ISOLATION OF YEAST GENES THAT SUPPRESS THE CHROMOSOME LOSS DEFECT OF `YAC STABILITY IN MITOSIS' MUTANTS

Brian Ruggle, Eve Rusdianto, and Heidi Sleister, Biology Department, Drake University, Des Moines, IA 50311

ABSTRACT

The eukaryotic cell cycle and processes that maintain genome stability occur with high fidelity. Mutations in genes important for the structure, replication, repair, and/or segregation of chromosomes are correlated with genome instability (*i.e.*, chromosome rearrangements, increased mutation rates, aneuploidy, abnormal chromatin structure, and/or abnormal gene expression). As part of an effort to identify proteins important for genome stability, we previously implemented a genetic screen in the yeast *Saccharomyces cerevisiae* that allows for visual detection of mutants with increased loss of an *ADE2*-marked yeast artificial chromosome (YAC). This screen resulted in 132 YAC stability in mitosis (*ysm*) mutants. Three mutants, *ysm76*, *ysm83*, and *ysm84*, have been further characterized for phenotypes related to genome instability. In addition, both high copy and single copy suppressors of these mutants' YAC loss defects are being isolated. Identification of these suppressors will contribute to our understanding of protein networks and processes important for eukaryotic genome stability.

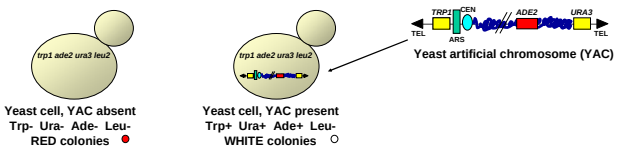
BACKGROUND

➢ **GOAL:** To identify gene products and processes important for accurate chromosome transmission.

➢ **IMPORTANCE:** Proper chromosome segregation is essential for genome stability and cell viability. Errors during chromosome transmission can lead to phenotypes ranging from cell death to uncontrolled cell growth (cancer).

➢ **EXPERIMENTAL STRATEGY:** To isolate plasmid suppressors of the chromosome loss defect (i.e., white/red colony sectoring) of YAC stability in mitosis (*ysm)* mutants. Suppressors will define gene products that are defective in *ysm* mutants and/or that genetically interact with *ysm* mutants.

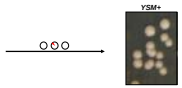
Visual Assay For YAC Loss



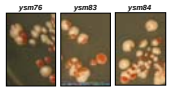
Yeast cell, YAC absent
Trp- Ura- Ade- Leu-
RED colonies

Yeast cell, YAC present
Trp+ Ura+ Ade+ Leu-
WHITE colonies

Wild-type (*YSM+*) yeast cells have very low rates of chromosome/YAC loss and produce white colonies with no/very few red sectors.



YAC stability in mitosis (ysm) mutants have increased rates of YAC loss and produce white colonies with many red sectors. Mutants ysm76, ysm83, and ysm84 were generated by exposure of yeast cells to the mutagen UV.

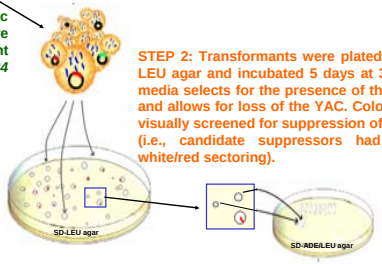


METHODS

Isolation of Suppressors of YAC Loss in *ysm* Mutants

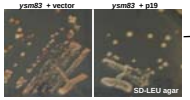
Yeast genomic plasmid library

STEP 1: Yeast genomic plasmid libraries were transformed into mutant *ysm*76, *ysm83*, and *ysm84* cells containing a YAC.



STEP 2: Transformants were plated onto SD-LEU agar and incubated 5 days at 30ºC. This media selects for the presence of the plasmid and allows for loss of the YAC. Colonies were visually screened for suppression of YAC loss (i.e., candidate suppressors had reduced white/red sectoring).

STEP 3: Candidate suppressors were re-picked to SD-ADE/LEU agar and re-screened two more times for the suppression phenotype by single colony purification on SD-LEU agar.

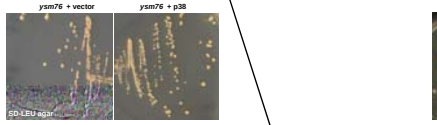


Plasmid p19 suppresses the sectoring defect (i.e., YAC loss) of *ysm83*.

STEP 4: Candidate suppressors were analyzed by one of two methods to confirm that the plasmid is responsible for the suppression phenotype.

STEP 4a: Plasmid DNA was isolated from *ysm* candidate suppressor cells and reintroduced into mutant yeast *ysm* cells. Was suppression gained (i.e., was sectoring absent)?

STEP 4b: The plasmid was removed from *ysm* candidate suppressor cells. Was suppression lost (i.e., was sectoring observed again)?



