The rhythm of yeast

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Abstract

Although yeast are unicellular and comparatively simple organisms, they have a sense of time which is not related to reproduction cycles. The glycolytic pathway exhibits oscillatory behaviour, i.e. the metabolite concentrations oscillate around phosphofructokinase. The frequency of these oscillations is about 1 min when using intact cells. Also a yeast cell extract can oscillate, though with a lower frequency. With intact cells the macroscopic oscillations can only be observed when most of the cells oscillate in concert. Transient oscillations can be observed upon simultaneous induction; sustained oscillations require an active synchronisation mechanism. Such an active synchronisation mechanism, which involves acetaldehyde as a signalling compound, operates under certain conditions. How common these oscillations are in the absence of a synchronisation mechanism is an open question. Under aerobic conditions an oscillatory metabolism can also be observed, but with a much lower frequency than the glycolytic oscillations. The frequency is between one and several hours. These oscillations are partly related to the reproductive cycle, i.e. the budding index also oscillates; however, under some conditions they are unrelated to the reproductive cycle, i.e. the budding index is constant. These oscillations also have an active synchronisation mechanism, which involves hydrogen sulfide as a synchronising agent. Oscillations with a frequency of days can be observed with yeast colonies on plates. Here the oscillations have a synchronisation mechanism which uses ammonia as a synchronising agent.

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Keywords: Oscillation; Oscillatory metabolism; Synchronisation; Signalling; Intercellular communication

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1. Introduction

Rhythmic or oscillatory behaviour is very common in biological systems. It is often related to day and night cycle, annual cycles, reproduction or other cycles. However, this review deals with rhythms which are not directly linked to such external or reproductive cycles, and which can be described as chemical oscillations. This review also focuses on yeast and mainly concerns \textit{Saccharomyces cerevisiae}. One of the earliest descriptions for a plausible mechanism for chemical oscillations is from Lotka [1]. This mechanism is represented by chemical equations and can be written as follows:

\[ G + A \rightarrow 2A \]  
\[ A + B \rightarrow 2B \]  
\[ B \rightarrow \]  

This can be illustrated by translating it to an ecological system where the grass \( G \) is constantly supplied and animal \( A \) eats grass and reproduces as represented by Eq. 1. Animal \( B \) eats animal \( A \) and reproduces in Eq. 2. In Eq. 3 animal \( B \) is dying. This system exhibits limit cycle oscillations. The concentrations of \( A \) and \( B \) oscillate out of phase, i.e. at a high concentration of \( B \), \( A \) is low. A low concentration of \( A \) causes \( B \) to decrease. Subsequently a low concentration of \( B \) allows \( A \) to increase, which is followed by an increase in \( B \), and this continues with a regular pattern. A more detailed introduction to oscillating chemical systems and their mathematical analysis is described e.g. in Prigogine [2]. Examples for oscillations in biological systems are listed by Rapp [3] and Hess and Boiteux [4]. Many of the chemical oscillating systems require an autocatalytic element (as in Eqs. 1 and 2 of the previous example). One can also say a system with an autocatalytic element is potentially unstable. An autocatalytic element is present in glycolysis, where two adenosine triphosphate (ATP) are invested and result in four ATP (Fig. 1). Indeed, glycolysis exhibits oscillatory behaviour under certain circumstances. The earliest reports of fluctuations in the concentrations of NADH date back to 1957, when Duyens and Amesz used fluorescence to detect reduced nicotinamide dinucleotides in intact yeast cells [5]. Oscillation in NADH in intact yeast cells was then described by Hommes [6] and Chance et al. [7] in 1964. Oscillations with a frequency of about 1 min were found for other metabolites of the glycolytic pathway as well [8]. The glycolytic oscillations could also be demonstrated in cell-free extracts [9–11]. In the 1970s a lot of research was done on glycolytic oscillations in yeast extracts, i.e. oscillations of metabolites in cell-free extracts of yeast when glucose was continuously added. This research had a profound impact on our understanding of the transient behaviour of multienzyme systems. There are several reviews covering this work [4,12–16]. Since that time no review has been published on yeast oscillations, although a significant amount of original research has been published. The recent research, however, is more focused on oscillations in intact cells. Oscillatory behaviour is not limited to glycolytic oscillations. In 1969 Küenzi and Fiechter [17] and von Meyenburg [18] found oscillations in aerobic glucose-limited continuous cultures. The carbon dioxide evolution and the percentage of budding cells oscillated with a frequency of about 1 h. In this review they are called respiratory oscillations since they are routinely monitored by following the dissolved oxygen. These oscillations are partly related to the reproductive cycle. However under certain conditions they are independent of the cell cycle [19] and called short period respiratory oscillations, because the frequency is normally higher. Recently also yeast colonies on plates were shown to have some oscillatory behaviour. These oscillations have frequencies of several days and were suggested to be involved in guiding the spatial growth of yeast colonies [20].

