Potential Binding Partners for H1 histone in the Yeast Species, *Saccharomyces cerevisiae*

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Abstract

The nucleosome is the basic unit of chromatin, a constituent of chromosomes, the final structure in which DNA is packaged in the cell. Nucleosomes consist of DNA wrapped around an octamer of four histone proteins, resulting in a bead on a string type structure. However, our focus is on a fifth histone, H1, which has two domains GI and GII. H1 functions as a linker histone that binds to the linker DNA between each nucleosome.[1] This research centers around unbiased screening of the 6000 genes of budding yeast, *Saccharomyces cerevisiae*, for physical interactions with H1 and its GII domain using the yeast two-hybrid assay. The assay currently shows 22 possible interactions with the full-length H1 protein and the GII domain. This research is a preliminary measure for further understanding the functions of the H1 protein and its GII domain. The proteins identified would undergo further screening using different methods to ensure an interaction with the H1 protein or its GII domain.

Introduction

H1 histone impacts nucleosome structure

- Binding of the H1 histone to linker DNA confers the nucleosome with a more compact and rigid structure.
- The H1 histone protects the linker DNA from degradation by nucleases.

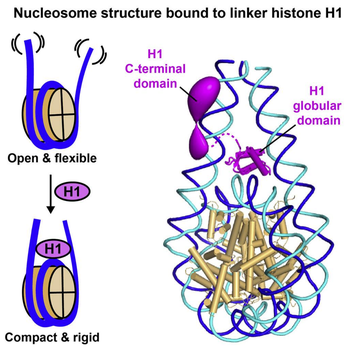

Fig 1: Function of the H1 histone.[2]

Structure of the H1 histone protein in the yeast species, *Saccharomyces cerevisiae*.

- The H1 histone protein in the yeast species, *Saccharomyces cerevisiae*, is unique in that it has two globular domains, GI and GII.
- Independent studies of the two domains found that the GI domain protects linker DNA from degradation by nucleases while the GII domain did not offer protection to the linker DNA.
- The GI domain is structurally more stable than the GII domain and is folded at lower anion concentration while the GII domain is not. The GI and GII domains are however structurally similar.
- Both the GI and the GII domains of the yeast H1 are homologous to the single globular domain of the H1 histone found in other eukaryotes.[2]
- The difference in structural stability between the GI and GII domains of the H1 histone protein in yeast is due to a loop between the helices II and III which is present in the GI domain and absent in the GII domain.[3]

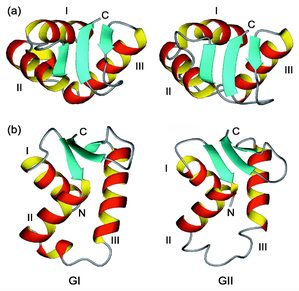

Fig 2: Structural differences between the GI and GII domains of yeast H1.[3]

Yeast two hybrid assay:

Identification of proteins that physically interact with H1 allows us to understand its role in yeast cells. To accomplish this we used the two hybrid assay.

- Screening method for protein binding partners
- Simple and can be high throughput
- Unbiased, sensitive, and based on physical interactions
- Exploits the ability of the downstream transcriptional factor GAL4 to have its DNA binding domain (BD) and activation domain (AD) fold independently and regain function when reconstituted
- The yeast strains used lack the *LEU2, TRP1*, and *ADE2* genes for selection.
- H1 and GII BD were cloned on a pOBD2 plasmid containing *TRP1*, and a library of yeast proteins was fused with the AD on a pOAD plasmid containing *LEU2*.

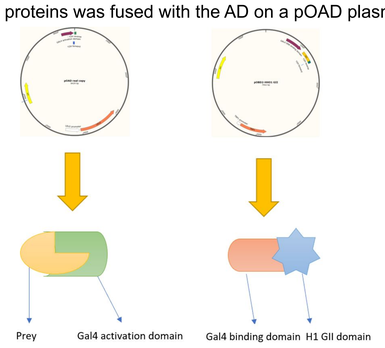

Figure 3: pOAD and pOBD2 plasmids carrying the *LEU2* and *TRP1* genes respectively, and responsible for expressing the AD and BD fusion proteins.

