The Yeast Metabolic Cycle: heterogeneous gene expression within synchronized cultures

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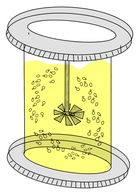
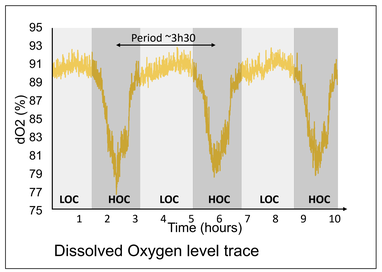
1. The Yeast Metabolic Cycle (YMC) A temporally compartmentalized system

Chemostat culture steps

Batch culture
Starvation > 6h
Continuous feeding in limiting [Glucose]



Synchronized cell population of prototrophic strains of *Saccharomyces cerevisiae*



Dissolved Oxygen level trace

Low **O**xygen **C**onsumption
Low energy charge
Storage accumulation

High **O**xygen **C**onsumption
High energy charge
Nutrient consumption

Features showing oscillating behaviour in the YMC

- Metabolites (e.g. Acetyl-CoA)
- Ions and osmolytes (e.g. H+ export and pH, K+)
- Peroxiredoxin oxidation/reduction
- Protein modifications (e.g. Phosphorylation, solid vs liquid biomolecular condensates)
- Histone modifications (e.g. Acetylation of H3 and H4 lysines)
- Transcription and transcript levels

Up to 60% of transcripts cycling at the mRNA level[1]

- Translation
- However, protein levels are constant

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

- Glycolytic and respiratory phenotype

"Growth vs Quiescence"

Modified from Feltham et al., *bioRxiv.*, (2019).[2]

2. Research Objective

How do patterns of gene expression at the individual cell level in a synchronous cell population vary over time?

Expectations:
Individual cells show coordinated increase in transcript levels to match the population

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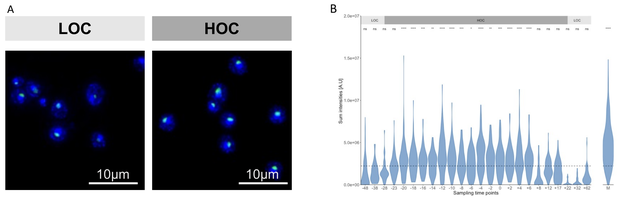
3. Testing the hypothesis

A. Measuring the rate of pre-ribosomal RNA production during the YMC via RNA FISH

- Rps6 phosphorylation peaks in the HOC phase of the YMC
- Rps6 phosphorylation reflects assembly of ribosomes

Is the production of pre-rRNA coordinated with ribosome assembly in the HOC phase of the YMC?



A. Representative RNA FISH images of samples taken during the LOC vs HOC of the YMC. Transcribed spacer regions of the 35S ribosomal RNA probed with DNA probes labelled in green. Images cropped and enlarged. DAPI: DNA, Quasar 570: pre-ribosomal RNA.
B. Violin plots showing the summed intensity of all rRNA foci pixels contained in the nucleus of each cell acquired via RNA FISH[2].

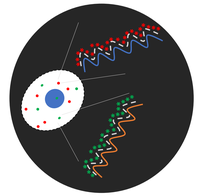
Pre-rRNA levels peak in the HOC of the cycle
Most cells express rRNA at the peak[2]

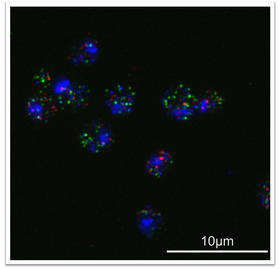
Variation in pre-rRNA transcript levels in single cells within YMC populations, supports cell-to-cell gene expression heterogeneity

B. Using multiplex smRNA-FISH to explore the distribution of antagonistically oscillating transcripts[3] in individual cells from a cycling population of cells at different stages of the YMC[4]

Workflow[5]

Enzymatic synthesis of smFISH probes
Knock-Out strains production
Optimizations: Spheroplasting,
Probe concentration
Growth/Sampling
Fixation
Spheroplasting
Attachment on coverslips
Membrane permeabilisation
Hybridization
Nucleus staining
Mounting
Imaging
Deconvolution,
Cells and Nuclei detection,
Thresholding, Quantification;
Semi-Automated Pipeline[5]
Downstream analysis