2. Glycolytic oscillations

2.1. The soluble oscillator

2.1.1. Glycolytic oscillations in a yeast extract

The earliest reports of glycolytic oscillations in cell-free extracts of yeast are from 1964 [9–11]. Glucose was fed at a continuous rate to a yeast cell extract and oscillations were observed in the concentrations of the glycolytic intermediates with a frequency of several minutes. The metabolites oscillate around phosphofructokinase, i.e. the metabolites which are in the glycolytic pathway before

![Glycolysis diagram](https://academic.oup.com/femsre/article-abstract/27/4/547/593696)
the phosphofructokinase oscillate with a phase shift of 180° with respect to the metabolites after the phosphofructokinase. Oscillations were also observed when glucose 6-phosphate or fructose 6-phosphate was continuously added instead of glucose. Continuous addition of a metabolite further down the glycolytic pathway did not result in oscillations. This already suggested that the phosphofructokinase plays a central role in the glycolytic oscillations. Phosphofructokinase was then identified as the oscillating enzyme and adenosine monophosphate (AMP) as the allosteric regulator. AMP oscillates with a phase shift of 90° with respect to glucose 6-phosphate (Fig. 2). At high AMP concentrations the phosphofructokinase activity is high, resulting in a depletion of fructose 6-phosphate and accumulation of fructose 1,6-bisphosphate. At low AMP concentrations the phosphofructokinase is less active, resulting in an accumulation of fructose 6-phosphate and depletion of fructose 1,6-bisphosphate. The regulation of phosphofructokinase by fructose 2,6-bisphosphate is not an essential component in the oscillations [21]. The frequency depends on the rate of glucose feed. At a high rate of glucose feed the frequency is also high. The various aspects of glycolytic oscillations in cell-free extracts were mainly studied in the 1970s and there are several reviews covering this work, e.g. [4,12–15].

A continuous glucose feed can be achieved by a discontinuous addition of trehalose or maltose. Here the enzymes trehalase or maltase release glucose at a constant rate. Boiteux and Hess [22] studied such a self-sustained dynamic system in a thin layer and monitored the NADH with a camera. They observed dynamic two-dimensional structures (see also [23]), however regular patterns such as spirals, as seen for example with the Belousov–Zhabotinsky reaction [24], were not observed.

The heat production in an oscillating yeast extract oscillates with an amplitude of 10% of the average heat production [25]. This amplitude is much lower than the amplitude of the glycolytic intermediates, which can be more than 100% of the average for some metabolites [26]. There is no information about the phase of the heat oscillations in relation to the metabolite concentrations in cell extracts, however it might be similar to the phase relations in intact cells [27].

The glycolytic oscillations are routinely followed by monitoring NADH through fluorescence or absorbance measurements. This allows non-invasive and continuous monitoring. However, what is monitored is mainly enzyme-bound NADH. In a typical experiment only about 10% of the NADH is free in solution, the rest is enzyme-bound, mainly to alcohol dehydrogenase [28]. The shape of the oscillations is different at different rates of glucose addition, varying from sinusoidal at the highest rate of glucose addition to spikes and sawtooth at lower rates of glucose addition [29,30]. The frequency is not only dependent on the rate of glucose addition but also on temperature, pH and protein concentration, i.e. an increase of all these parameters led to an increase in frequency [29]. The highest frequency was obtained with fructose and was about 0.5 min⁻¹, the lowest frequency was about 0.003 min⁻¹ corresponding to a period of 6 h [29,31]. In intact cells the frequency is generally higher, the highest value obtained at 40°C is 4.6 min⁻¹. Under comparable conditions at 30°C the extracts have the highest frequency of about 0.5 min⁻¹, whereas in intact cells the frequency is about 2 min⁻¹ [32].

