Poster Presentation
Quantitative BioImaging Conference
06 – 09 January 2020
University of Oxford, Oxford, UK

Relating the 3D organisation of chromatin to transcription and metabolism
Combining quantitative experiments with predictive theoretical models

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Keywords: Yeast Metabolic Cycle, Chromatin Architecture, Fluorescence In Situ Hybridization

The Yeast Metabolic cycle (YMC) is a model system of temporal compartmentalization of cellular and metabolic processes. After starvation and continuous glucose feeding, *Saccharomyces cerevisiae* undergo synchronized glycolytic and respiratory oscillations, commencing the YMC. The 300 minute cycle can be divided into two phases characterized by the level of oxygen consumption; low vs high. Additionally, during the cycle, oscillating transcription and transcript levels of genes associated with particular pathways can be clustered. Although widely studied, little is known about chromatin structure dynamics and its correlation with gene expression upon metabolic changes within this model system. Furthermore, most experiments aiming to understand how synchronicity is achieved during the YMC have been performed on whole cell populations. Consequently, there is a poor understanding of what is happening at the single cell level in the YMC.
To this end, we will perform RNA Fluorescence In Situ Hybridization (RNA-FISH) on antagonistically oscillating transcripts in cells sampled from a synchronous cell population i.e. undergoing the YMC. Using the results we aim to answer the questions: Does synchronous behavior allow heterogeneity in cell populations? Are antagonistically oscillating transcripts strictly exclusive?
A second objective is to understand if/how long-range chromatin contacts change during the metabolic cycle and how it relates to gene expression. As localization of the chromatin plays an important role in its compaction, we will inspect the spatial localization of cycling transcripts and their respective DNA coding regions, scoring specific DNA loci positions relative to the nuclear periphery. To do so we will use different microscopic techniques such as DNA-FISH and IF.
Finally, we aim to derive a stochastic mathematical model describing relationships and determining cause and effect between higher order chromatin structure and cell phenotype. By doing so we hope to apply what we discover in yeast to mammalian cells and apply our discoveries to problems such as ageing or disease.

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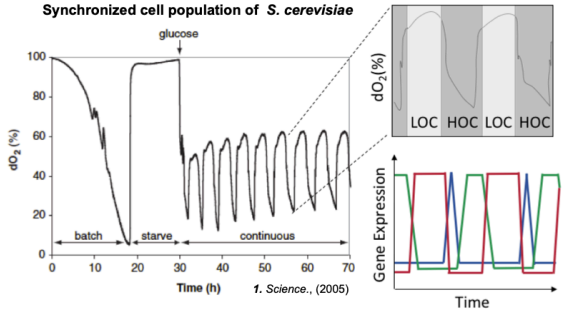
1. The Yeast Metabolic Cycle

The YMC: a temporally compartmentalized synchronized system

Synchronized cell population of *S. cerevisiae*



1. Science., (2005)

Low Oxygen Consumption
Low energy charge
Storage accumulation

High Oxygen Consumption
High energy charge
Nutrient consumption

Under oscillation
Key metabolites
Ions
Gene expression
Biochemical oscillators
Histone modifications

2. Measuring the rate of ribosomal RNA transcription during the YMC via RNA FISH

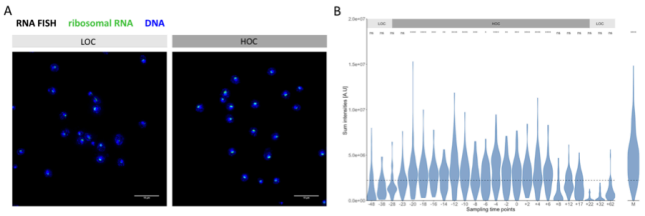
A multi-omics analysis of the YMC

Transcriptional changes are regulated by metabolic pathway dynamics but decoupled from protein levels[2].

Metabolites and PTMs regulate protein activity

- Enrichment of Phospho-Rps6p, a ribosomal protein also signaling ribosome assembly, in HOC-phase

=> Cycling of rDNA transcription and thus ribosome biogenesis



Ribosome assembly varies throughout the YMC

A. Representative RNA FISH images of samples taken during the LOC vs HOC of the YMC. Fluorescent labeling; DNA: DAPI, transcribed spacers regions of the 35S ribosomal RNA probed with DNA probes labelled with Quasar 570 in green. **B.** Violin plots showing the summed intensity of all rRNA foci pixels contained in the nucleus of each cell acquired via RNA FISH. rRNA production levels peak in the HOC of the cycle. Most cells express rRNA at the peak.

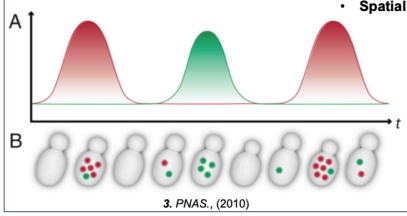
Ribosomes assembly is coordinated during the YMC.

3. RNA FISH on antagonistically oscillating transcripts

Does synchronous behaviour allow heterogeneity in cell population?

Oscillations of antagonist transcripts levels in single cell from asynchronous population. The concept of intrinsic clock[3].

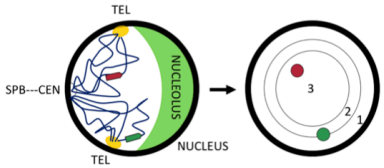
- **Single cell level**
- **Spatial localization**



3. PNAS., (2010)

4. Spatial localization of DNA loci

Inert sub-telomeric genes expressed under specific nutrient conditions



"YMC GENES"
Antagonistically expressed

Scoring position relative to nuclear periphery

Adapted from *4. CSH Perspect Biol.*, (2010). *5. Nature.*, (2006).

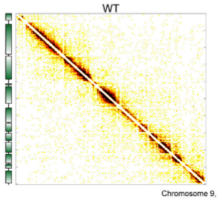
5. Mapping chromosome interactions

Identify Metabolic-state-specific CIDs

- Microarray
- 3C methods

Boundaries and TF

- ChIP-Seq



6. Cell. (2015).

Information

Understanding

6. Mathematical modelling

Prediction

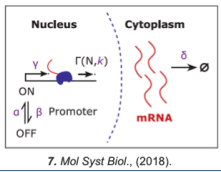
- Change in metabolism e.g. feeding rate

-> Chromatin architecture outcome?

- Chromosome folding at "YMC genes"
- Yeast -> Mammalian cells
- Quiescent cells

Fit our stochastic model of transcription to the data

- Extract unknown parameters



7. Mol Syst Biol., (2018).

7. How do long range chromatin contacts change during metabolic cycling?

Underlying mechanisms?
Metabolism and chromatin regulation impact on gene expression?
Understand biological oscillations
Apply discoveries

References: 1. Tu et al., *Science.*, (2005). 2. Feltham et al., *bioRxiv.*, (2019). 3. Silverman et al, *PNAS.*, (2010). 4. Taddei et al., *Cold Spring Harb Perspect Biol.*, (2010). 5. Taddei, et al., *Nature.*, (2006). 6. Hsieh et al., *Cell.* (2015). 7. Brown et al., *Mol Syst Biol.*, (2018).

This project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under the Marie Skłodowska-Curie Grant Agreement PEP-NET