Loss of histone **H2A.Z** creates defects in **Non-homologous end joining (NHEJ)** and **chromosome stability** in *S. cerevisiae*.

Demonstrating a Role for Histone H2A.Z in DNA Double Strand Break Repair and Chromosome Stability

Ye Jun Kim, Scott G. Holmes
Molecular Biology and Biochemistry Department, Wesleyan University

Introduction:

- Histone H2A.Z (coded by *HTZ1*) is a variant of histone H2A and can replace H2A in nucleosomes. H2A.Z containing nucleosomes are associated with altering chromatin structure, nucleosome stability, and transcription[1,2].
- Histone H1 (coded by *HHO1*) is the histone present on linker DNA, outside the histone octamer[3].
- Non-homologous end joining (NHEJ) is the pathway that repairs DNA double strand breaks (DSB) in the absence of a homologous template.
- Previous studies in the Holmes Lab found a genetic interaction between H1 and H2A.Z (silencing, condensation).

Objectives/Questions of Interest:

Does **Δ*htz1*** cause **defects** in **NHEJ-mediated DSB repair** or **chromosome stability** measured by plasmid retention rates?
Does additional **deletion of H1 suppress** Δ*htz1 defects*.

Methods:

Plasmid Retention Assay:

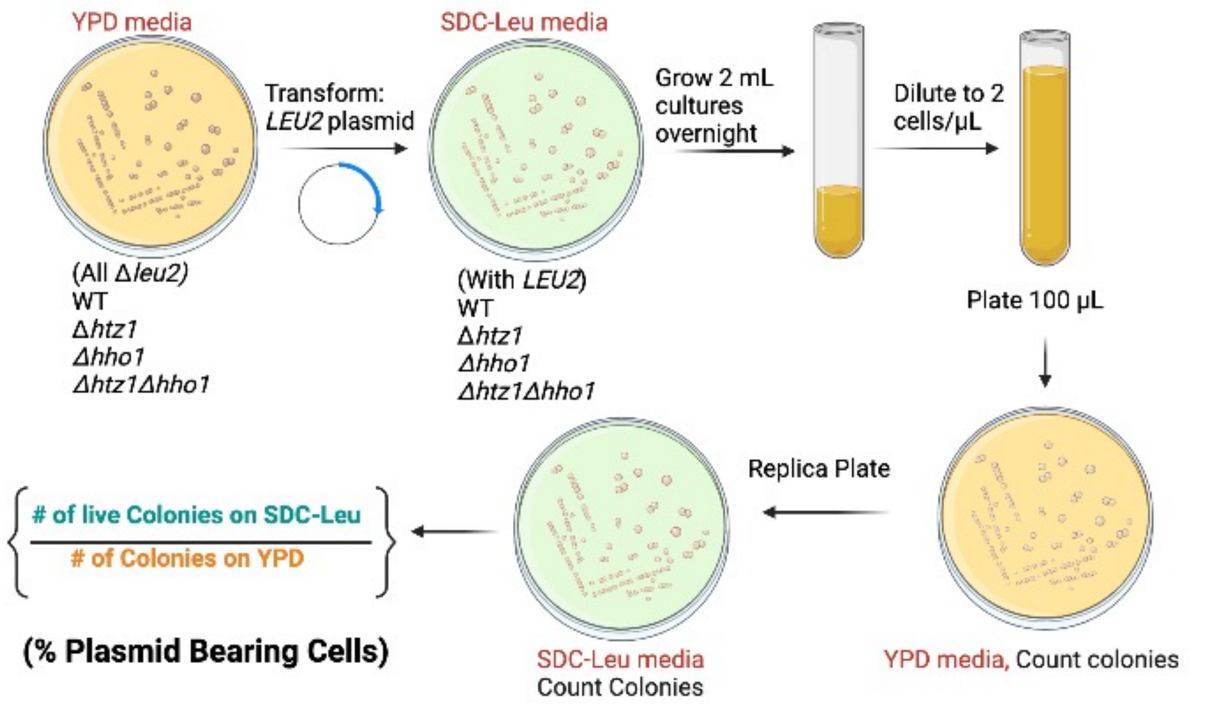

Figure 1: Figure describing the plasmid retention assay. Δ*leu2* strains were transformed with pRS415 (*LEU2* plasmid), grown, and diluted to a density of 2 cells/µL. 100 µL of diluted cells were plated on YPD and replica plated to SDC-Leu, selecting for cells that retained pRS415. Calculation of the % of plasmid bearing cells shown above quantified plasmid retention rates for each strain. Created with BioRender.com