2.2. Chaos

Oscillation in yeast extracts is observed when glucose is fed at a constant rate. Boiteux et al. [33] changed the constant glucose feed to a non-constant glucose feed and observed irregular waveforms. Markus et al. [34–36] used a sinusoidal rate of glucose feed. Different frequencies of sinusoidal glucose addition were studied. The frequencies of the glucose additions were higher than the frequency of the NADH oscillations at constant glucose feed. The average rate of glucose addition was kept constant. Depending on the frequency of glucose addition, regular, quasiperiodic, and even apparently chaotic oscillations in NADH were observed (Fig. 3) (see also [37]).

2.3. Oscillations in a cellular system

2.3.1. Oscillations in intact yeast cells

An early report on oscillations in NADH in intact cells is from Chance et al. [10] (Fig. 4). To starved cells a glucose pulse was given followed by a shift to anaerobiosis, which led to a train of about five cycles of oscillations in the metabolites. This behaviour can also be observed when the cells are immobilised [38]. Betz and Chance [39] analysed the oscillations of the other metabolite concen-
trations in intact yeast cells. Glucose 6-phosphate and fructose 6-phosphate oscillated in phase and fructose 1,6-bisphosphate oscillated with a phase shift of 180°. AMP and adenosine diphosphate (ADP) oscillated with a phase shift of 90° and ATP with a phase shift of 270°. Glucose 6-phosphate and fructose 6-phosphate oscillated, translated to cytosolic concentrations, between 2 and 6 mM and between 0.2 and 0.6 mM respectively. Fructose 1,6-bisphosphate oscillated between 2 and 8 mM. AMP, the effector of phosphofructokinase, exhibited the highest relative amplitude, oscillating between 60 and 600 μM [40].

The cytosolic NADH oscillated between 200 and 400 μM [41]. The heat production in intact cells oscillated with an amplitude of 5–10% of the average heat production and in phase with NADH [27].

There is a similar pattern as in cell extracts, i.e. the oscillations are around phosphofructokinase with AMP as a potent allosteric activator of phosphofructokinase. A difference to cell extracts is that the oscillations of the metabolites in intact cells are always sinusoidal. Close to 50% of the glucose during oscillatory metabolism in cells is converted to storage carbohydrates, according to Betz and Hinrichs [42]. The production of storage carbohydrates is also the process where, during oscillatory metabolism, most of the ATP that is produced in glycolysis, is used [42].

2.4. Frequency

The rate of sugar transport has an effect on the frequency as reported by Becker and Betz [43]. Oscillations are not only observed when using glucose as a carbon source but also with mannose and fructose. With fructose the frequency is 1.5 to 2 times lower. This correlates with a lower fructose uptake rate, or in other words the frequency is controlled by sugar transport [43]. Reijenga et al. [44] quantified the control of sugar uptake on the frequency. By titrating with maltose, an inhibitor of glucose transporter, a control coefficient for the frequency of 0.6–0.9 was calculated.

2.5. Oscillations are cell density dependent

The glycolytic oscillations in whole yeast cells are induced at one time point by adding glucose. The individual cells start to oscillate in synchrony with the other cells. However, when the individual cells oscillate with a slightly different frequency the macroscopic oscillations will die out. Support for this scenario was given by Aon et al. [45] who attempted to monitor the NAD(P)H oscillations at the single cell level and observed that single cells oscillated even after the macroscopic oscillations had died out. In several reports it was observed that the macroscopic oscillations last longer at a high cell density [45–47]. This can be interpreted as an indication of an active synchronisation mechanism, i.e. the cells are not only oscillating individually, but there is a mutual interaction and the strength of this interaction is dependent on the cellular proximity or cell density.

