Role of the ribosomal DNA repeats on chromosome segregation of *Saccharomyces cerevisiae*

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ABSTRACT

Chromosome segregation is a highly conserved process that progresses with great accuracy. Failure of proper segregation can lead to genetic disorders, such as Down syndrome in humans. Interestingly, segregation errors found in human genetic disorders and associated with spontaneous abortions or stillbirths are frequent in the chromosomes containing the ribosomal RNA gene repeats (rDNA). The rDNA is essential for cell viability and growth as it encodes ribosomal RNA, a major component of ribosomes. In yeast, the rDNA locus has a unique cohesin-independent cohesion mechanism to hold sister chromatids together before separation, and behaves in unique ways with respect to replication, recombination and transcription. These rDNA-specific features may promote a chromosome segregation mechanism distinct from the rest of the genome. Therefore, the aim of this thesis was to test the hypothesis that the rDNA affects chromosome segregation.

To test this hypothesis I focused on mitotic chromosome segregation, and used the model genetic organism, *Saccharomyces cerevisiae*. *S. cerevisiae* offers many advantages for testing this hypothesis, including its tolerance to aneuploidy and systems that have been developed to genetically manipulate the rDNA. I developed and optimized a chromosome loss assay (CLA) that measures the rate of chromosome loss during mitosis in *S. cerevisiae*. I modified a number of strains that had alterations associated with the rDNA, including strains deleted for the chromosomal rDNA repeats, with a reduction in rDNA copies, and with the rDNA translocated to a different chromosome, with specific phenotypic markers for detection of chromosome loss events. I then tested the chromosome loss rates of these strains using the CLA. My results demonstrate that the rDNA affects mitotic chromosome segregation fidelity at two levels. First, the rDNA increases the segregation fidelity of the rDNA-containing chromosome, defining a local chromosome segregation role for the rDNA. I found that this local effect is mediated by the rDNA binding protein Fob1, and I propose three potential mechanisms for how Fob1 mediates this role: (1) through establishment of rDNA recombination-intermediates that may help to stabilize the long rDNA locus; (2) through recruitment of condensin to establish intra-chromatid linkages that promote timely condensation of sister chromatids; or (3) through recruitment of a silencing complex to achieve an appropriate rDNA chromatin state for chromosome segregation. Second, I show that the rDNA has

a global effect on chromosome segregation fidelity, with rDNA deletion or reduction in rDNA copies influencing the segregation of many or all chromosomes. Curiously, heterozygosity of rDNA state, regardless of what states are present, confers wild type missegregation rates. I rule out trivial explanations for this global effect, and instead propose that the rDNA affects segregation through changes in nucleolar structure and overall nuclear organization that impact spindle polarity and thus the fidelity of chromosome segregation. Together, these results define a new role for the rDNA in facilitating chromosome segregation, and one that acts at two different levels. This work provides insights into a novel beneficial role of the rDNA in chromosome segregation of *S. cerevisiae,* and the conserved mechanism of chromosome segregation across eukaryotes suggests the rDNA may play similar roles in more complex organisms. It will be interesting to determine if the rDNA also has beneficial role in meiosis, where the rDNA has been associated with missegregation.

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Finally I'd like to end with a short quote that I feel summarizes well my PhD: **"Success is the sum of small efforts, repeated day in and day out" -Robert Collier**

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LIST OF ABBREVIATIONS

5FOA: 5-Fluoroorotic Acid

APC: anaphase promoting complex

CAR: cohesin association region

Chr: chromosome

CEN: centromere

CF: core factor

CHEF: contour-clamped homogeneous electrophoresis

CIN: chromosome instability

CLA: chromosome loss assay

Cp: Cross point cycle

DF: dense fibrillar component

EDTA: ethylenediaminetetraacetic acid

FC: fibrillar center

fdr: false discovery rate (p-value adjustment)

G418: geneticin, an aminoglycoside antibiotic

GC: granular component

gDNA: genomic DNA

GFP: green fluorescent protein

HP: helper plasmid

IGS: intergenic spacer

INMS: Institute of Natural and Mathematical Sciences (Massey University)

KU: CLA tags *kanMX2* and *URA3*

lacI-GFP: *lac* operator/ *lac* repressor system coupled to GFP

lacO: *lac* operator

MCS: multiple cloning site

nm: nanometer (wavelength unit for OD measurements)

NOR: nucleolar organizer region

OD: optical density

O/N: overnight

pGAL-*FOB1*: galactose inducible *FOB1* plasmid

PIC: pre-initiation complex

Pol I: RNA Polymerase I

Pol II: RNA Polymerase II

Pol III: RNA Polymerase III

rARS: rDNA origin of replication

RDN1: ribosomal DNA gene

rDNA: ribosomal RNA gene repeats

RENT complex: regulator of nucleolar silencing and telophase complex

RFB: replication fork block

rRNA: ribosomal RNA

RT: room temperature

SD: synthetic dextrose medium

SGal: synthetic galactose medium

SM: starvation medium

SMC: structural maintenance of chromosome

Sp*ADE6*: *ADE6* gene from *S. pombe*

SPB: spindle pole body

t_0: CLA time 0

t_1: CLA time 1

UAF: upstream activation factor

WT: wild type

YPD: yeast peptone dextrose

YPGal: yeast peptone galactose

YPGly: yeast peptone glycerol