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GENOMES IN SPACE AND TIME:

INSIGHTS INTO THE FUNCTIONAL THREE-DIMENSIONAL ORGANIZATION OF PROKARYOTIC AND EUKARYOTIC GENOMES IN RESPONSE TO ENVIRONMENTAL STIMULI AND CELL CYCLE PROGRESSION

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ABSTRACT

The specific three-dimensional organization of prokaryotic and eukaryotic genomes and its contribution to cellular functions is increasingly being recognized as critical.

Bacterial chromosomes are highly condensed into a structure called the nucleoid. Despite the high degree of compaction in the nucleoid, the genome remains accessible to essential biological processes such as replication and transcription. Here I present the first high-resolution Chromosome Conformation Capture based molecular analysis of the spatial organization of the *Escherichia coli* nucleoid during rapid growth in rich medium and following an induced amino-acid starvation that promotes the stringent response. My analyses identified the presence of origin and terminus domains in exponentially growing cells. Moreover, I observe an increased number of interactions within the origin domain and significant clustering of SeqA binding sequences, suggesting a role for SeqA in clustering of newly replicated chromosomes. By contrast, “Histone-like” protein (*i.e.* Fis, IHF, H-NS) binding sites did not cluster suggesting that their role in global nucleoid organization does not manifest through the mediation of chromosomal contacts. Finally, genes that were down-regulated after induction of the stringent response were spatially clustered indicating that transcription in *E. coli* occurs at transcription foci.

The successful progression of a cell through the cell cycle requires the temporal regulation of gene expression, the number and condensation levels of chromosomes and numerous other processes. Despite this, detailed investigations into how the genome structure changes through the cell cycle and how these changes correlate with functional changes have yet to be performed. Here I present the results of a high resolution study in which we used synchronized Fission yeast (*Schizosaccharomyces pombe*) cells to investigate changes in genome organization and transcription patterns during the cell cycle. The small size of the Fission yeast genome makes this organism particularly amenable to studies of the spatial organization of its chromosomes. I detected cell cycle dependent changes in connections within and between chromosomes. My results show that chromosomes are effectively circular throughout the cell cycle and that they remain connected even during the M phase, in part by the co-localization of

repeat elements. Furthermore, I identified the formation and disruption of chromosomal interactions with specific groups of genes in a cell cycle dependent manner, linking genome organization and cell cycle stage specific transcription patterns. Determining the structure and transcript levels for matched synchronized cells revealed: 1) that telomeres of the same chromosome co-localization throughout the cell cycle, effectively circularizing the chromosomes; 2) that genes with high transcript levels are highly connected with other genomic loci and highly expressed genes at specific stages of the cell cycle; 3) that interactions have positive and negative effects on transcript levels depending on the gene in question; and 4) that metaphase chromosomes assume a 'polymer melt' like structure and remain interconnected with each other. I hypothesize that the observed correlations between transcript levels and the formation and disruption of cell cycle specific chromosomal interactions, implicate genome organization in epigenetic inheritance and bookmarking.

Over the course of mitochondrial evolution, the majority of genes required for its function have been transferred and integrated into nuclear chromosomes of eukaryotic cells. The ongoing transfer of mitochondrial DNA to the nucleus has been detected, but its functional significance has not been fully elucidated. To determine whether the recently detected interactions between the mitochondrial and nuclear genomes (mt-nDNA interactions) in *S. cerevisiae* are part of a DNA-based communication system I investigated how the reduction in interaction frequency of two mt-nDNA interactions (COX1-MSY1 and Q0182-RSM7) affected the transcript level of the nuclear genes (MSY1 and RSM7). I found that the reduction in interaction frequency correlated with increases in MSY1 and RSM7 transcript levels. To further investigate whether mt-nDNA interaction could be detected in other organisms and characterize their possible functional roles, I performed Genome Conformation Capture (GCC) on Fission yeast cell cycle synchronized in the G1, G2 and M phases of the cell cycle. I detected mt-nDNA interactions that vary in strength and number between the G1, G2 and M phases of the Fission yeast cell cycle. Mt-nDNA interactions formed during metaphase were associated with nuclear genes required for the regulation of cell growth and energy availability. Furthermore, mt-nDNA interactions formed during the G1 phase involved high efficiency, early firing replicating origins of DNA replication. Collectively, these results implicate the ongoing transfer of regions of the mitochondrial genome to the nucleus in the regulation of nuclear gene transcription and cell cycle progression following exit from metaphase. I propose that these

interactions represent an inter-organelle DNA-mediated communication mechanism.

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TABLE OF CONTENT

FIGURES

TABLES

ABBREVIATIONS

h	hour
min	minute
s	second
mmol	millimole
temp	temperature
Rm	room
O/N	overnight
OD	optical density
nm	nano-meter
MQ	milliQ
H_2O	water
µl	microliters
rpm	revolutions per minute
ºC	degrees celsius
µg	micrograms
U	units
v/v	volume per volume
w/v	weight per volume
ml	milliliters
g	g-force
Amp	ampicillin
PCR	polymerase chain reaction
V	volts
bp	base pair
Kb	kilo-base pair
Mb	mega-base pair
NTC	no template control
EM	electron microscopy
µm	micro-meter
GCC	genome conformation capture
LTR	long terminal repeat
DNA	Deoxyribonucleic Acid
mtDNA	mitochondrial DNA
nDNA	nuclear DNA
mt-nDNA	mitochondrial – nuclear DNA interactions

3C	chromosome conformation capture
4C	circular chromosome conformation capture
5C	chromosome conformation capture carbon copy
6C	combined 3C-ChIP-cloning
ChIA-PET	chromatin interaction analysis with paired-end tag sequencing
G1 phase	growth one phase of the cell cycle
S phase	DNA synthesis phase of the cell cycle
G2 phase	growth two phase of the cell cycle
M phase	metaphase of the cell cycle
PE	paired-end