2.6. Sustained oscillations in intact yeast cells

Hess and Boiteux [48] and Pye [49] have described how to treat yeast cells in order to observe longer trains of glycolytic oscillations. Betz and Chance [32] showed longer trains of oscillations when adding cyanide after a glucose pulse instead of just switching to anaerobiosis. The longest trains of oscillations were observed when cells were harvested at the transition from using glucose to using ethanol as a carbon source. For sustained oscillations cells had to be harvested at the growth phase transition from using glucose to using ethanol and subsequently starved for a
period of a few hours. The cells were then concentrated and exposed to a glucose pulse followed by an addition of cyanide to a final concentration between 2 and 8 mM. The oscillations then lasted until all the glucose was consumed [41,50]. Dano [51] showed that these oscillations were indeed sustained in a system where glucose, cells and cyanide were continuously provided. Oscillations with a frequency of about 1 min were observed for up to 14 h or about 840 cycles.

2.7. Synchronisation of glycolytic oscillations in intact yeast cells

Indications that there is an active synchronisation mechanism are given by the cell density dependence of synchronised oscillations and the fact that sustained oscillations can be observed. Another indication is that synchronised oscillations can occur spontaneously [47]. The most convincing demonstration of active synchronisation, however, comes from experiments where two cell populations, which are oscillating out of phase, are mixed and the oscillations reoccur [52,53] (Fig. 5). The first observation of synchronisation after mixing intact cells which were oscillating out of phase was made by Ghosh et al. [52]. Here the oscillations were not sustained but damped. The authors also found that the oscillations were even more damped in the absence of aldehyde traps, such as KCN or extracellularly added NADH and alcohol dehydrogenase, which let to the speculation that acetaldehyde secreted by the cells is a desynchroniser. On the contrary, Betz and Becker [54] showed that phase shifts could be induced by adding acetaldehyde or pyruvate, which suggested that these compounds might be involved in the signalling. Richard et al. [50] showed that for sustained oscillations an environment which removes the acetaldehyde at a certain rate is essential. These authors also showed that the concentration of free acetaldehyde was oscillating in the medium between 40 and 100 µM [53].

In this concentration range acetaldehyde was also effective in inducing phase shifts. For any intercellular signalling it would be required that (a) the signal is sensed by the cell, (b) the signal is emitted, and (c) the signal is transmitted through the extracellular medium. Since acetaldehyde met all these requirements it was concluded that this is the synchronising agent for sustained glycolytic oscillations [53].

2.8. Kinetic simulations of glycolytic oscillations

The Lotka system [1], described in Section 1, is so simple that the differential equations describing the kinetics can be solved analytically [55]. The glycolytic system, however, is far too complicated for an analytical solution. The differential equations of the kinetics can only be solved numerically and with the aid of computers. Attempts to solve these equations, i.e. to make a kinetic simulation of the glycolytic oscillations, are nearly as old as the computers themselves. The earliest simulations were performed in 1967 by Higgins on an analog computer [56]. The simulations included inhibition of phosphofructokinase by fructose 1,6-bisphosphate and activation by AMP. The simulations did not give a quantitative description of the experimental results, but gave a qualitative description of the phase relations of the metabolites around phosphofructokinase. When analog computers became less fashionable, digital computers were used for the simulations. Richter et al. [57,58] presented a model in 1974 including all enzymes of the glycolytic pathway. The rate equations of the individual enzymes were obtained from the literature. The maximal rates of the enzymes were adjusted. A myokinase reaction was included to catalyse the reaction from AMP and ATP to two ADP as well as an ATPase to remove the accumulating ATP. This numerical model exhibited oscillations, which were in accordance with the experimental data.

A similar approach was followed by Termonia and Ross [59,60]. Simplified models were described by Goldbeter [61,62], based on the product activation of phosphofructokinase, and Sel’kov [63], based only on the autocatalytic properties of glycolysis. The simplest model is probably from Bier et al. [64], and a more complex one from Hynne et al. [65]. In all of these models the frequency and the amplitude are not controlled by a single step, but the control is shared by all enzymes [66]. In recent work the intercellular synchronisation between glycolysing cells [67–69] and the synchronisation of short period oscillations [61] were analysed with kinetic models. Kinetic models for the oscillations can be found on different web pages, e.g. http://www.cellml.org/examples/repository/index.html or http://www.jjj.bio.vu.nl/.

