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Systems approaches for the study of metabolic cycles in yeast

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Abstract

Over four decades ago, the first oscillations in metabolism in yeast cells were reported. Since then, multiple forms of oscillatory behavior have been observed in yeast grown under a variety of continuous culturing environments. The remarkable synchrony of cells undergoing such oscillations has made them ideal subjects for investigation using systems-based approaches. Herein, we briefly summarize previous work on the characterization of such oscillations using systems approaches, and present the long-period, Yeast Metabolic Cycle as an excellent model system for deciphering the temporal organization of fundamental cellular and metabolic processes at unprecedented resolution.

Ultradian oscillations in yeast

In the 1960s, rapid periodic fluctuations in reduced pyridine nucleotide concentrations were among the earliest oscillations in metabolism observed in yeast cells [1,2]. Subsequently, numerous groups have studied yeast under continuous, steady-state growth conditions at high cellular density and limiting glucose levels using chemostats. Under these conditions, budding yeast could be observed to undergo robust oscillations as measured by oxygen utilization. The period of such oscillations ranged from as short as ~40 min to over 10 h, depending on the strain and culturing conditions [3–5,6*,7*,8*,9]. In short, such oscillations depict the metabolic behavior of a yeast cell population under these continuous growth environments. The oscillation period of these cultures was often highly sensitive to the chemostat dilution rate [4,5], which is the proportion of media in the culturing vessel that is replaced per hour. With a given medium composition, higher dilution rates generally reduce the period of oscillations, while lower dilution rates increase the period of oscillations. The synchronous behavior of these cycling cell populations has revealed that a variety of metabolic parameters also oscillate, though not necessarily in phase with the dissolved oxygen utilization. The emergence of key technologies in the past decade, such as genome-wide expression profiling and global metabolite profiling methods, has enabled investigations into the temporal changes in transcription, metabolism, and other cellular outputs that occur as a function of these robust cycles of oxygen consumption. These studies have started to reveal an underlying logic in such oscillatory behavior in yeast that may prove to be very useful for the investigation of metabolism and numerous fundamental biological processes.

Genome-wide periodic gene expression and regulation

Beginning in the 1990s, Kuriyama, Klevecz, Murray and colleagues pioneered the study of short-period, 40-min oscillations observed during continuous culture of an industrial

fermentation strain of *S. cerevisiae* [6[•],10,11]. By sampling populations of cycling cells at frequent intervals, low-amplitude, genome-wide fluctuations in transcription and numerous metabolic parameters were detected during these short-period oscillations [7^{••},11]. Periodic changes in gene expression were subsequently observed during the longer-period, 4–5 h oscillations [8^{••}]. Importantly, both the short-period (~40 min) and long-period (~4–5 h) cycles revealed that the majority of yeast genes appeared to be cyclically regulated as a function of the oscillations in oxygen consumption [7^{••},8^{••}]. However, there was little correlation between the two datasets with respect to the phases during which particular classes of transcripts peaked [12]. This suggested that the long-period and short-period cycles are quite different, at least by the criteria of periodic transcription and gene expression.

The short-period cycles suggested that the temporal separation between the oxidative (oxygen-consuming) and reductive phases is propagated through the yeast transcriptome [7^{••}]. The temporal segregation of biological processes was more apparent in the long-period cycles, where over half the yeast genome showed high-amplitude, periodic expression, with different genes being expressed at their highest levels at completely different times during these oscillations [8^{••},12]. Furthermore, the genes that were highly overrepresented in the set of periodic genes were predominantly involved in metabolism and protein synthesis, with gene products that localize to the mitochondria also significantly overrepresented [8^{••}]. These gene expression studies from the long-period cycles (hereon referred to as the Yeast Metabolic Cycle, or YMC) also suggested why the genes that peak in the oxygen-consuming phase (ribosomal proteins, translation initiation factors, genes involved in amino acid biosynthesis, etc.) may be significantly upregulated in this phase: these processes are energetically demanding, and their expression correlates perfectly with a burst of mitochondrial oxidative phosphorylation [8^{••}].

