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Oscillations in glycolysis in *Saccharomyces cerevisiae*: The role of autocatalysis and intracellular ATPase activity

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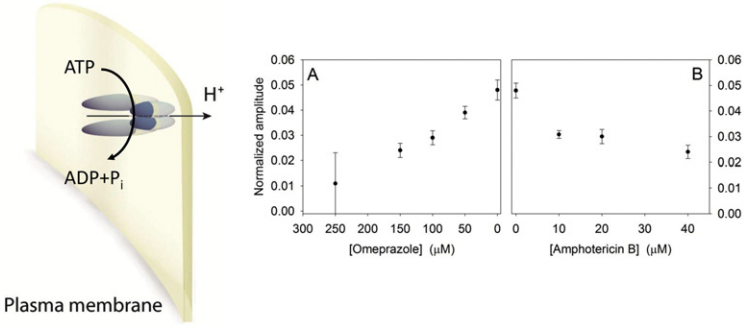
HIGHLIGHTS

- ► We studied the role of ATPase activity on the glycolytic oscillations in yeast.
- ► Our study includes modulation of ATP activity and mathematical modelling.
- ► Both inhibiting and stimulating ATPase activity suppress glycolytic oscillations.
- ► Simulations of a simple autocatalytic model of glycolysis support our data.

GRAPHICAL ABSTRACT



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ABSTRACT

We have investigated the glycolytic oscillations, measured as NADH autofluorescence, in the yeast *Saccharomyces cerevisiae* in a batch reactor. Specifically, we have tested the effect of cell density and a number of inhibitors or activators of ATPase activity on the amplitude of the oscillations. The amplitude dependence on cell density shows the same behavior as that observed in cells in a CSTR. Furthermore, the amplitude decreases with increasing inhibition of the three ATPases (i) F_0F_1 ATPase, (ii) plasma membrane ATPase (Pma1p) and (iii) vacuolar ATPase (V-ATPase). The amplitude of the oscillations also decreases by stimulating the ATPase activity, e.g. by FCCP or Amphotericin B. Thus, ATPase activity strongly affects the glycolytic oscillations. We discuss these data in relation to a simple autocatalytic model of glycolysis which can reproduce the experimental data and explain the role of membrane-bound ATPases . In addition we also studied a recent detailed model of glycolysis and found that, although this model faithfully reproduces the oscillations of glycolytic intermediates observed experimentally, it is not able to explain the role of ATPase activity on the oscillations.

1. Introduction

Oscillatory dynamics are found in many different biological contexts [1]. Oscillations may serve various purposes such as time keepers [2,3], signaling and synchronizing purposes [4,5] and as protection of enzymes against harmful products [6].

Glycolytic oscillations in cells of the yeast *Saccharomyces cerevisiae* kept under anaerobic or semianaerobic conditions have been studied for over 50 years, both in intact cells and in cell extracts [1]. The function of these oscillations is not known, but recently it was shown, using mathematical modeling, that oscillations may appear as an inevitable side effect in the tradeoff between hard robustness and efficiency of metabolic pathways [7]. It has become clear that in suspensions of yeast cells all the cells oscillate in phase [8]. Synchronization of the cells' metabolism is mediated by acetaldehyde [9–11] and possibly also to a lesser extent by other glycolytic intermediates [4]. Furthermore,