3. Respiratory oscillations

Yeast cells growing aerobically can exhibit oscillations with a frequency of 1–40 h. In the literature they are some-
times called ‘oscillations in continuous culture’, ‘metabolic oscillations in continuous culture’ or ‘ultradian oscillations in continuous culture’; ‘ultradian’ indicates that the cycles are shorter than 24 h. (The only report about circadian oscillations in continuous culture’ or ‘ultradian oscillations’ is from Klippert [70] who described circadian oscillations of heat tolerance in Schizosaccharomyces pombe.) In this review I refer to them as respiratory oscillations, since they are respiration dependent and are routinely followed by measuring the dissolved oxygen. There are two distinctly different types of respiratory oscillations in continuous culture, one partly related to the cell division cycle, the other not. Since both types were sometimes called ultradian oscillations [71,72], it might be confusing to use this term for either of them. In this review I will refer to oscillations which are partly related to the cell cycle as ‘metabolic oscillations in continuous culture’ according to [73] and to oscillations which are not related to the cell cycle and have in general a shorter period as ‘short period oscillations’ according to [74].

3.1. Metabolic oscillations in continuous culture

The first reports were from Küenzi and Fiechter [17] and von Meyenburg [18]. These oscillations were observed in continuous culture under aerobic conditions, as oscillations in e.g. dissolved oxygen, pH or carbon dioxide evolution rate. The oscillations were suggested to be at least partly related to the cell cycle [18,75–77]. Porro et al. [75] observed that in an aerobic glucose-limited continuous culture the budding index oscillated between 10 and 60%. An explanation for the relation between cell cycle and oxygen demand was that before budding ethanol production was enhanced and then the cells switched to growth on ethanol. Chen and McDonald [76] also observed oscillations, which were synchronised with the cell cycle. They found the specific glucose uptake rate to be highest during the budding phase. Münch et al. [77] analysed the cells from an oscillating continuous culture by using flow cytometric methods to estimate the fractions of cells in different states of the cell cycle. They could confirm the synchronisation with the cell cycle, e.g. the fraction of cells in the G1 state of the cell cycle oscillated between 40 and 80%. The cellular resistance to stresses such as heat, \( \text{H}_2\text{O}_2 \) or menadione also oscillated, as described by Wang et al. [71]. This might be connected to the trehalose content of the cell since the best resistance is seen at a high trehalose content, however it doesn’t seem to be as simple as that. The gene product of \( \text{GTS1} \), which influences the heat tolerance of \( \text{S. cerevisiae} \), affects not only glycolytic oscillations [78] but also respiratory oscillations [79]. When the \( \text{GTS1} \) gene was deleted the oscillations disappeared. The expression of \( \text{GTS1} \) under its own promoter could rescue the oscillations; however, they could not be rescued by expressing \( \text{GTS1} \) under a constitutive promoter. In an oscillating continuous culture the gene product of \( \text{GTS1} \) was found to oscillate with a phase shift to the dissolved oxygen of about 180° [79]; see also [73]. Liu et al. [80] used the two-hybrid system to screen for proteins interacting with the \( \text{GTS1} \) gene product. In this screen the genes \( \text{TDH1} \) to \( \text{TDH3} \) were found, which code for glyceraldehyde 3-phosphate dehydrogenase, an enzyme in the Embden–Meyerhof pathway which is active in glycolysis as well as in gluconeogenesis. The mRNA level of \( \text{TDH1} \) oscillated in continuous culture, but not the levels of \( \text{TDH2} \) and \( \text{TDH3} \). \( \text{TDH1} \) is known to be expressed in stationary phase but not during exponential growth on glucose [81]. The oscillations disappeared when \( \text{TDH1} \) was deleted, but did not appear again when \( \text{TDH2} \) or \( \text{TDH3} \) were expressed under the \( \text{TDH1} \) promoter, indicating that the interaction of the proteins Gts1p and GAPDH1 plays an important role in the respiratory oscillations. Information about intracellular metabolite concentrations during the oscillations is lacking, as is information about a possible mechanism to synchronise these oscillations.

