Does DNA topography coordinate intra- and inter-chromosomal Galactose (*GAL*) gene expression?

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Abstract

For a long time, DNA had been considered as a stabilized, rigid, and “linear” structure, which acts as a platform for molecular regulators to function. However, genome structure in living cells is far more complex than the linear representation of the primary DNA sequence implies. This thesis aims to investigate whether the position of a gene within the genome plays a role in the regulation of its activity. The galactose (*GAL*) gene family of *Saccharomyces Cerevisiae* is used as a model. This gene family enables yeast cells to utilize galactose as an alternative carbon source; and it consists of structural and regulatory genes. Structural genes *GAL1*, *GAL10* and *GAL7* exist in a cluster on yeast chromosome II. The products of the regulatory genes, *GAL3*, *GAL4*, and *GAL80*, regulate the expression of the *GAL* structural genes, depending on the availability of carbon sources. Specifically, *GAL* gene expression is repressed by glucose, paused for induction (noninduced) by glycerol/lactate, and fully induced by galactose.

The aim of this project was to study the relative position of the *GAL* structural genes within the nucleus, and whether any chromosomal interactions at the *GAL* locus help to regulate their activation. These were tested in accordance with the expression status of the *GAL* genes *(i.e.* repressed, noninduced or induced). Followed confirmation of the existence of any chromosomal interactions, protein/protein complexes that mediate these interactions were attempted to identify.

The methods applied in this project were Chromosome Conformation Capture (3C) and Circular Chromosome Conformation Capture (4C), which applied in combination to map the positions of the GAL genes in the context of the overall genome structure. The results indicated that the GAL locus on chromosome II was divided into two “interaction zones”. DNA loops formed around these interaction zones to form an S-shape structure in a carbon source-independent manner. Two novel inter-chromosomal interactions between chromosomes II and XVI, *i.e. SVL3-GAL7* and *HOS1-GAL10*, were also identified. Although these interactions occurred regardless of the *GAL* gene activities, it was suggested by real-time PCR that the interaction frequency for *SVL3-GAL7* declined as the *GAL* genes being activated. Unfortunately no protein/protein complexes were identified to play an important role in mediating either intra- or inter-chromosomal interactions.

Future work will be needed to identify the protein/protein complexes that play a role in mediating the S-Shape structure at the *GAL* locus and the two inter-chromosomal interactions. Additional works could also focus on the understanding of the functional implication of the interactions between chromosomes II and XVI.

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Abbreviations

In addition to the chemical symbols shown in the Periodic Table of Elements and the *Systeme international d'unites* (SI), the following abbreviations are used:

SC	Synthetic complete
DNA	Deoxyribonucleic acid
DTT	Dithiothreitol
DMSO	Dimethyl sulfoxide
EDTA	Ethylenediaminetetraacetic ccid
PCR	Polymerase chain reaction
PBS	Phosphate buffered saline
PMSF	Phenylmethanesulfonyl flouride
SDS	Sodium dodecyl sulfate
SOC	Super optimal catobolite
BSA	Bovine serum albumin
TE	Tris EDTA
TBE	Tris borate EDTA.
TEMED	Tetramethylethylenediamine
LPA	Linear acrylamide
H	Histidine
Kb	Kilobase
w/v	Weight/volume
v/v	Volume/volume
RT	Room temperature
O/N	Overnight
Min	Minute
S	Second
U	Unit
3C	Chromosome Conformation Capture
4C	Circular Chromosome Conformation Capture
CHO	Carbohydrate
C	Cut/restriction enzyme digested sample
CL	Cut/restriction enzyme digested and ligated sample

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