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Interrogating the Functions of the Histone H2A Repression Domain in Relation to Cell-Cycle Progression in *Saccharomyces cerevisiae*

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Abstract

In eukaryotic cells, DNA is wrapped around histone proteins to form the basic repeating unit of chromatin, the nucleosome core particle. Most nucleosomes consist of two copies each of histones: H2A and H2B in a heterodimer and histones H3 and H4 in a heterotetramer. Because of their intimate association with DNA, histones regulate all DNA-templated processes such as transcription, DNA replication, and DNA damage response and repair. Several studies have illustrated the importance of a small portion of the histone H2A amino terminal domain which regulates global transcription. This domain is responsible for the repression of ~4% of the yeast genome. This domain, termed the histone **H2A Repression** (H.A.R.) domain, consists of a small, conserved portion (amino acids 16-20) of histone H2A. The H.A.R. domain has also been shown to be an important regulator of DNA damage response and repair. In this study, we find that deletion of the H.A.R. domain leads to a defect in cell proliferation. Indeed, deletion of the domain leads to a marked sensitivity to hydroxyurea, indicating a defect in either DNA damage response or DNA replication machinery. Intriguingly, we find that the RNR2 gene (which regulates the transition of nucleoside triphosphates into deoxynucleotide triphosphates) is down regulated in these mutants. Finally, we find that deletion of the H.A.R. domain leads to an abnormal number of cells that show an apparent delay in the transition out of S-phase. Taken together, these data illustrate the importance of this domain in proper progression through the cell cycle.

Figure 1: H.A.R. sequence conserved among species.

Figure 1: Sequence alignment highlighting the H.A.R. domain in different species. The H.A.R. domain is well conserved indicating the importance of this sequence. We were curious if the H.A.R. domain regulated other DNA-templated process such as DNA replication and cell cycle progression.

Figure 2: Growth data comparing wild-type to the H.A.R. deletion mutant.

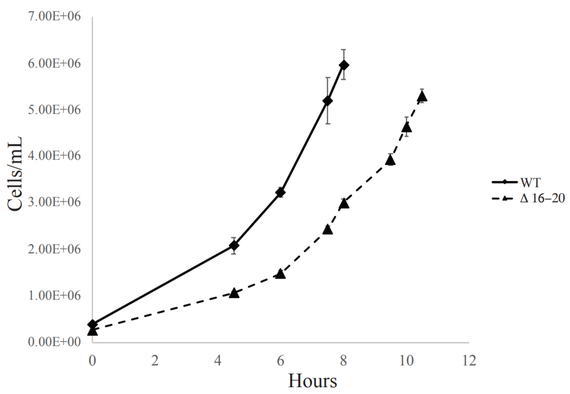

Figure 2: Wild-type and the deletion of the H.A.R. domain growth data in liquid YPD monitored over 10 hours. These data indicate that the mutant is a slow grower which is likely due to the H.A.R. domain having an impact in the cell cycle process. We then tested various genotoxic stressors in order to determine if the mutant strains had sensitivities.

References

1. Michael A. Parra and John J. Wyrick, *Mol. Cell. Biol.*, (2007) ;27:7641-7648

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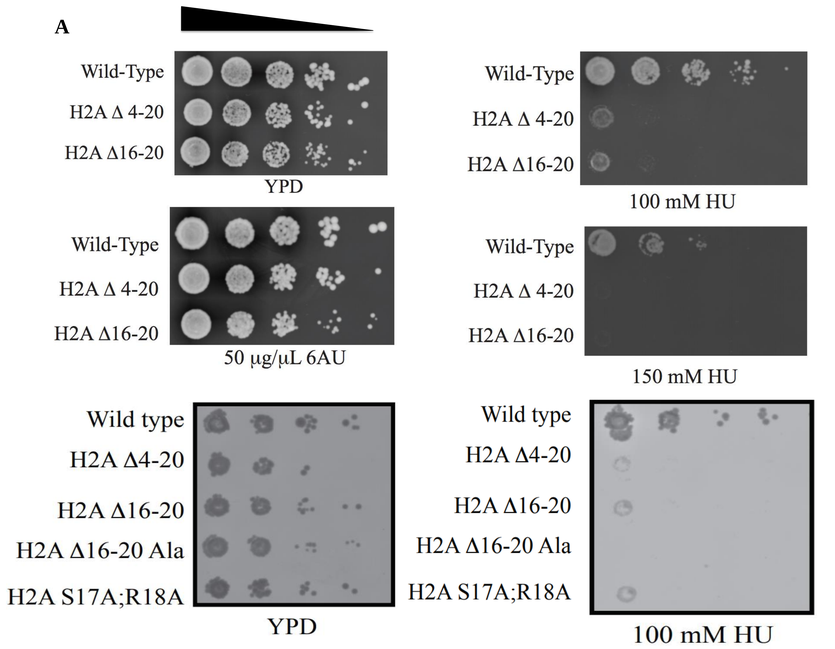
Figure 3: H.A.R. deletion leads to hydroxyurea sensitivity.