3.2. Short period oscillations

Satrudinov [19] described a different type of respiratory oscillation which is apparently not related to the cell cycle. An indication of this was that the trehalose level was constant, in contrast to the partially cell cycle synchronised oscillation, where trehalose also oscillated [75]. This new type of oscillation was called short period synchronised metabolic oscillation. It appeared at dilution rates between 0.06 and 0.11 h\(^{-1} \) (glucose was about 20 g l\(^{-1} \)). At lower dilution rates the oscillations were cell cycle related; at higher dilution rates no oscillations occurred. In the short period oscillations dissolved oxygen, \( \text{CO}_2 \), acetate, pyruvate, ATP, NADH and glycogen oscillated. Cell number and budding index were constant (budding index 40–45%) [19]. The short period oscillation also appeared in an aerobic continuous culture in the absence of glucose, when ethanol was the only carbon source [82]. The phase relations of the different metabolites are described by Lloyd et al. [83]. The dissolved oxygen, ethanol and NADH oscillated in phase and acetaldehyde about 180° out of phase. Peaks of hydrogen sulfide were observed at ascending dissolved oxygen (Fig. 6). The electrochemical potential difference across the plasma membrane remained steady, while the mitochondria were in ‘orthodox’ conformation during the lower rate of respiration and in ‘condensed’ conformation at high rate of respiration [83]. The oscillations are not a result of periodic carbon depletion. It is not the acetaldehyde which synchronises the oscillations, as occurs in glycolytic oscillations [84]. The short period oscillations are not affected by changes in temperature unlike glycolytic oscillations. Between 27 and 34°C the frequency is constant [85]. A possible mechanism for the short period oscillations, which included (i) sulfur metabolism and biosynthetic processes, (ii) oxidative phosphorylation, and (iii) ATP utilising processes, was presented by Wolf et
In this model the oscillatory dynamics arise from a reaction chain with feedback inhibition (no autocatalytic element), i.e. the inhibition of sulphate uptake by cysteine leads to sustained oscillations. The kinetic simulation of this model gives phase relations of the different intermediates which are in good agreement with the experimental observations.

3.3. Synchronisation of short period oscillations

When Keulers et al. [74] investigated the short period oscillations in continuous culture on glucose, it was observed that the aeration rate had an effect on the oscillations. However, it seemed that the effect was not related to an enhanced oxygen concentration, but instead to an increased gas flow rate which could efficiently stop the oscillations. The interpretation [74] was that a volatile compound, which was synchronising the oscillation, was efficiently removed at high gas flow rates which could efficiently stop the oscillations. CO₂ was suggested as a possible candidate since it also influences the pH. Murray et al. [86] showed that these oscillations could be disturbed by NO⁺ and glutathione, suggesting that the synchronisation is somehow related to a change in the redox potential. It was suggested that the synchronising agent was a volatile compound with a thiol group [87].

Sohn et al. [72] identified this compound as hydrogen sulfide. The hydrogen sulfide concentration oscillated in the medium between 0 and 1.5 μM and additions of hydrogen sulfide could stop the short period oscillations. A mathematical model for the short period oscillations, which included the synchronisation through hydrogen sulfide could reproduce most of the experimentally observed features [61]. Hydrogen sulfide oscillations with a similar amplitude but much slower frequency are also observed in a completely different context, i.e. in anaerobic brewing fermentations. However, these were suggested to be related to the cell cycle since they correlated with oscillations in the budding index [88].