Relative to the vector control, *ysm76* re-transformed with plasmid p38 displayed the wild-type, white-colony phenotype (i.e., reduced YAC loss). This indicates that plasmid p38 is responsible for suppression of *ysm76*'s YAC loss.

In the absence of the candidate suppressor plasmid p83, increased levels of sectoring were observed. This indicates that p83 is responsible for suppression of *ysm76*'s YAC loss.

STEP 5: The yeast DNA sequences of plasmids testing positive for suppression were determined and compared to the yeast genome database by a BLAST search. This allowed for identification of genes within the suppressor plasmids.

RESULTS

STEP 1	STEP 1	STEP 2	STEP 2
Mutant Yeast Strain	Yeast Genomic Library	Number of Transformants	Number of Nonsectored Transformants (Suppressor Candidates)
ysm76	LEU2, CEN (single copy)	1,566	>183
ysm83	LEU2, 2-micron (high copy)	3,377	>71
ysm84	LEU2, 2-micron (high copy)	595	>98

STEP 3	STEP 3	STEP 4a	STEP 4b	STEP 4	STEP 5
Candidate Plasmid Suppressor	What was the phenotype of candidate suppressors upon re-screening?	Did mutant *ysm* cells gain suppression upon retransformation with the candidate suppressor plasmid?	Did mutant *ysm* cells lose suppression upon loss of the candidate suppressor plasmid?	Is the plasmid a confirmed suppressor of YAC loss?	Which yeast gene(s) are contained within the suppressor plasmid?
ysm76-p38	no sectoring	YES	*not determined*	YES	*in progress*
ysm76-p46	no sectoring	*in progress*	*not determined*	?	*in progress*
ysm76-p67	no sectoring	*not determined*	YES	YES	*in progress*
ysm76-p73	no/little sectoring	*not determined*	YES	YES	*in progress*
ysm76-p74	no/little sectoring	*in progress*	*not determined*	?	*in progress*
ysm76-p83	no sectoring	*not determined*	YES	YES	*in progress*
ysm76-p103	no sectoring	*in progress*	NO	NO	*in progress*
ysm76-p135	no sectoring	*in progress*	*not determined*	?	*in progress*
ysm76-p152	no sectoring	*in progress*	*not determined*	?	*in progress*
ysm76-p154	no sectoring	*in progress*	*not determined*	?	*in progress*
ysm76-p160	no sectoring	*not determined*	YES	YES	*in progress*
ysm76-p165	no sectoring	*in progress*	*not determined*	?	*in progress*
ysm76-p183	no/little sectoring	*in progress*	*not determined*	?	*in progress*
ysm83-p19	no/little sectoring	*not determined*	YES	YES	*DTD1, YDL218W, TIM22*
ysm83-p26	no/little sectoring	*not determined*	YES	YES	*DTD1, YDL218W, TIM22*
ysm83-p41	no/little sectoring	*not determined*	YES	YES	*OCA1, RAS2, YNL097C-B, PHO23, tL(CAA)N*
ysm83-p67	no/little sectoring	*not determined*	YES	YES	*YDR003W-A, RAD57, MAF1, SOK1, TRP1*
ysm83-p68	no/little sectoring	*in progress*	*not determined*	?	*in progress*
ysm83-p71	no/little sectoring	*not determined*	YES	YES	*RRP3, SSF1, HTD2*
ysm84-p11	no/little sectoring	YES	*not determined*	YES	*CAK1, AGX1*
ysm84-p33	no/little sectoring	*not determined*	YES	YES	*in progress*
ysm84-p57	no/little sectoring	YES	YES	YES	*SMF1*
ysm84-p61	no/little sectoring	*in progress*	*not determined*	?	*RRF1, MSC7*
ysm84-p76	no/little sectoring	*in progress*	*not determined*	?	*in progress*
ysm84-p98	no/little sectoring	*in progress*	*not determined*	?	*in progress*
ysm84-p114	no/little sectoring	*not determined*	YES	YES	*AAT2, SNR30, ADE16, RPL15A*

CONCLUSIONS

➢ Single-copy gene suppressors of YAC loss were isolated for *ysm76*, and high-copy gene suppressors were isolated for *ysm83* and *ysm84*. Identification of these suppressors may reveal processes and proteins important for accurate chromosome transmission: cell cycle regulation (*CAK1, OCA1*), regulation of transcription (*PHO23*), translation (*DTD1, RPL15A*), protein transport (*TIM22*), ion transport (*SMF1*), and recombination (*MSC7*).

➢ For suppressor plasmids (e.g., ysm83-p19) containing multiple genes, each gene (e.g., *DTD1*) will be re-tested individually for its ability to suppress YAC loss.

➢ The sequence of each suppressor gene in the *ysm* mutant cells and wild-type cells will be analyzed to determine the genetic mechanism by which suppression occurs (i.e., dosage suppression vs complementation).

ACKNOWLEDGEMENTS

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