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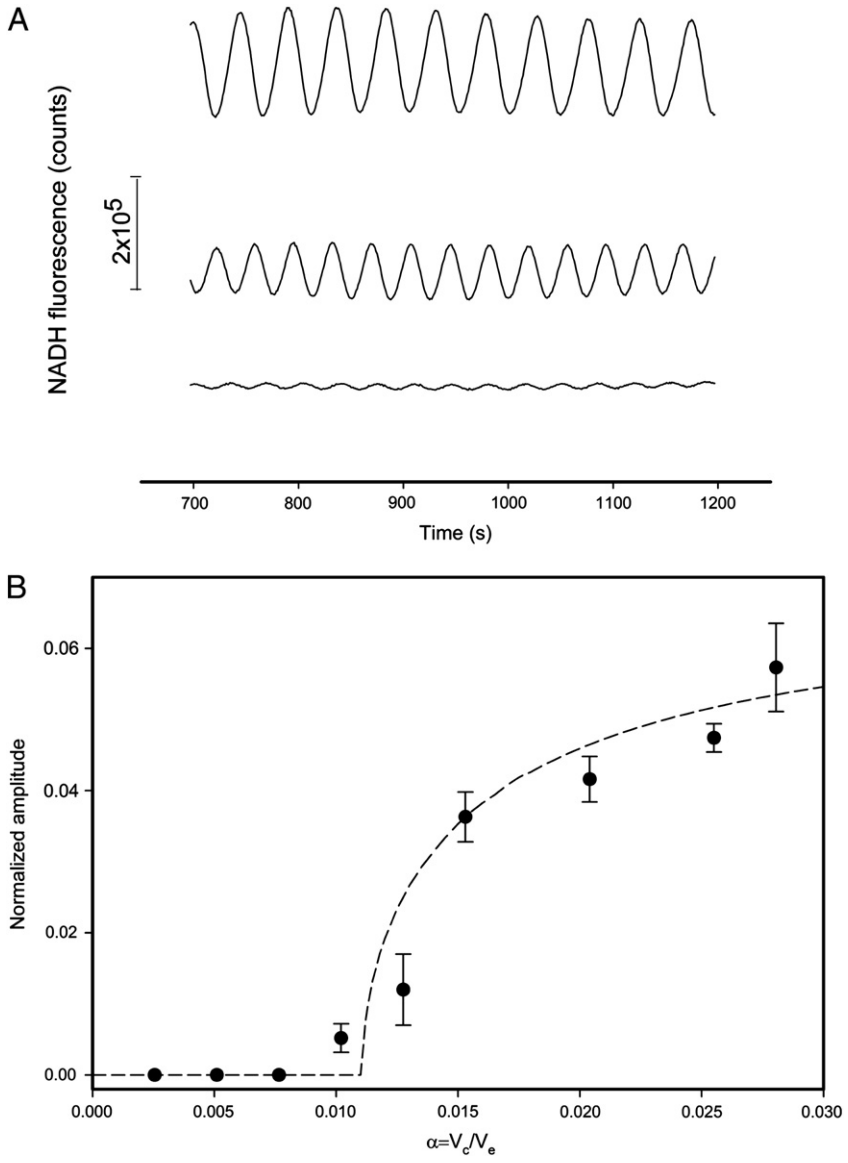

Fig. 1. Effect of cell density on the amplitude of the NADH oscillations. A: NADH oscillations at three different cell densities corresponding to a) 2.72, b) 4.08 and c) 7.48 mg protein/ml, respectively. B: Plot of the amplitude of oscillation versus the ratio of the cytoplasmic volume to the exernal volume (α). The amplitude is normalized to the average fluorescence at a particular density. The dashed line represents the predicted relationship between oscillation amplitude and α obtained by solving Eq. (3) in the text using the following parameters w_0=0.17 s^{-1} (omega$_0$=0.17 s^{-1}) $\tau = 0.16\ s^{-1}$, $g = -3.6\ s^{-1}$, $c = 850$ and $\lambda_0 = 0.017\ s^{-1}$.

the glycolytic oscillations represent a collective phenomenon, that is, while a suspension of cells will show oscillations when the cell density surpasses a certain critical density [12–14], oscillations in glycolysis in cells in suspensions at low cell density are either absent or desynchronized [14,15].

Oscillations in yeast glycolysis in intact cells are usually detected as oscillations in NADH autofluorescence. However, direct measurements of other metabolites in intact cells or analyses of cell extracts have shown that other glycolytic intermediates, including ATP and ADP, oscillate as well [16–18]. Furthermore, other variables, such as the mitochondrial membrane potential and intracellular pH were also found to oscillate with periods similar to that of NADH [19,20]. It was suggested that the latter oscillations could be ascribed to the activity of intracellular ATPases such as the mitochondrial F_0F_1 ATPase and the plasma membrane ATPase (Pma1p). This suggestion was supported by studies of the effects of inhibitors and activators of these two ATPases on the glycolytic oscillations [18,20] and by studies of the dynamics of glycolysis in yeast strains devoid of these two ATPases [21]. It was found that the amplitude of the oscillations, but not the frequency, apparently depends on the activity of intracellular ATPases [20].