The data from these studies suggested an overall logic underlying the long-period YMC, where cellular processes are not just separated by subcellular spatial compartmentalization of metabolic enzymes, but are also tightly regulated in time [8^{••},12]. The oscillating transcripts of the YMC fall within three distinct, temporally separated phases organized about the cycles of oxygen consumption [8^{••}]. These were designated as the Oxidative phase (OX), the Reductive-Building phase (RB), and the Reductive-Charging phase (RC) [8^{••}]. In the OX phase, cells rapidly consume molecular oxygen in the form of a burst of mitochondrial respiration. This phase is characterized by the sudden transcriptional upregulation of genes involved in ribosomal biogenesis, the translational machinery, amino acid biosynthesis, sulfur metabolism and numerous other genes involved in growth. In the RB phase, cells enter S phase and complete the cell division process. This activity correlates with an increase in transcripts of genes involved in the cellular building machinery, including DNA replication, spindle pole components, and other genes involved in the cell cycle. The majority of gene products encoding mitochondrial proteins also peak in this phase. In the RC phase, transcripts of many genes associated with starvation, stress, and cell survival increase before the cells prepare to enter another OX phase. Thus, the OX phase can be likened to ‘growth’, the RB phase to ‘division’, and the RC phase to ‘survival/quiescence’. Cells continuously alternate among these three metabolic phases, each associated with particular transcriptional programs, during continuous growth. One of the most interesting and significant examples of temporal compartmentalization is the finding that cell division is gated to the RB phase during which the rate of oxygen consumption decreases [8^{••},13]. Such gating of cell division was also reportedly observed during short-period 40 min oscillations, despite the lack of a corresponding upregulation of cell cycle genes [7^{••}].

A separate series of comprehensive gene expression studies was performed on yeast cells grown in various nutrient-limited chemostat cultures under changing growth rates [14,15,16[•]]. These studies aimed to determine groups of genes that are either positively or negatively correlated with growth rate. Strikingly, there was a substantial overlap in the genes that are positively correlated with growth rate and those that peak during the OX, growth, phase of the long-period YMC [16[•]]. Moreover, many genes that were negatively correlated with increasing growth rate in this study peaked during the quiescent-like RC phase of the YMC [16[•]]. Thus, through two independent experimental means, similar groups of genes were found to be co-regulated with respect to growth and cellular metabolic state. Collectively, these data predict that cells might undergo such metabolic cycles outside of a steady-state glucose-limited growth environment, and that the chemostat merely facilitates the establishment of a unique synchrony that enables the macroscopic observation of these oscillations and their systematic dissection. Figure 1 illustrates the organization of the different phases of the long-period YMC, with different functionally related biological processes temporally separated in these phases.

The periodic gene expression data collected over three consecutive long-period metabolic cycles was used to investigate the tightly controlled sequence of transcriptional events that span the budding yeast cell cycle [17[•]]. Eukaryotic cell division is a highly complex and precisely orchestrated process involving specific gene transcription, translation, protein localization and turnover. Using deconvolution of the unique profile shapes of cell cycle genes, the peaks of expression of cell cycle regulated genes were timed at an unprecedented resolution of ~2–3 min [17[•]]. Such precise timing was perhaps enabled by the slower growth of cells in the YMC (compared to cells growing in high-glucose stationary cultures) as well as the extraordinary synchrony of cells undergoing such metabolic oscillations, which often persists for weeks. These data revealed a previously unanticipated wave of gene transcription around the G1 to S transition of the cell cycle, and a just-in-time transcription of genes involved in the initiation of DNA replication [17[•]]. These studies suggest that the YMC may be an excellent system to investigate the temporal sequence of events that comprise the budding yeast cell cycle program because it imposes an unusually synchronous progression of cells through the cell cycle.