3.4. Yeast colony oscillations

According to Palková et al. [20,89,90] individual yeast colonies can also exhibit a periodic behaviour, i.e. oscillate. Individual colonies exhibit a periodical behaviour, i.e. change the pH of their surroundings from acidic to nearly alkaline and vice versa. In the acid phase the colonies are growing, whereas in the alkaline phase growth is transiently inhibited. Ammonia is released during the alkaline phase which acts as a long-range signal between neighbouring colonies, influencing their periodicity of acidification or

Fig. 6. Phase relations of short term oscillations. a: Dissolved oxygen (dashed line), NADH (continuous line) and ethanol concentration (open symbols). b: Dissolved oxygen (dashed line), acetaldehyde (full triangles) and hydrogen sulfide (empty triangles). From [83].
alkalification and their growth. Colonies exposed to volatile ammonia respond by enhancing their own ammonia production, regardless of their current developmental phase. Ammonia production in neighbouring colonies is thus amplified for a few hours, especially in mutually adjacent regions. As a result, the growth of neighbouring colonies becomes concurrently inhibited. Subsequently, ammonia production gradually declines, and colonies start to grow again and consequently enter the next acid period. This induced ammonia production synchronises the acid/alkali pulses in neighbouring colonies and directs their growth to the free space. Within a colony intercellular communication might be facilitated through fibrils which connect the cells [91].

4. Open questions

In 1973 Pye listed some unsolved problems related to glycolytic oscillations [92]. This listing stimulated further research and most of the areas where the knowledge was limited at that time are now better understood. However a few problems still remain.

How common are oscillations at the single cell level? Cells that are harvested during the growth phase transition, starved, concentrated to a certain cell density and subsequently presented with glucose and cyanide, exhibit sustained oscillations, i.e. the many millions of individual cells in the sample oscillate in concert. At lower cell concentrations, after harvesting at different growth phase or in the absence of cyanide, macroscopic oscillations can be induced, but die out after a few cycles. The two possible interpretations are (i) the macroscopic and microscopic oscillations die out and switch to a steady state metabolism or (ii) the microscopic oscillations continue but cannot be observed macroscopically because the individual cells have slightly different frequencies and desynchronise due to the absence of a synchronisation mechanism. It might be that the oscillations on a single cell level are normally overlooked and are much more common than we are aware.

Why is the frequency different in cell extracts and intact cells? The frequency of glycolytic oscillations in yeast extracts depends on the rate of glucose feed. At a higher glucose feed rate the frequency is faster, however the frequency never reaches the frequency of intact cells. Under comparable conditions the frequency in intact cells is higher by a factor of two to four.

What is the role of glycolytic and respiratory oscillations? The glycolytic oscillations in cell-free extracts and intact cells are pronounced when the cells are harvested around the growth phase transition from using glucose to ethanol [30,49]. This is also the phase where most storage carbohydrates are synthesised [93] and in intact cells about half of the glucose is converted to storage carbohydrates during the oscillatory metabolism [42]. This might suggest that glycolytic oscillations are related to storage carbohydrates. Oscillatory versus steady state metabolism might have a role in directing the metabolism towards different products. Another role for the glycolytic oscillations was suggested by Goldbeter [94] to be related to the energy charge. The energy charge oscillates between values that alternately activate or inhibit enzymes of the biosynthetic or ATP-regenerating pathways. The role of the oscillations is then to switch between the opposite pathways and thereby avoiding futile cycles. In intact cells during glycolytic oscillations the energy charge oscillates between 0.6 and 0.9 [40]. Information about the energy charge during respiratory oscillations is not available.

Do glycolytic oscillations have benefits over steady state glycolysis with respect to speed or efficiency? A possible benefit of a different mode of metabolism is a faster utilisation of glucose in order to use the glucose before a possible competitor. That the oscillatory metabolism is faster was suggested by Pye [92] who reported slower ethanol production after the oscillations had died out. Another possible benefit of an oscillatory metabolism is that the efficiency of free energy conversion is higher, or in other words glucose metabolism yields more chemical energy such as ATP and less dissipation of heat. It was suggested by Richter and Ross [95,96] that an oscillatory mode would have a 5–10% increased efficiency, however any experimental evidence is lacking. It might be that glycolytic oscillations are just a metabolic accident that results from the autocatalytic nature of glycolysis and the regulatory properties of phosphofructokinase as suggested by Goldbeter [16] and that the possibility of oscillatory metabolism was not eliminated during evolution because it is not unfavourable for the cell.

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References


volvement of glutathione in the regulation of respiratory oscillation during a continuous culture of Saccharomyces cerevisiae. Microbiology 145, 2739–2745.