Glycolysis encompasses 10–15 enzymatic reactions, and many mathematical models of varying complexity have been proposed to explain and simulate the oscillations obtained experimentally [1,22–25]. Analyses of these models and of experimental data suggest that the oscillatory behavior cannot be ascribed to a single reaction of glycolysis [24,26], but is a property of the entire biochemical pathway. Most models focus on the central regulatory role played by phosphofructokinase [1], but other models also include the regulatory properties of hexokinase and pyruvate kinase [23–25]. However, in glycolysis ATP metabolism is also autocatalytic, i.e. two molecules of ATP are consumed in the catabolism of one hexose molecule, but four molecules of ATP are produced in the subsequent conversion of the hexose bisphosphate to the ultimate products (lactate in mammals and ethanol in yeast) in anaerobic glycolysis. It is well known that autocatalysis may lead to oscillatory behavior [27–29] and autocatalysis has previously been explored in simple models of yeast glycolysis [30–33]. These studies revealed that autocatalysis may, at least to some extent,

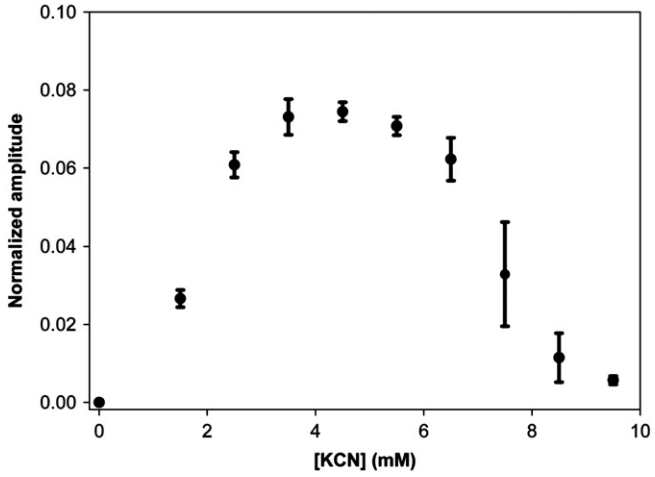

Fig. 2. Effect of the concentration of KCN on the normalized amplitude of oscillations. 30 mM glucose and various concentrations of KCN were added to a dense suspension of starved yeast cells, corresponding to 6.8 mg protein/ml. Oscillations were recorded as NADH fluorescence and the amplitude was normalized to the mean NADH fluorescence. The normalized amplitude was subsequently plotted against the KCN concentration. Temperature 25 °C.

account for the oscillatory behavior and metabolic waves in yeast cell suspensions and in yeast cell layers.

Here we present new experimental data on glycolytic oscillations in intact yeast cells, e.g. the dependence of oscillation amplitude on the activity of intracellular ATPases. We then explore a simple autocatalytic model for its ability to simulate some of the experimental observations of glycolytic oscillations. Our simulations show that this simple model is indeed capable of simulating and explaining most of the experimental data. Specifically, the model supports the earlier conclusions about the importance of membrane-bound ATPases in regulating the oscillatory dynamics [18,20,21]. In addition, we have also studied a recently proposed detailed model of oscillations in yeast glycolysis. Although this model is capable of simulating many of the experimental measurements of glycolytic intermediates it fails to simulate the regulation of intracellular ATPases. Hence, we conclude that much work still needs to be done in order to establish a credible detailed mechanism for the glycolytic oscillations in the intact yeast cell.

2. Materials and methods

2.1. Reagents

All reagents were purchased from Sigma-Aldrich (Munich, Germany).

2.2. Yeast cells

Yeast cells, *S. cerevisiae* diploid strain X2180, were grown and harvested as described in Poulsen et al. [34]. The cells were starved for three hours at room temperature in 100 mM potassium phosphate buffer, pH 6.8, before use. In all measurements cells were suspended in 100 mM potassium phosphate buffer, pH 6.8. The cells' protein content was determined according to Lowry et al. [35]. It was found that a suspension of yeast of 10% wet weight corresponded to 6.8 mg protein/ml.

