Unravelling the genomic structure of *Saccharomyces cerevisiae*

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Abstract

Advances in high-throughput sequencing technology have enabled the sequencing of genomes for many organisms. But the ability to describe the linear arrangement of genetic elements on the chromosomes does not tell us much about how all of these elements work in concert to form and maintain a functional cell. To get some way towards a more holistic understanding of how this is achieved requires the elucidation of three-dimensional genome organisation.

The association of chromosomes with each other and other nuclear components plays a critical role in nuclear organisation and genome function. Interactions, which can be structural or functional in nature, form between different parts of the genome. Chromosomal interactions can be broadly divided into two groups, inter- and intra-chromosomal interactions, depending upon whether the interaction forms between different chromosomes or within a single chromosome, respectively.

Here I describe a methodology capable of capturing these interactions on a global scale, Genome Conformation Capture (GCC), and reveal the interaction network for *Saccharomyces cerevisiae.* The inter- and intra- chromosomal interactions detected by GCC are non-random and include contacts between the nuclear chromosomes, 2-micron plasmid, and the mitochondrial genome. These results formed the first global map of chromosomal interactions in a eukaryotic nucleus and demonstrated the highly connected nature of the yeast genome.

I subsequently performed GCC on *S. cerevisiae* cells grown on glucose, galactose, and glycerol lactate to investigate how genome organisation alters depending upon the metabolic regime being employed. I describe the difference in the numbers and types of interactions that form in the three conditions and investigate interactions involving transfer RNAs in detail. Interactions between the mitochondrial and nuclear genomes undergo significant changes depending upon the carbon source on which the yeast is grown.

The nuclear and mitochondrial organelles must maintain a communication system in order to respond effectively to environmental conditions. Previous studies have identified mitochondrial DNA inside the nucleus and interacting with the nuclear chromosomes. How this transfer occurs and what the function of the mitochondrial

DNA is once inside the nucleus remains unclear. Here I isolate interactions between the mitochondrial and nuclear genomes and demonstrate dependence upon mitochondrial encoded reverse transcriptase machinery. Furthermore, the nuclear gene transcript level is altered when the interaction frequency between the mitochondrial and nuclear genome is reduced. I conclude that mitochondrial DNA interactions with the nuclear genome are biologically relevant and that the results argue for a role for reverse transcription in inter-organelle DNA mediated communication.

The results presented in this thesis have significant implications for our understanding of eukaryotic genome organization.

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Abbreviations

amp	Ampicillin
bp	Base Pairs
Da	Daltons
°C	Degrees Celsius
df	Degrees of freedom
FPR	False positive rate
g	G-Force
hr	Hour
hrs	Hours
kb	Kilobases
µg	Micrgrams
µl	Microlitres
ml	Millilitres
mmol	Millimole
mQ	MilliQ
min	Minute
nm	Nanometres
NTC	No Template Control
O/D	Optical Density
O/N	Overnight
PCR	Polymerase Chain Reaction
rpm	Revolutions per Minute
rm	Room
SDS	Sodium dodecyl sulphate
temp	Temperature
U	Units
V	Volts
v/v	Volume per Volume
H_2O	Water
w/v	Weight per Volume
mtDNA	Mitochondrial DNA
Mito-gDNA	Mitochondrial genome to nuclear genome interaction
Mito-Plas	Mitochondrial genome to 2-micron plasmid interaction
gDNA-gDNA	Interaction between loci within the nuclear genome