Periodic changes in metabolism and metabolites

These robust oscillations in oxygen consumption had suggested that periodic changes in cellular metabolites involved in energy metabolism, particularly glucose metabolism, respiration, and carbohydrate reserves might accompany such cycles [8[•],9,12,18]. Earlier studies had reported that NADH levels oscillate with oxygen levels, and ATP levels are lowest in the respiratory phase [9]. These studies suggested that the synthesis and breakdown of carbohydrates as well as the NAD⁺/NADH ratio might play an important role in regulating such respiratory cycles in yeast. GC–MS metabolite analysis of cells undergoing the ~40-min short-period cycles in oxygen consumption showed that the majority of the metabolites oscillate, with metabolite concentrations peaking with NAD(P)H concentrations [19]. However, it remained unclear whether there exists a hierarchical control of metabolism in relation to gene expression fluctuations during such short-period cycles. In the long-period YMC, the gene expression data that reveal the highly periodic transcriptional output could be used to predict oscillations in the metabolic output of the cell. Indeed, comprehensive metabolite profiling across the YMC using a combination of liquid chromatography (LC-MS/MS) and gas chromatography–mass spectrometry (GC × GC–TOFMS) based methods showed that many metabolites change periodically as a function of the YMC [20[•],21]. The phase in which particular metabolites increase generally matches the oscillations of corresponding gene transcripts observed in the YMC. For example, many amino acid precursors, amino acids, nucleotides and TCA cycle metabolites increase during

the OX growth phase [20^{*}], suggesting that the OX phase provides cells with a temporal window dedicated to biosynthesis and energy production coupled to increased respiration rates. The metabolites that increase during OX phase can be predicted to be crucial for cell growth and proliferation. These metabolite analyses also suggested a defined logic for the regulation of the sulfur metabolism pathway [20^{*}]. Moreover, the metabolite profiling studies also showed numerous changes in metabolism that were not immediately predictable from inspection of periodic gene expression profiles. Nevertheless, these metabolomic studies of the YMC have revealed the actual biological outputs of the cell at different times of the oscillations, and future studies could unveil the precise mechanisms by which these outputs are specified in terms of gene expression and protein activities. The changes in metabolism and cellular metabolic state that govern these yeast cycles may also provide clues towards understanding changes in metabolism that occur during the course of other biological cycles [12,22].

Visualizing metabolic cycles

A further understanding of such metabolic cycles has so far been restricted by the unavailability of robust reporters for visualizing cells undergoing respiratory oscillations in real time. When developed, these reporters will enable the observation of specific events during temporally segregated biological processes at the level of a single cell, as well as in a large population of synchronized cells. This visualization would facilitate the study of those factors that enable, control or regulate metabolic oscillations, and allow the observation of the real-time responses of cells to various environmental, nutritional and other perturbations. A series of recent developments hold promise to ameliorate this limitation. One study monitored the cycling cell population with destabilized luminescence reporters developed using the firefly luciferase system [23]. These transcription reporters of the cell cycle enabled the real-time monitoring of cell cycle positions of the cell population undergoing the long-period YMC. One limitation of this reporter is that low oxygen tensions tend to mask the luminescence signal; however, this property can be exploited to use luciferase as real-time reporter of oxygen tension [23]. A second report used quantitative *in situ* hybridization of fluorescent nucleotide probes to visualize the expression of different YMC phase-specific reporters in asynchronous cells that were limited for various nutrients [24^{*}]. This study suggested the occurrence of metabolic oscillations as an intrinsic property of yeast metabolism under a variety of low nutrient conditions, including low phosphate, in addition to low glucose [24^{*}]. More recently, fluorescent reporters to monitor phase-specific gene expression in single cells as well as large populations of cells across the YMC were constructed [25^{*}]. Using these reporters, the YMC could be observed in populations of living cells, which subsequently suggested the requirement of low glucose concentrations for sustaining synchronized metabolic oscillations in large populations of cells. These studies also showed that robust, temporally controlled oscillations of transcripts and metabolites occur in spite of some heterogeneity within the system in terms of subpopulations of cells that both grow and divide, and those that do not [25^{*}]. However, while these recent developments have provided additional insight into such metabolic cycles, there remain limitations in using the various reporter systems developed thus far, which include reporter stability, turnover and reporter signal intensity. Future work requires the development of multiple, real-time reporters that will faithfully and precisely represent the expression of different phase-specific genes during the YMC. This in turn will enable the visualization and investigation of the precise events that occur in, and the factors that control, metabolic cycling.

