Defining a Minimal Yeast Centromere

Aryan Kokkanti, Daniel Kolbin, Kerry Bloom

Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-3280, USA

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

Abstract

A minimal centromere is defined as the smallest sequence that allows for proper segregation of chromosomes in anaphase. To determine the minimal centromere of *S. cerevisiae*, we engineered multiple centromere VIII (CEN8) sequences that have their 118 base pair consensus sequence along with additional DNA flanking the 118 bp upstream, downstream, or both. We inserted the CEN8 sequences into chromosome III under the control of the GAL promoter to conditionally activate a dicentric chromosome. Analysis of colony growth on dextrose (centromere active) and galactose (centromere inactive) shows that the 118 bp consensus does not function as a centromere. Flanking sequences downstream of the 118 bp consensus confers centromere function. Adding DNA upstream of the 118 bp consensus has no significant effect on function. The distribution of repairs events arising from broken dicentric chromosomes shows an increased frequency of NHEJ and HR with the latter dominating as more DNA is added to either flank. When the engineered CEN8 sequences are introduced into yeast plasmids with selection markers, there is an increase in centromere function with increased DNA downstream of the 118 bp consensus. We conclude that CEN8 requires another 19 bases pairs downstream of the 118 bp consensus to function as a centromere.

Background

- Previous literature suggests that the minimal length of a functional CEN8 in *S. cerevisiae* is 118 bp.[2]
- We engineered CEN8 sequences with different amounts of additional upstream and downstream homology and integrated them into chromosome 3 of yeast strains that have all their centromeres replaced with the unadjusted CEN8 sequence
- These dicentric (two centromeres on one chromosome) strains were examined to test for functionality of centromere by looking at viabilities and repair event distributions.[1,3]
- The engineered centromeres were introduced into yeast plasmids and stabilities were tested to further corroborate function.

Methods

Viability Assay

Dicentric yeast strains were grown on YPD and YPG plates at 24 °C and total counts were taken after 72 hours to determine viability of each strain.

Repair Event Assay

Single colonies on YPD plates after a 72-hour time course were grown in liquid media at 24 °C. After 24 hours, DNA was extracted, and PCR was performed and scored to determine repair events.

Plasmid Stability Assay

Yeast strains with centromere plasmids were grown in selective YCAU media for 24 hours at 24 °C and then plated on YCAU and YPD and incubated. Colony counts were taken after 72 hours to determine plasmid stability.

Results

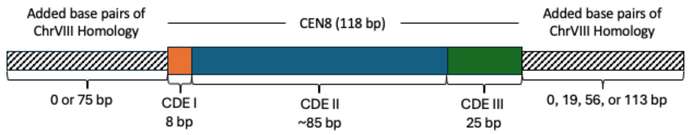

Figure 1: Schematic of Engineered CEN8 Structure. Additional base pairs of homology to ChrVIII were added to CEN8 upstream, downstream, or both. Upstream additions were either 75 bp or no addition. Downstream additions were either 0, 19, 56, or 113 bp of ChrVIII homology.

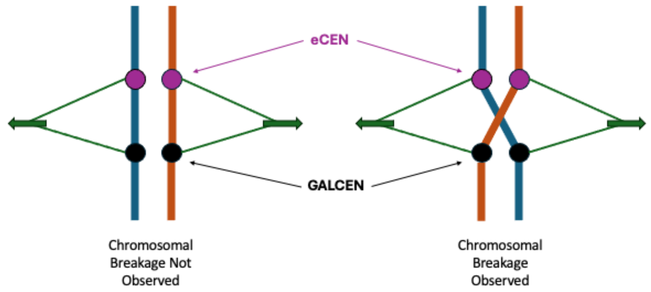

Figure 2: Dicentric Chromosome Breakage. Dicentric chromosomes are more likely to break in anaphase than monocentric chromosomes. Force exerted by the microtubules is shown with the green arrows. Dicentric chromosomes used in this experiment have an endogenous centromere (eCEN) and the conditional centromere, GALCEN, containing GAL promoter and an engineered CEN8 sequence. The GAL promoter inactivates the centromere when yeast is grown on galactose.

Table 1: Engineered CEN8 Sequences with Viabilities and Plasmid Stabilities.

ChrVIII Homology Sequence	Upstream Chromosome Homology (bp)	Downstream Chromosome Homology (bp)	Total ChrVIII Homology Including CEN8 (bp)	Viability (# of YPD Colonies/# of YPG Colonies)	Plasmid Stability (# of YCAU Colonies/# of YPD Colonies)
0 - 0	0	0	118	92.9%	39.3%
0 - 19	0	19	137	48.8%	51.4%
0 - 56	0	56	174	47.8%	69.6%
75 - 0	75	0	193	95.8%	41.6%
0 - 113	0	113	231	23.7%	61.7%
75 - 113	75	113	306	33.3%	58.0%

Results

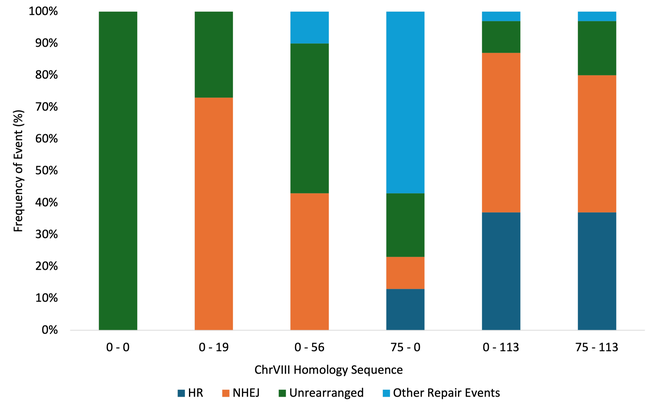

Figure 3: Distribution of Repair Events. Single colonies of dicentric yeast w were checked for repair products including presence of eCEN, GALCEN, rearrangement, and reciprocation. Different combinations of products indicate different repair pathways, most notably homologous recombination (HR) and non-homologous end joining (NHEJ).

Conclusions

- An increase in the amount of ChrVIII homology added downstream of the 118 bp consensus significantly decreases viability, thus increasing function. DNA addition upstream had an insignificant effect on viability.
- Similarly, as the DNA added downstream of the 118 bp consensus increased, plasmids generally became more stable. DNA addition upstream had negligible effects on plasmid stability.
- As the total homology to ChrVIII increased, repair events occurred more often. Furthermore, as the amount of homology increased, the proportion of HR increased and NHEJ decreased.
- We conclude CEN8 requires another 19 bp downstream to be functional.

Acknowledgements

We are grateful to the members of the Bloom Lab for providing equipment, discussion, and assistance with this study. We would also like to thank Dr. Wing Lee Karen Yuen for providing the homologous centromere 8 background strain. This research was supported by NIH RO1 GM32238 to K.B.

References

1. Cook D, Long S, Stanton J, Cusick P, Lawrimore C, Yeh E, et al. Behavior of dicentric chromosomes in budding yeast. PLoS Genet. 2021 Mar;17(3):e1009442.
2. Lauer S, Luo J, Lazar-Stefanita L, Zhang W, McCulloch LH, Fanfani V, et al. Context-dependent neocentromere activity in synthetic yeast chromosome VIII. Cell Genom. 2023 Nov 8;3(11):100437.
3. Cook D, Kozmin SG, Yeh E, Petes TD, Bloom K. Dicentric chromosomes are resolved through breakage and repair at their centromeres. Chromosoma. 2024 Jan 2;