complementation tests: are the recessive suppressors in the five already known genes?

Table 1: Results of complementation tests

Complements as:	Number of Isolates
unknown gene – complements all known mutations	5
mutation in one known gene – fails to complement 1 of the 5 known mutations	36
unclear – fails to complement >1 of the 5 known mutations	58
Total	99

We performed whole-genome sequencing of 25 *spn1Δ* suppressor strains.



Summary of whole-genome sequencing results

-1-3 mutations in transcription or chromatin-associaed genes
-New mutations in the 5 previously identified genes
-Mutations in 12 additional genes with roles in transcription/chromatin

Table 2: Candidate mutations identified in 25 *spn1Δ* suppressor strains that target coding regions of transcription and chromatin-associated proteins

Gene (complex)	Number of new mutations found	Protein/complex function
SET2	1	methyltransferase
RCO1 (Rpd3S)	2	deacetylase
EAF3 (Rpd3S)	2	deacetylase
SPT16 (FACT)	5	histone chaperone
POB3 (FACT)	2	histone chaperone
CHD1	11 (all found in combination with mutations in other genes)	chromatin remodeler
SNF2 (Swi/Snf)	1	chromatin remodeler
RTT109	5 (1 mutation found in two strains)	acetyltransferase
SPT10	3	acetyltransferase
AHC1 (ADA)	1	acetyltransferase
JHD2	2	demethylase
ASH1 (Rpd3L)	4 (found in 4 strains)	deacetylase
UBP8 (SAGA DUB)	1	deubiquitination (catalytic)
SGF73 (SAGA DUB)	1	deubiquitination (structure)
TFG1 (TFIIF subunit)	1	pre-initiation factor
RPB8 (RNAPII)	1	pre-initiation factor
YTA7	2	chromatin factor

Future directions

-Gene replacements to confirm *spn1Δ* suppresors
-Identify additional 75 *spn1Δ* suppresors
-Choose subset of suppressors for further study

References

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