B

Gene name	Description
CBL6	B-type cyclin involved in DNA replication during S phase
MCM7	primes origins of DNA replication in G1
NOP7	required for exit from G_0 and the initiation of cell proliferation
RNR1	regulated by DNA replication and DNA damage checkpoint pathways via localization of small subunits
PSF1	complex is localized to DNA replication origins and implicated in assembly of the DNA replication machinery

C Transcriptional Activation

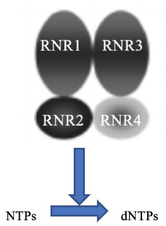


Figure 3: **(A)** Yeast cells were grown to log phase, serially diluted, and spotted on plates with the indicated genotoxic stressors. The plates were incubated for three days. Yeast cell dilution on un-drugged YPD. 6-azauracil has little effect on cell viability. Hydroxyurea shows a marked effect on cell viability. Hydroxyurea was then tested with the H.A.R. alanine mutation and the mutation of the two key residues, S17 and R18. **(B)** We examined the DNA microarray data from the H2A Δ4-20 mutant[1]. We queried the gene ontology term finder on the yeast genome database (www.yeastgenome.org) to identify processes affected in this mutant. Several genes whose expression are altered in the H2A Δ4-20 and are involved in DNA replication. This is a potential explanation for the the for the HU sensitivity.[1] **(C)** Ribonucleotide reductase 1 (RNR1) is a protein involved in DNA replication. RNR1 is a part of the cascade that catalyzes the transformation of nucleoside triphosphates, NTPs, to deoxynucleoside triphosphates, dNTPs (the building blocks of DNA). Down regulation of this gene may lead to a depletion of dNTP pools which may explain the sensitivity to HU.

Figure 4: Analyzing the RNR genes with duplex PCRs.

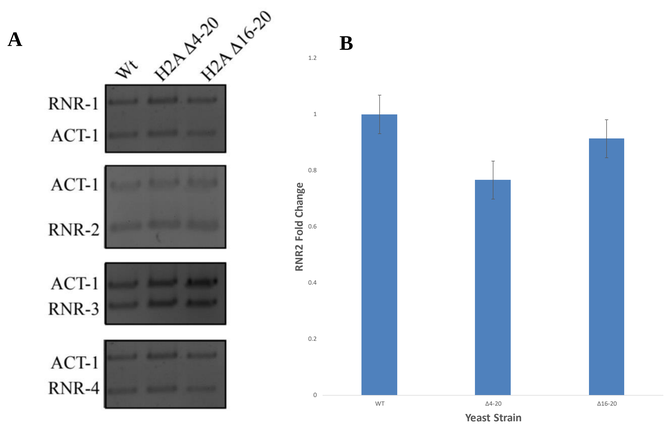


Figure 4: **(A)** Reverse-transcription polymerase chain reaction was performed on RNR 1-4 genes with each corresponding ACT1 primer set. cDNA was extracted from the RNA from the mutant strains and wild-type. **(B)** The RNR gene expressions were compared to the wild-type expression via the band quantification software program AzureSpot. The gene expression of RNR2 was the most significant differentiation from wild-type to the mutant strains. When wild-type is considered a 1.0 fold, mutant Δ4-20 has a 0.766 fold change and Δ16-20 has a fold change of 0.913. This fold change indicates that the deletion of the H.A.R. domain shows a down regulation of the RNR2 gene which could be the reason this domain has a role in DNA replication.

Figure 5: Bud morphology analyzing cell cycle progression in mutants.

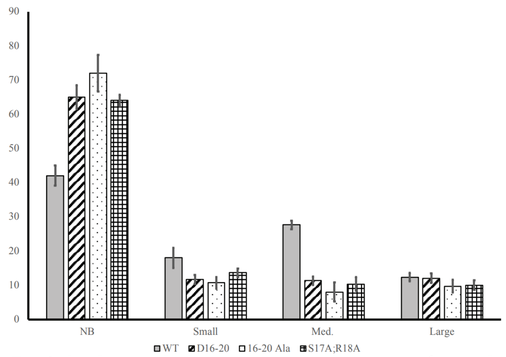


Figure 5: A bud morphology procedure was performed with the wild-type, H.A.R. deletion, H.A.R. alanine mutation, and the alanine mutation of S17 and R18. Cells were grown in YPD liquid media overnight and the cell cycle phases were recorded in triplicate. No buds were found in higher concentrations compared to the other phases, but there was a significant increase in no buds seen in the mutant strains. This indicates that there is a cell arrest in the mutant strains entering the S phase. The H.A.R. domain is an important regulator of entering the S phase of the cell cycle as well as the two main residues, S17 and R18.

Conclusions

Two novel functions of the Histone H.A.R. domain were identified. We find that the H.A.R. domain likely regulates DNA replication and cell cycle progression through the RNR gene cascade specifically the RNR2 gene. The H.A.R. domain, more so the two key residues S17 and R18, are important regulators of cell cycle progression.

Future Work

We are planning on investigating more of the cell cycle genes such as NOP7 and NRM1 through RT-PCRs. Cell growth will also be analyzed in the presence of hydroxyurea compared to wild-type growth. We are also doing a flow cytometry analysis of our mutants to confirm the cell cycle defects.