FUNCTION™ american physiological society

FUNCTION, 2021, 2(5): zqab039

https://doi.org/10.1093/function/zqab039
Advance Access Publication Date: 3 September 2021
Perspectives

PERSPECTIVES

Multiple Rediscoveries and Misconceptions; the Yeast Metabolic Oscillation

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In the 1950s, parallel to revolutions in our understanding of DNA and the cell division cycle (CDC), there were also huge developments in biotechnology. As some of the first domesticated microbes, *Saccharomyces* spp. have been at the vanguard of these developments. Specifically, the development of continuous culture has shaped cell growth laws and our understanding of metabolism. As early as 1954, Finn and his coworkers observed that during the continuous growth of *Saccharomyces pastorianus* (lager yeast) an autonomous oscillation in fermentation products, pH and dissolved oxygen developed during steady-state conditions, providing a first taste of metabolic and CDC integration.[1]

As technology advanced, Finn's observations were rediscovered and added to, using the stalwart of biotechnology bakers' yeast (*Saccharomyces cerevisiae*). Von Meyenburg refined culture conditions and precisely measured growth kinetics, gas exchanges and energetics to define the relationship between growth, the CDC and respiration (budding commences at high respiration rates).[2] The Fiechter group closely tracked DNA synthesis using flow cytometry to show that S-phase occurred during high respiration.[3] The periods observed during the oscillation were about half the doubling time of the culture (4∼6h) so the oscillation was thought to be an inherent property of the CDC and respiratory capacity.

In 1991, the Kuriyama group showed that a much shorter-period respiratory oscillation (usually ranging between 40∼60 min) could develop under similar growth conditions.[4] In the following years, there were many insights from the groups of Kuriyama, Lloyd and Murray. [5] Further studies used growth with ethanol, measurement of glutathione levels and by inhibitors of its synthesis, and inhibition by nitrosation reagents. Continuous monitoring of NAD(P)H and flavin fluorescence showed that the oscillation could be entirely respiratory and did not require either glucose, fermentation or glycogen accumulation.

Therefore by 2005, the oscillation phases could be defined according to flux through the mitochondrial electron transport chain (ETC), showing respiratory control (ADP-acceptor control), and also responsive to uncouplers of mitochondrial energy conservation. The respiratory/oxidative (high oxygen consumption; HOC) phase has a lower residual dissolved oxygen concentration (Figure 1A) than the reductive phase (low oxygen consumption; LOC). Residual dissolved oxygen concentration exactly tracks the oxygen uptake rate (and thus ETC flux) because continuous culture is an open system (Figure 1 B), *i.e.*, nutrients such as oxygen, are continuously perfused into the reactor, so any changes in nutrient concentration in the reactor are indicative of flux changes.

Population synchrony was shown to be mediated by small molecules such as acetaldehyde, as shown by monitoring phase response curves. The period of the oscillation is temperature compensated, and period lengthening occurs with Li^+, and A-type monoamine oxidase inhibitors (Phenalzine and Iproniazid). These psychotropic drugs are also well known to prolong period circadian timing, and are effectors of phospho-inositide signaling; this suggests a shared pathway between longer and shorter time domains. Most significantly, it became evident that the metabolic oscillation, was not solely a downstream function of the CDC, but a rhythm (an ultradian clock).

Up until this point, the oscillation had largely been published in specialist biotechnology and microbiology journals. However,

Submitted: 6 August 2021; **Revised:** 10 August 2021; **Accepted:** 9 August 2021