YMC sample, image cropped and enlarged
DAPI: DNA, Atto633: SHM2, Atto565: IDH1

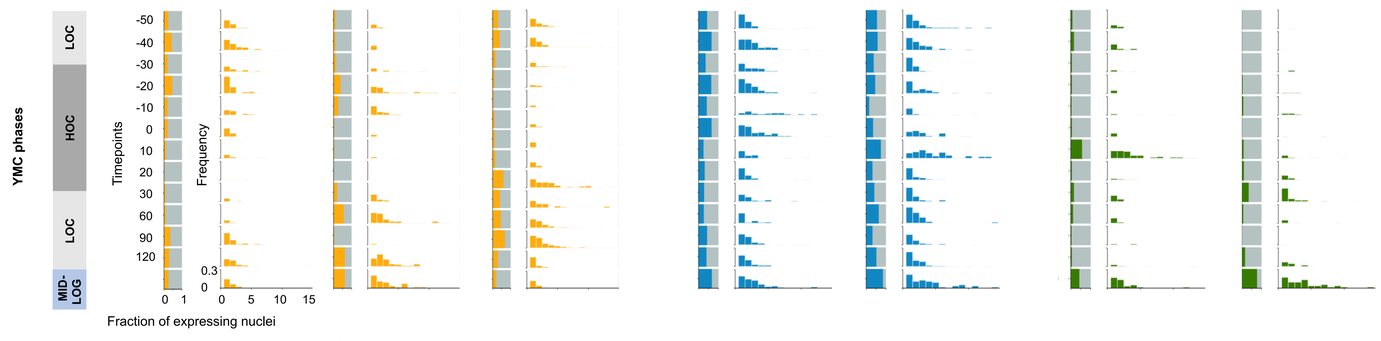
Experiments n=4,
~2000 cells per experiment,
~150 cells per timepoint

Global changes in transcript levels along the YMC correspond to oscillations in transcription and transcript levels within cells, combined with a fluctuation in the number of cells within the population expressing these particular transcripts at variable levels

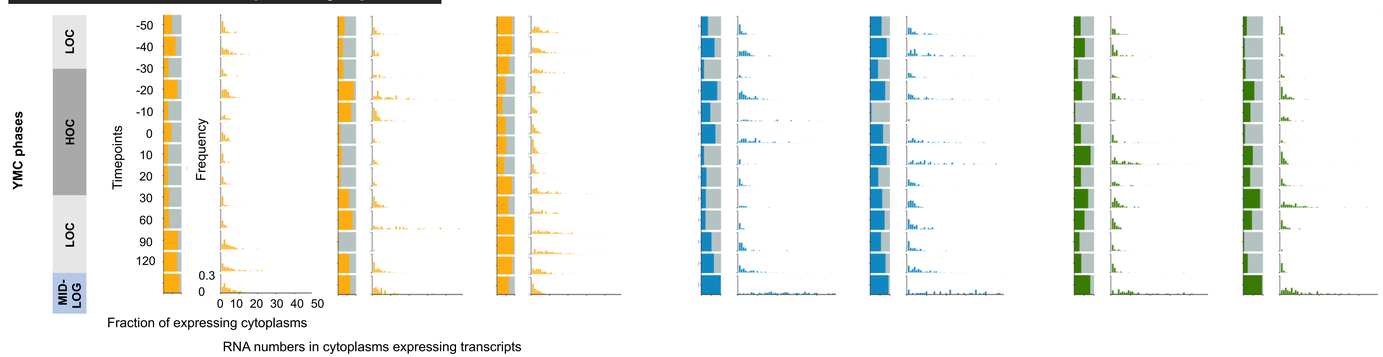
Target transcripts

ATO3 n=3 | **SHM2** n=2 | **IDH1** n=2

Distributions of transcripts in nuclei

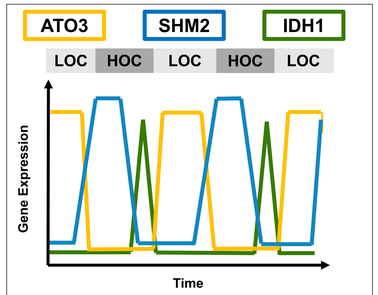


Distributions of transcripts in cytoplasms

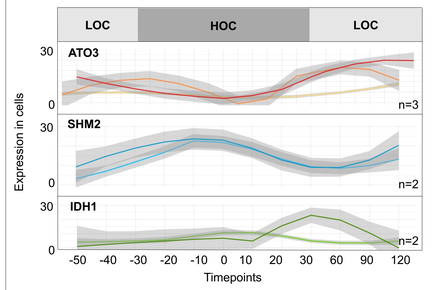


Overall transcript levels cycle

Similar trends in mean transcript levels compare to measures formerly acquired by microarray and sequencing studies



Schematic of the cycling transcript profiles of 3 genes in the synchronised population of cells[3]



Smoothed conditional means of RNA numbers in expressing cells

4. Conclusions

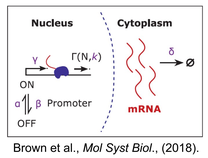
The presence of non-expressing cells within the YMC population supports a model where transcription itself is not heavily involved in the control of the Yeast Metabolic Cycle

Individual cells show a high degree of variability in levels of expression of individual mRNA transcripts over time in the YMC yet together as a population the synchronous behaviour emerges.

5. Future directions

Combining quantitative experiments with stochastic mathematical modelling of transcription to identify transcript and transcription underlying dynamics

- Based on existing models of transcription
- Fit the data acquired throughout the YMC
- Stochastic simulations of RNA distributions
- Identify key parameters



Brown et al., *Mol Syst Biol.*, (2018).

References: 1. Tu et al., *PNAS.*, (2007). 2. Feltham et al., *bioRxiv.*, (2019). 3. Tu et al., *Science.*, (2005). 3. 4. Silverman et al, *PNAS.*, (2010). 5. Brown et al., *Mol Syst Biol.*, (2018).