The future

These ultradian yeast metabolic cycles represent remarkably robust systems where the growth of a cell population is both highly synchronized and ‘slowed down’. This combination provides a system wherein the temporal sequence of various transcriptional and metabolic events that lead to different biological outputs can be probed with an unmatched precision. Figure 2 illustrates a range of systems-level questions that the YMC may be able to uniquely address. Comprehensive microarray analyses of gene expression have already been conducted with other biological cycles, most notably the circadian cycle [26–29]. However, some of these studies have been limited to a 4 h resolution due to the feasible frequency of sampling during these long cycles. The YMC in theory can be sampled at minute to minute intervals, providing very high resolution snapshots of any temporally regulated process of interest. Additionally, the transcriptome of the YMC suggests an extreme economy and efficiency of transcription and metabolism specific for each set of biological processes. This coordination immediately predicts an equally efficient, economical and temporally regulated ‘just in time’ proteome in these cells, controlling specific biological outputs. Examples have already been observed in the studies of cell cycle timing and progression [17*]. Moreover, stores of the carbohydrate trehalose were observed to be metabolized preferentially by cells exiting quiescence [30]. Using the YMC, the major trehalase enzyme, Nth1p, was observed to substantially peak in activity at a single ~20-min interval during the OX phase [30], showing how specific and temporally controlled a protein activity can be within the YMC. Immediate next steps include utilizing the YMC for specific proteomic analysis, to probe the temporal sequence and regulation of different proteins and protein complexes, as well as the post-translational modifications that regulate their activity during specific biological and signal transduction events. Future studies could use the YMC to study the molecular mechanisms by which gene expression, protein abundance, post-translational modifications and metabolism are specified at a minute to minute temporal resolution. The outstanding temporal resolution of the YMC could provide a systems-level platform to finely dissect the mechanisms driving diverse biological processes such as growth control, the cell cycle, quiescence, autophagy, and how fundamental cellular processes are coupled to metabolism and the metabolic state of the cell.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
 - of outstanding interest
1. Chance B, Estabrook RW, Ghosh A. Damped sinusoidal oscillations of cytoplasmic reduced pyridine nucleotide in yeast cells. *Proc Natl Acad Sci U S A*. 1964; 51:1244–1251.
 2. Chance B, Schoener B, Elsaesser S. Control of the waveform of oscillations of the reduced pyridine nucleotide level in a cell-free extract. *Proc Natl Acad Sci USA*. 1964; 52:337–341. [PubMed: 14206600]
 3. von Meyenburg HK. Energetics of the budding cycle of *Saccharomyces cerevisiae* during glucose limited aerobic growth. *Arch Microbiol*. 1969; 66:289–303.