NHEJ Assay:

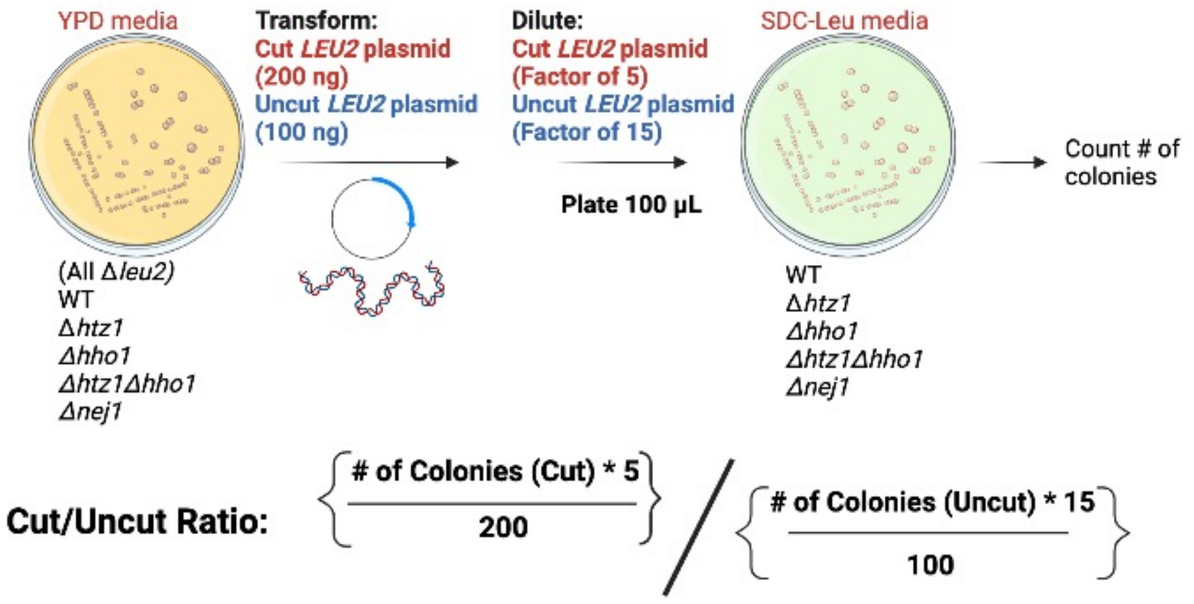

Figure 2: Figure describing the NHEJ Assay. Δ*leu2* strains were transformed with 100 or 200 ng of uncut or HindIII-linearized pRS415 (*LEU2* plasmid) respectively. 100 µL of transformants, diluted by a factor of 5 (linearized) or 15 (uncut), were plated on SDC-Leu media. This selects for cells that contained uncut or repaired the cut *LEU2* plasmid through NHEJ, allowing propagation of the *LEU2* plasmid and colony growth on media lacking leucine. Calculation of a Cut/Uncut ratio shown above quantified each strain's NHEJ efficacy. Created with BioRender.com

Results:

