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Minireview

The temporal architecture of eukaryotic growth

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Abstract **Coherence of the time structure of growing organisms depends on a metronome-like orchestration. In a continuously perfused culture of *Saccharomyces cerevisiae* the redox state of the cell shows a temperature-compensated oscillation manifest in respiratory cycles, which are measured by continuous and non-invasive electrodes of probes such as dissolved oxygen and probes such as fluorometric NAD(P)H. Although the entire transcriptome exhibits low-amplitude oscillatory behaviour, transcripts involved in the vast majority of metabolism, stress response, cellular structure, protein turnover, mRNA turnover, and DNA synthesis are amongst the top oscillators and their orchestration occurs by an intricate network of transcriptional regulators. Therefore cellular auto-dynamism is a function of a large ensemble of excitable intracellular components of that self-organized temporally and spatially that encompasses mitochondrial, nuclear, transcriptional and metabolic dynamics, coupled by cellular redox state.**

Keywords: Yeast; Redox regulation; Biological clock; Respiration; Mitochondrion

1. Overview

The importance of cyclic mechanisms at every level in the organization of living organisms has been long evident [1]. An obsessive pre-occupation with homeostatic regulation [2] has however obscured the basic autodynamic principles that define the living state [3]. As non-invasive methods achieve greater sensitivity and specificity as well as faster response time for studies of intact organisms and cells, it becomes evident that steady-state operation is rare [4]. Ideas that the progression of the cell division cycle may be regulated by thiol–disulfide interconversion dates back to 1931, when it was shown that the total acid-soluble thiol levels in synchronously dividing sea urchin eggs, as measured by the nitroprusside reaction, varied cyclically each inter-division time [5]. In a similar system, the rapid cell division in the absence of cell growth, initiated by fertilization, were shown to be characterized by a changing thiol/disulfide ratio thought to be the glutathione redox couple [6,7]. In a formal model, a limit cycle oscillator was postulated [8] to account for the cell division cycle, where the state variables are XSH and XSSH, and where both low mol. wt. thiols and protein sulfhydryl groups are implicated. Thus at any time in the cycle, the state of the organism will be represented by a unique combination of the reduced and oxidized forms of thiol redox buffering capacity. Subsequent emphasis on the importance of the intracellular redox state as a central principle of bioenergetics, accompanied by direct access to its measurement by continuous monitoring of NAD(P)H fluorescence [9,10] even at the single cell level [11] has produced an extensive literature on bacteria, lower eukaryotes, plants as well as animal tissues and cells.

Although biochemical redox oscillations have been observed in many systems, temperature-compensation of the period defines a special class of oscillators that are especially relevant as timekeepers [12]. Unlike glycolytic oscillations, where Q_{10} values as high as 3 were noted in experiments with suspensions of intact yeasts [13], some respiratory oscillations in mitochondria in situ in *Acanthamoeba castellanii* were characterized as ADP-controlled [14] and with a Q_{10} close to unity [15].

The oscillating respiration observed in continuous cultures of the budding yeast, *Saccharomyces cerevisiae* has a circa-40 min period (Fig. 1). Here growth (the mean cell cycle time) is controlled by the dilution rate with inflowing growth medium [16,17], but the respiratory oscillation shows timekeeping characteristics [18], in that step-wise changes of temperature reveal temperature-compensated periodicity. The nicotinamide nucleotide [19] and glutathione [20] pools oscillate with distinct phase-relationships with the respiratory cycles, as do mitochondrial energy states [21,22]. Conserved signalling pathways are implicated as indicated by the period-lengthening effects of Li^+ [23] and phase shifting by H_2S [24]. Cell–cell communication involves acetaldehyde as an extremely mobile (highly diffusible) but evanescent (oxidizable) secreted metabolite [25] as well as other small molecules with similar characteristics.

Genome-wide expression levels revealed the pervasive nature of the 40 min time-frame [26], where the entire transcriptome showed a low amplitude oscillation. Transcripts either maximally expressed the oxidative (low respiration; ~10% of all transcripts) or the reductive (high respiration; ~90% of all transcripts) states of the cycle. Therefore, gene expression shows two major blocks of redox superclusters that extend beyond central metabolism and mitochondrial energetics to other

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Abbreviations: CCCP, *m*-chlorocarbonycyanide phenylhydrazone; S_{13}, 5-chloro-3-*t*-butyl-2-chloro-4′-salicylhydroxamic acid; ROS, reactive oxygen species