4. Parulekar SJ, Semones GB, Rolf MJ, Lievens JC, Lim HC. Induction and elimination of oscillations in continuous cultures of *Saccharomyces cerevisiae*. *Biotechnol Bioeng*. 1986; 28:700–710. [PubMed: 18555381]
5. Porro D, Martegani E, Ranzi BM, Alberghina L. Oscillations in continuous cultures of budding yeast: a segregated parameter analysis. *Biotechnol Bioeng*. 1988; 32:411–417. [PubMed: 18587737]
- 6•. Satroutdinov AD, Kuriyama H, Kobayashi H. Oscillatory metabolism of *Saccharomyces cerevisiae* in continuous culture. *FEMS Microbiol Lett*. 1992; 77:261–267. An early study describing short-period oscillations in yeast, where numerous metabolic parameters were observed to change cyclically. [PubMed: 1334018]
- 7••. Klevecz RR, Bolen J, Forrest G, Murray DB. A genomewide oscillation in transcription gates DNA replication and cell cycle. *Proc Natl Acad Sci USA*. 2004; 101:1200–1205. This study describes the low-amplitude, genome-wide oscillations of transcription observed in the short-period cycles, with a restriction of DNA replication to the reductive phases of these short cycles. [PubMed: 14734811]
- 8••. Tu BP, Kudlicki A, Rowicka M, McKnight SL. Logic of the yeast metabolic cycle: temporal compartmentalization of cellular processes. *Science*. 2005; 310:1152–1158. Gene expression analysis of long-period cycles (YMC) is described in this study, where over half the yeast genome was expressed periodically, and genes encoding proteins with similar functions showed similar temporal expression profiles. [PubMed: 16254148]
9. Xu Z, Tsurugi K. A potential mechanism of energy-metabolism oscillation in an aerobic chemostat culture of the yeast *Saccharomyces cerevisiae*. *FEBS J*. 2006; 273:1696–1709. [PubMed: 16623706]
10. Sohn HY, Murray DB, Kuriyama H. Ultradian oscillation of *Saccharomyces cerevisiae* during aerobic continuous culture: hydrogen sulphide mediates population synchrony. *Yeast*. 2000; 16:1185–1190. [PubMed: 10992282]
11. Lloyd D, Murray DB. Ultradian metronome: timekeeper for orchestration of cellular coherence. *Trends Biochem Sci*. 2005; 30:373–377. [PubMed: 15935677]
12. Tu BP, McKnight SL. Metabolic cycles as an underlying basis of biological oscillations. *Nat Rev Mol Cell Biol*. 2006; 7:696–701. [PubMed: 16823381]
13. Chen Z, Odstreil EA, Tu BP, McKnight SL. Restriction of DNA replication to the reductive phase of the metabolic cycle protects genome integrity. *Science*. 2007; 316:1916–1919. [PubMed: 17600220]
14. Saldanha AJ, Brauer MJ, Botstein D. Nutritional homeostasis in batch and steady-state culture of yeast. *Mol Biol Cell*. 2004; 15:4089–4104. [PubMed: 15240820]
15. Brauer MJ, Saldanha AJ, Dolinski K, Botstein D. Homeostatic adjustment and metabolic remodeling in glucose-limited yeast cultures. *Mol Biol Cell*. 2005; 16:2503–2517. [PubMed: 15758028]
- 16••. Brauer MJ, Huttenhower C, Airoidi EM, Rosenstein R, Matese JC, Gresham D, Boer VM, Troyanskaya OG, Botstein D. Coordination of growth rate, cell cycle, stress response, and metabolic activity in yeast. *Mol Biol Cell*. 2008; 19:352–367. In this study, yeast were grown continuously in a variety of nutrient- limiting conditions, and the relationships between growth rate, gene expression, and cell-cycle progression were studied. The gene expression results of these experiments correlated with gene expression profiles observed in the YMC. [PubMed: 17959824]
- 17•. Rowicka M, Kudlicki A, Tu BP, Otwinowski Z. High-resolution timing of cell cycle gene expression. *Proc Natl Acad Sci USA*. 2007; 104:16892–16897. Deconvoluting gene expression data from the long-period YMC, the authors were able to time the peaks of gene expression of cell cycle genes to a ~2–3 min resolution, and predict a fine sequence of events during the yeast cell cycle. [PubMed: 17827275]
18. Fitcher B. Metabolic cycle, cell cycle, and the finishing kick to Start. *Genome Biol*. 2006; 7:107. [PubMed: 16677426]
19. Murray DB, Beckmann M, Kitano H. Regulation of yeast oscillatory dynamics. *Proc Natl Acad Sci USA*. 2007; 104:2241–2246. [PubMed: 17284613]