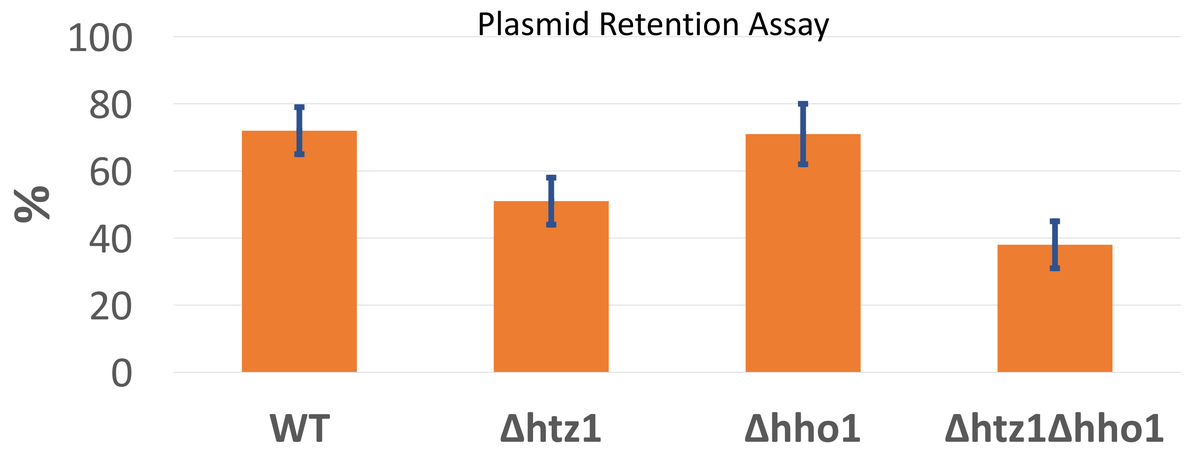

Figure 3: Bar graph showing the avg. plasmid retention rates for WT (72%), Δ*htz1* (51%), Δ*hho1* (71%), and Δ*htz1*Δ*hho1* (38%) yeast strains. Error bars depict one standard deviation above and below the mean. A statistically significant ($p<0.05$) difference was found between WT and Δ*htz1* ($p= 5.63E-07$), WT and Δ*htz1*Δ*hho1* ($p= 2.41E-10$), and Δ*htz1* and Δ*htz1*Δ*hho1* ($p<0.00015$) using a one tailed, two-sample t-test assuming unequal variances.

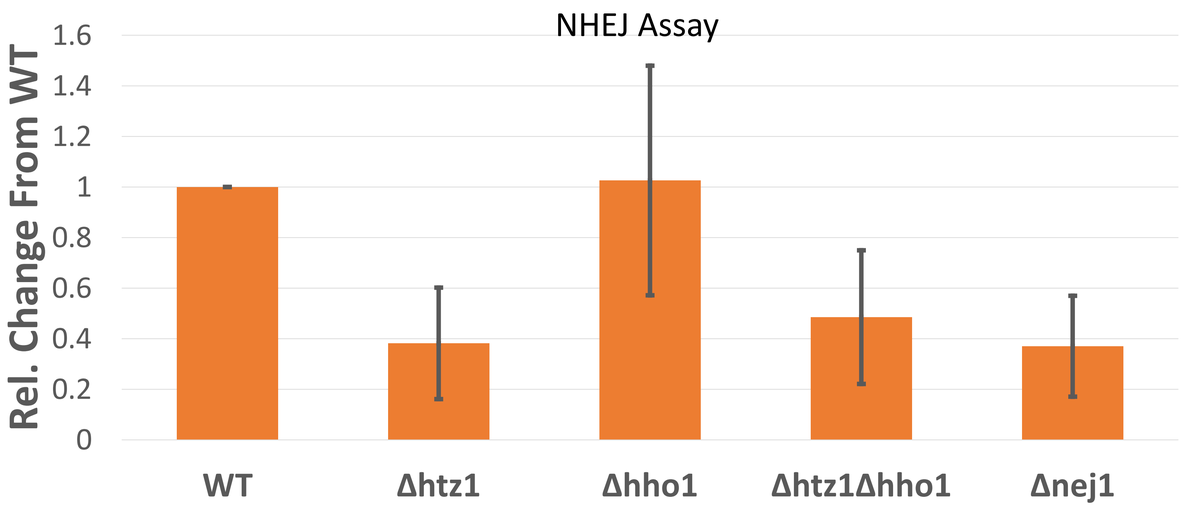

Figure 4: Bar graph showing the relative change in Cut/Uncut ratio from WT (set to 1) for Δ*htz1* (0.38), Δ*hho1* (1.03), Δ*htz1*Δ*hho1* (0.48), and Δ*nej1* (0.37) yeast strains. Error bars depict one standard deviation above and below the mean. A statistically significant ($p<0.05$) difference was found between WT and Δ*htz1* ($p=0.00015$), WT and Δ*htz1*Δ*hho1* ($p=0.00105$), and WT and Δ*nej1* ($p=0.00106$) using a one tailed, two-sample t-test assuming unequal variances.

Discussion/Future Directions:

1. Plasmid Retention defect in Δ*htz1,* worse in Δ*htz1*Δ*hho1* yeast ➔ H2A.Z is necessary for proper structural maintenance , replication, and/or segregation of chromosomes in budding yeast.
   - H1 may share a redundant, but less important role than H2A.Z in plasmid retention.
2. NHEJ defect in Δ*htz1*, Δ*htz1*Δ*hho1*, and Δ*nej1* (positive control) yeast[4].
   - H2A.Z is necessary for proper functioning NHEJ in DNA repair.
   - H2A.Z exchange from nucleosomes may promote a chromatin structure more permissive for access by proteins involved in NHEJ at the site of DNA damage[1].

References:

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