- The reporter gene, *ADE2*, is under the transcriptional control of the GAL4 transcription factor which is only functional when there is a protein interaction.
- The use of mutant strains lacking *ADE2* allows for color selection on low adenine plates due to the red color from the buildup and oxidation of P-ribosylaminoimidazole or AIR in the adenine monophosphate pathway. When the reporter gene *ADE2* is transcribed, the yeast cells can complete the pathway and regain their white color.[4]

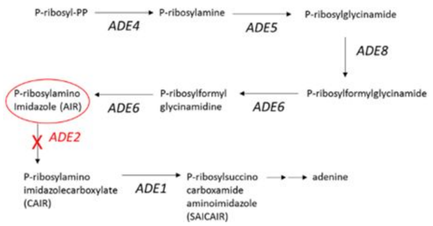

Figure 4: The adenine biosynthesis pathway and the precursor responsible for the red color.[4]

Method

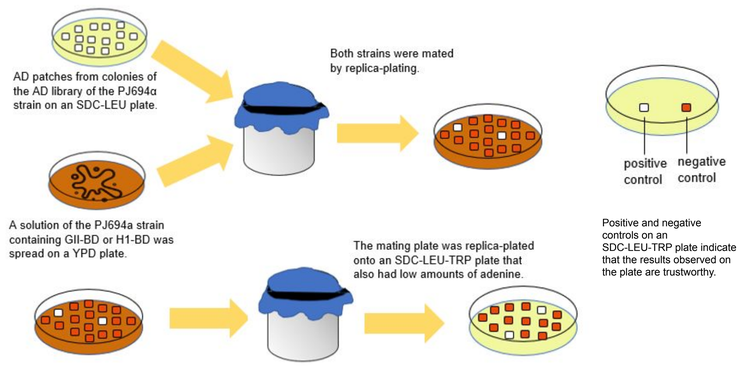

Figure 5: The method used in performing the Yeast Two Hybrid Screening Assay

Results

- A yeast transformation was successful in inserting the GI-BD and the pOBD2 plasmid into the PJ694a yeast strain for future matings with strains containing the GI domain of the H1.

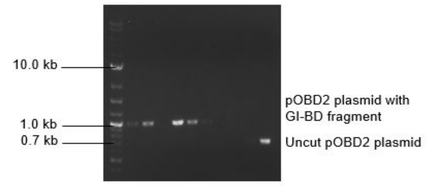

Fig 6: Gel showing the results of the PCR to identify successful transformations.

- White patches on the SDC-LEU-TRP plate indicate possible binding partners for the full length H1 or the GII domain of the H1.
- There are currently 22 white patches in total that were observed on the SDC-LEU-TRP plates after completing a total of 3432 matings.

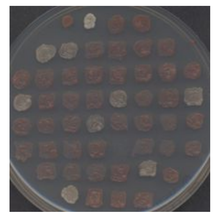

Fig 7: SDC-LEU-TRP plate showing the white patches which suggest an interaction between the GII-BD or H1-BD and the AD of another yeast protein.

Discussion

- Our results showed an acceptable value of 22 potential positives with a percentage of about 0.6%.
- The adenine biosynthetic pathway has multiple steps. Any mutation that knocks out genes that catalyze precursors before *ADE2,* or any mutation that results in a gain of function for *ADE2* will allow for the appearance of white colonies. These mutations possibly account for some of the potential positive cases.
- We have screened 3432 AD-protein fusions for interactions with H1 and GII. However, the probability of screening the same AD-protein fusions more than once necessitates screening more than 6000 AD-protein fusions.

Future Directions

1. Re-patching the candidates on FAA plates then on SDC-LEU plates will be used to test the candidates to determine whether they are true positives.
2. FAA plates select for the loss of the TRP1 gene and therefore, the loss of the pOBD2 plasmid from the mated cells. This would essentially prevent the formation of white patches on SDC-LEU and low adenine plates caused by the activation of the reporter gene, ADE2, because of a physical interaction of the AD and BD.
3. Persistence of the white color indicates a mutation in the adenine biosynthesis pathway but does not guarantee a false positive.
4. True positives will be sequenced to identify the protein binding partners.

References and Acknowledgements

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