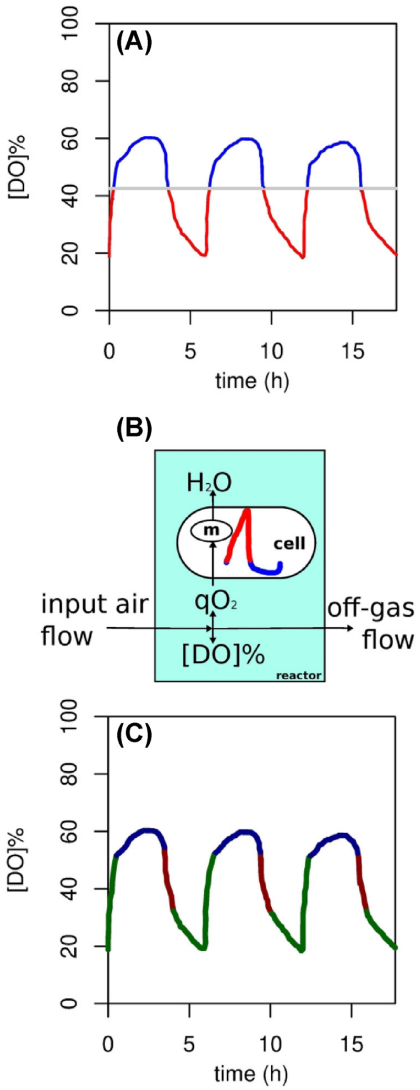

Figure 1. Phase definition of the respiratory oscillation in continuously grown yeast (A). The oxidative and reductive phases are shown in red and blue, respectively. Phases can be defined in a number of ways, for example, mean amplitude[8] (in this figure), min and max first derivative,[5] or using measured gas exchanges and respiratory quotient calculations.[10] The exact method used only affects the fringes of the phases. Measured residual dissolved oxygen ([DO]%; B) represents the amount of unused oxygen after the cell has consumed what it needs in the mitochondrion (m), i.e., oxygen consumption flux (qO_2). As continuous culture is an open system that is constantly perfused with air, the [DO]% is the inverse of qO_2.[4,5,8,10] Compare this to the arbitrary phase definitions of Tu et al.[7] (C), where the phases oxidative, reductive building and reductive charging are represented in dark red, dark green and dark blue, respectively. Even though they originally tried to define these phases according to mitochondrial activity, the oxygen flux through the ETC does not tally with the given phase definitions.

with the full sequence of *S. cerevisiae* being available in 1996 and the concurrent development of microarray transcriptome technologies, Klevecz's group published a breakthrough publication that showed the transcriptome was remarkably dynamic during the short-period oscillation and that the cohorts of transcripts (encompassing the vast majority of transcripts) have phase relationships with respiratory activity.[6] Moreover, they highlighted the intricate relationship between the CDC and metabolism by showing that progression through the CDC was gated to the reductive phase of the oscillation, and they also postulated that this possibly might be to protect DNA from hazardous reactive oxygen species (ROS) generated from respiration, akin to existent theories about circadian timing. However, the authors did not take into account the previous measurements where budding and S-phase coincides with high respiration.[2,3]

A PNAS editor McKnight (previously assigned to oversee the reviewers of the Klevecz paper[6]), conducted very similar experiments, these data were published in Science[7] (November 18, 2005) and February 17, 2006 (with many corrections)[†]. On the surface, this paper confirmed the work of Klevecz. However, their data also showed that the HOC phase was where maximum DNA synthesis occurred, thus providing further evidence the ROS-DNA partitioning hypothesis was wrong[†]. Rather than address this, the authors arbitrarily shifted phases to draw the same erroneous conclusion as Klevecz (Figure 1C) [‡]. Slavov et al.[8] highlighted these inconsistencies and showed metabolic cycling even occurred in the absence of a measurable CDC oscillation during the long-period oscillation. Even though the timing of S-phase in the long and short period oscillation is completely different, an extensive reanalysis of both datasets showed overwhelming commonality in both the transcripts involved, and their phase relationship with dissolved oxygen.[9] However, such is the impact of a publication in Science that McKnight's research has become the default discovery paper for many readers, and the misrepresentation of the oscillation phases has propagated throughout the field.

Until now, the erroneous ROS-DNA hypothesis remains to be considered one of the main explanations for the function of the oscillation. However, it is still far from clear what role, if any, the oscillation has. Several alternative functional interpretations were suggested in recent years (in summary[10]): differential durations of the metabolic phases may directly explain the relation of growth rate to the ribosomal biomass fraction, and the cycle may underpin spatio-temporal protein homeostasis.[10] That the oscillation is an emergent property of a spatially and temporally coherent system of mathematically complex networks comprised of large number of coupled feedback loops has also been repeatedly suggested since 2006.[5] Thus, the normally stable respiratory oscillation can undergo spontaneous period halvings and doublings and other chaos-like behaviour.[5,10]

Before he passed away (13.05.2008), Klevecz had published papers since 1976 confirming that "the cell is a multi-oscillator, the cell cycle a developmental process." Functions for ultradian clocks have also been determined in other yeasts (e.g., *Schizosaccharomyces pombe* and *Candida utilis*), seven protists (i.e., *Crithidia fasiculata, Tetrahymena pyriformis*, *Dictyostelium discoedeum, Acanthamoeba castellanii*, *Paramecium tetraurelia, Euglena gracilis, and Chlamydomonas reinhardtii*), as well as in cultured mammalian cells. We suggest that ultradian timekeeping is a basic universal necessity for the maintenance of low entropy and coordinated intracellular coherence.

[†]Please see the PubPeer discussion: (https://pubpeer.com/publications/1177CD2AA25CCB523C74A0A9CA7AF7). Tu et al.[7] have declined to comment.

[‡]Klevecz et al.[6] was not cited as a source for this.

Acknowledgment

We thank all our coworkers for advice and laboratory help, and we are grateful to Dr Rainer Machné for critically reviewing this manuscript.

Funding

DBM was initially funded The Royal Society, and recently by a partnering award from Japan Science and Technology Agency, Yamagata prefectural government and the City of Tsuruoka. Grants to DL were from The Royal Society, MRC, SRC, BBSRC, KTP, Sêr Cymru and Cardiff University

Conflict of Interest statement

None declared

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