Effects of kinetochore depletion in *Candida albicans*

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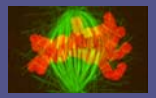
Abstract

Centromere DNA and the numerous associated kinetochore proteins, are found in our cells attaching sister chromatids together and are essential for normal chromosomal segregation. The objective of this project is to characterize kinetochore mutants in *Candida albicans* and determine which kinetochore proteins are essential and which are necessary for chromosome stability. With GRACE (gene replacement and conditional expression) strain analysis using a tet-off promoter to regulate gene expression, we have concluded that the gene products of Dad2, Spc19 and Cse4 are absolutely essential for the growth of *C. albicans*, while depletion of Nuf2 and Skp1 gene products resulted in a growth defect. The growth phenotypes following depletion of other kinetochore proteins are yet to be determined. We quantified cell viability with FUN-1 vital stain and found that after prolonged depletion of Nuf2, Dad2, Skp1, and Cse4 gene products, most, but not all, cells have undergone cell death rather than remaining in a dormant state. As a second method of obtaining a knock-out strain to determine essentiality, we are transforming a Uridine-Arginine-Uridine (UAU) construct, selecting for the integration of this construct into both genome copies, and determining if all surviving cells still contain a wild-type allele resulting from triosomy or a gene duplication event. This phase is currently in progress. We also plan to determine cell size as a measure of polyploidy which could give insight as to how some cells are adapting and escaping cell death. This work will provide a better understanding of how cells react to stresses, such as kinetochore malfunction.

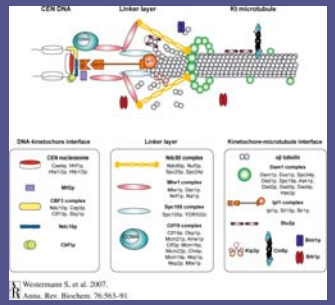
Introduction

•All of our genetic information is contained in chromosomes composed of two sister chromatids connected at the centromere. The centromere and associated kinetochore protiens are essential for cell division.

•The kinetochore is made up of numerous proteins that function to attach spindle fibers to the centromere. This phenomenon happens during the phase of the cell cycle termed metaphase.

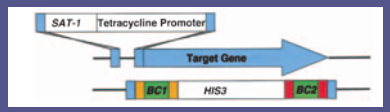


•Many of the proteins comprising the kinetochore have been identified as well as their composition.



•This work focuses on the microtubule attachment proteins in the fungal pathogen *C. albicans*.

Results

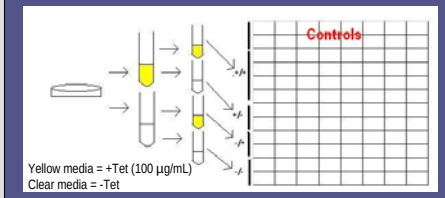


Roemer *et al.*, 2003

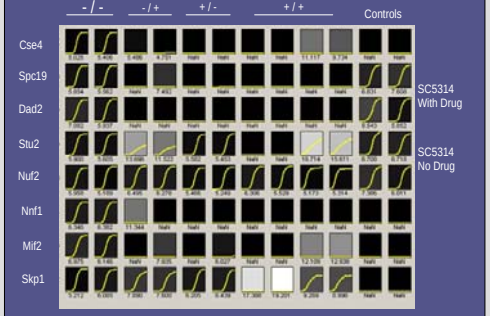
GRACE (gene replacement and conditional expression). In the presence of tetracycline the transactivator is unable to bind to the target gene, therefore repressing its transcription.

We obtained GRACE strains in which a tet-off promoter has been inserted in front of the Cse4, Spc19, Dad2, Stu2, Nuf2, Nnf1, Mif2 and Skp1 genes.

Determination of growth phenotypes



Preparation of 96-well plate for growth curve analysis. Growth of the cells in the well plate was analyzed by OD at 15 minute intervals for 36 hours.



The absence of some kinetochore proteins affects the growth of GRACE strains. *C. albicans* with a non functional Stu2 protein were profoundly affected little growth was observed when in tetracycline. Skp1 suppressed strains experienced a slightly variety of growths presence of tetracycline. Cse4 appears to be essential as the Cse4$^-$ cells did not grow in the presence of tetracycline. The same phenotype is observed in Spc19, Dad2, Nnf1 and Mif2 knockout cells.. The absence of Nuf2 in *C. albicans* appears to have no effect.

Vital Stain

Fun-1 staining allows for determination of live and dead cells. After growth in the presence of tetracycline or in the absence of, cells were isolated from the culture, stained with FUN-1 and observed under a fluorescent microscope.


Live cells

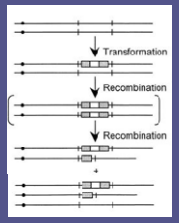

Dead cells

	-/-	+/-	-/+	+/+
SC5314	74/95	79/103	13/140	1/11
Skp1	41/49	12/25	ND	ND
Nuf2	199/211	183/120	23/214	ND
Dad2	34/37	---	ND	---
Spc19	145/203	---	40/103	ND

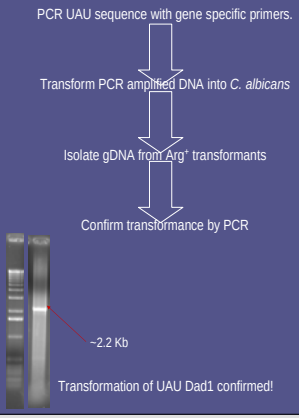
Fraction of live cells/total cells ND = no live cells detected --- = no cells detected

The remaining strains (Spc19, Stu2, Nnf1, and Mif2) have not been analyzed further with the vital stain.

UAU



Generation of DASH knock-outs to confirm gene essentiality. By the insertion of a uridine-arginine-uridine (UAU) contruct into one loci of the target gene, recombination knocking out gene function at the other loci leads to complete loss of protein function. If the gene is essential, all Arg$^+$Ura$^+$ cells will have a third, wild-type copy remaining.



Conclusions

•Dad2 is essential for growth and is inviable by FUN-1 staining.

•Stu2 is essential for growth.

•Skp1 and Nuf2 do not appear essential for growth, although in the absence of their function hindered growth is observed.

•Wild-type (SC5314) experienced 22.1-90.9% death in the presence of tetracycline measured by FUN-1 staining. This is unexpected because wild type does not contain a tet-off promoter for any genes indicating all genes should be fully functional. This could be due too high a concentration of tetracyline for *C. ablicans* to survive in.

•UAU constructs specific to genes Dad1 and Dad2 have been confirmed to be transformed into *C. ablicans* SN76.

Future Directions

•The growth curve experiment will have to be repeated to determine why wild type is not growing in the tetracycline. The experiment will be done with varying concentrations of tetracycline in the culture.

•Analysis of cell size in the vital stain pictures will be done to determine if a change in cell size has occurred under the suppression of certain genes. A change in cell size can indicate polyploidy.

•The UAU transformed strains will be check for recombination events that knock out the second loci of the gene by their selection on SCD arg$^-$ ura$^-$ plates.

•After transformed and recombined strains are obtained we will look for the presence of the wild type allele. If the later exists along with Arg$^+$Ura$^+$ isolated cells we will have futrher evidence the DASH proteins are essential.

Acknowledgements

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Works Cited

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