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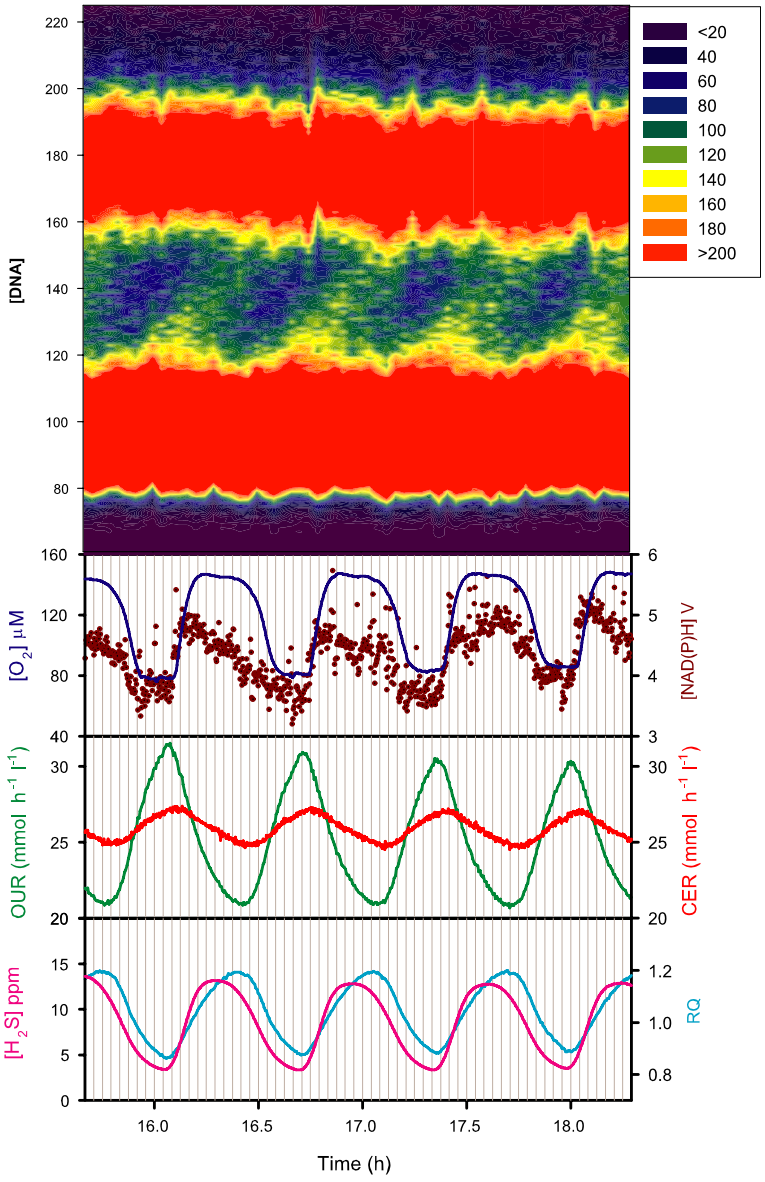

Fig. 1. The respiratory oscillation found during continuous growth of *Saccharomyces cerevisiae*. Measured outputs include S-phase (top panel; [DNA] 120 through 160); the contour plot was constructed from 64 flow cytometry histograms. Continuous online parameters include NAD(P)H, H_2S, O_2 Uptake (OUR), and CO_2 Excretion (CER) rates, and derived respiratory quotient (RQ). The grey vertical lines indicate the sampling interval. Acetaldehyde oscillates 180° out of phase with dissolved oxygen [17]. The measurements used methods outlined previously [17,24,26,45].

highly conserved cellular processes, including, autophage and peroxisome functions and protein regulation, RNA and DNA synthesis, repair and turnover.

Of the core transcripts, 35% have a significant sequence homology to higher eukaryotes; this suggests that what we observe is a basic universal necessity to provide temporal coherence of all higher living systems. At its core is the oscillatory intracellular redox state, but the entire intracellular reaction network entrains to this fundamental rhythm.

2. Mitochondrial respiratory dynamics

The respiratory oscillations in yeast growing in continuous culture represent a high-amplitude variation in the structure and function of the organelles [21,22]. The mechanism had been previously elucidated in the cell-division synchronized cultures of the soil amoeba, *A. castellanii*, where measurements of adenine nucleotide pools clearly indicated a process of in vivo mitochondrial respiratory control (i.e. restriction of respiratory chain activity by the availability of ADP, which in turn depends on biosynthetic ATP utilization rates) [14]. This research led to the suggestion that the control of mitochondrial respiratory rates lies in an epigenetic control circuit with a slow ($\tau = 69$ min for *A. castellanii*) dynamic. Thus the mitochondria dance to a "slow drum beat" played elsewhere [12]. The yeast system obeys similar rules, but in this case the ultradian period is about 40 min, again the observed cycles are dictated by the slower time-scales of the energy-requiring processes of biosynthesis, so that the changing respiration rates we observe in cultures of the growing cells is a consequence of the ultradian clock-driven cycles