- 20•. Tu BP, Mohler RE, Liu JC, Dombek KM, Young ET, Synovec RE, McKnight SL. Cyclic changes in metabolic state during the life of a yeast cell. *Proc Natl Acad Sci USA*. 2007; 104:16886–16891. This study shows the extensive, periodic oscillations of metabolites across the long-period YMC. [PubMed: 17940006]
21. Mohler RE, Tu BP, Dombek KM, Hoggard JC, Young ET, Synovec RE. Identification and evaluation of cycling yeast metabolites in two-dimensional comprehensive gas chromatography–time-of-flight-mass spectrometry data. *J Chromatogr A*. 2008; 1186:401–411. [PubMed: 18001745]
22. Tu BP, McKnight SL. The yeast metabolic cycle: insights into the life of a eukaryotic cell. *Cold Spring Harb Symp Quant Biol*. 2007; 72:339–343. [PubMed: 18419291]
23. Robertson JB, Stowers CC, Boczek E, Johnson CH. Real-time luminescence monitoring of cell-cycle and respiratory oscillations in yeast. *Proc Natl Acad Sci USA*. 2008; 105:17988–17993. [PubMed: 19004762]
- 24•. Silverman SJ, Petti AA, Slavov N, Parsons L, Briehof R, Thiberge SY, Zenklusen D, Gandhi SJ, Larson DR, Singer RH, et al. Metabolic cycling in single yeast cells from unsynchronized steady-state populations limited on glucose or phosphate. *Proc Natl Acad Sci USA*. 2010; 107:6946–6951. Using *in situ* hybridization, this study suggested the presence of metabolic cycles in single cells in unsynchronized yeast cultures. [PubMed: 20335538]
- 25•. Laxman S, Sutter BM, Tu BP. Behavior of a Metabolic Cycling Population at the Single Cell Level as Visualized by Fluorescent Gene Expression Reporters. *PLoS ONE*. 2010; 5:e12595. This study visualizes live cells undergoing metabolic oscillations using phase specific fluorescent reporters. [PubMed: 20830298]
26. Harmer SL, Hogenesch JB, Straume M, Chang HS, Han B, Zhu T, Wang X, Kreps JA, Kay SA. Orchestrated transcription of key pathways in Arabidopsis by the circadian clock. *Science*. 2000; 290:2110–2113. [PubMed: 11118138]
27. Claridge-Chang A, Wijnen H, Naef F, Boothroyd C, Rajewsky N, Young MW. Circadian regulation of gene expression systems in the *Drosophila* head. *Neuron*. 2001; 32:657–671. [PubMed: 11719206]
28. McDonald MJ, Rosbash M. Microarray analysis and organization of circadian gene expression in *Drosophila*. *Cell*. 2001; 107:567–578. [PubMed: 11733057]
29. Panda S, Antoch MP, Miller BH, Su AI, Schook AB, Straume M, Schultz PG, Kay SA, Takahashi JS, Hogenesch JB. Coordinated transcription of key pathways in the mouse by the circadian clock. *Cell*. 2002; 109:307–320. [PubMed: 12015981]
30. Shi L, Sutter BM, Ye X, Tu BP. Trehalose is a key determinant of the quiescent metabolic state that fuels cell cycle progression upon return to growth. *Mol Biol Cell*. 2010; 21:1982–1990. [PubMed: 20427572]

Figure 1.

The Yeast Metabolic Cycle (YMC). A schematic illustration of the long-period cycles of oxygen consumption during continuous growth that have been termed the Yeast Metabolic Cycle. Cell growth, which is accompanied by the increased expression of growth genes, occurs in the oxygen-consuming oxidative phase (OX, shown in red). Cell division is tightly restricted to the lower oxygen-consuming reductive-building phase (RB), where cells reduce their oxygen consumption (shown in green). The reductive-charging phase (RC) is characterized by the upregulation of genes involved in stress, starvation, and cell-survival processes, with the genes expressed in this phase being negatively correlated with growth (shown in blue) [16].

Figure 2.

Dynamic processes amenable for systems-level investigation using the YMC. The YMC enables the study of the dynamics of transcription, protein function, and metabolism as a function of the growth and metabolic state of a cell.