2.3. Fluorescence measurements of NADH and ATP

NADH autofluorescence (excitation 366/3 nm, emission 450/10 nm) was measured in a FL920 Spectrofluorometer (Edinburgh Instruments, Edinburgh, Scotland) or in a SPEX Fluorolog spectrofluorometer (HORIBA Scientific, Kyoto, Japan). The cells were suspended in a stirred 2 ml sample in a 1 cm × 1 cm × 4.5 cm quartz cuvette containing 100 mM potassium phosphate, pH 6.8. Oscillations were induced by addition of 30 mM glucose and 5 mM KCN, unless otherwise indicated in figure legends. In all experiments presented, except for those in Fig. 1, the cell density was 10% wet weight corresponding to a protein content of 6.8 mg/ml.

Inhibition or activation of ATPase activity was carried out by incubating the yeast suspension at 25 °C with the indicated amount of inhibitor for 3–5 min before adding glucose and KCN, except for inhibition with omeprazole which was carried out as described in [20] and inhibition with concanamycin A was done for 30 min at 30 °C.

Measurements of intracellular ATP using an aptamer-based nanobiosensor were done essentially as described earlier [18,21]. However, here the ATP concentration was estimated from a calibration curve constructed by measuring the fluorescence from the ATP sensor in mixtures of ATP and ADP where the total concentration of ATP plus ADP is 4 mM (Fig. S3 in the supplementary material) to match the total concentration of these two nucleotides in yeast cells [17,18].

Measurements of intracellular glucose 6-phosphate were made by quenching the cells with boiling buffered ethanol and subsequently extracting the metabolites as described by Gonzales et al. [36]. The concentration of glucose 6-phosphate was then measured by addition of $NADP^+$ and glucose 6-phosphate dehydrogenase to the extract as described in Gonzales et al. [36]. The concentration of glucose 6-phosphate in the extract was determined from the concentration of formed NADPH using standard curves measured on solutions with known concentrations of glucose 6-phosphate. In the estimation of the intracellular concentration of glucose 6-phosphate we assumed that 1 mg protein corresponds to a cytoplasmic volume of 3.7 μl ([17]).

In all experiments the temperature was 25 ± 0.01 °C. Experiments shown in the graphs represent the average of 3–6 individual runs.

2.4. Experimental results

Oscillations in NADH autofluorescence were induced by addition of 30 mM glucose and 5 mM KCN, unless otherwise indicated, to a dense suspension of yeast cells. A typical trace is shown in Fig. S1 in the supplementary material. First, we determined the dependency of the oscillation amplitude on the cell density.

Fig. 1 shows the effect of cell density, expressed as cytoplasmic volume over external volume ($\alpha = V_c/V_e$), on the amplitude of the oscillations. α was determined from the measured protein concentration (in mg/ml) and using that 1 mg of protein corresponds to a cytoplasmic volume of 3.7 μl. We note that below a threshold corresponding to about $\alpha = 0.011$ only damped oscillations are obtained. De Monte et al. [14] demonstrated that for glycolytic oscillations observed in yeast cells in a CSTR the equation for a population of synchronized cells can be reduced to a single oscillator coupled to an external medium. This can be described by the following reduced equation:

$$\frac{dz}{dt} = \frac{\alpha c}{\alpha c + 1} f(z) - \frac{\tau z}{\alpha c + 1} \tag{1}$$

where z is the variable (complex) in the oscillating plane and f(z) describes the intracellular dynamics in the plane of oscillations and the parameter τ measures the half life of an external perturbation to the steady state. The parameter c introduces a rescaling of the density. The value of c depends e.g. on the interaction of the diffusing metabolite responsible for the synchronization of cells with the core oscillator. Eq. (1) assumes that cells are identical and synchronized. To describe the intracellular dynamics De Monte et al. [14] used the following Hopf normal form

$$f(z) = \left(\lambda_0 + i\omega_0 + g|z|^2\right) z \tag{2}$$

where $\lambda_0 \pm i\omega_0$ are the eigenvalues associated with the eigenvectors spanning the plane of oscillations and g is a constant that can be fitted.

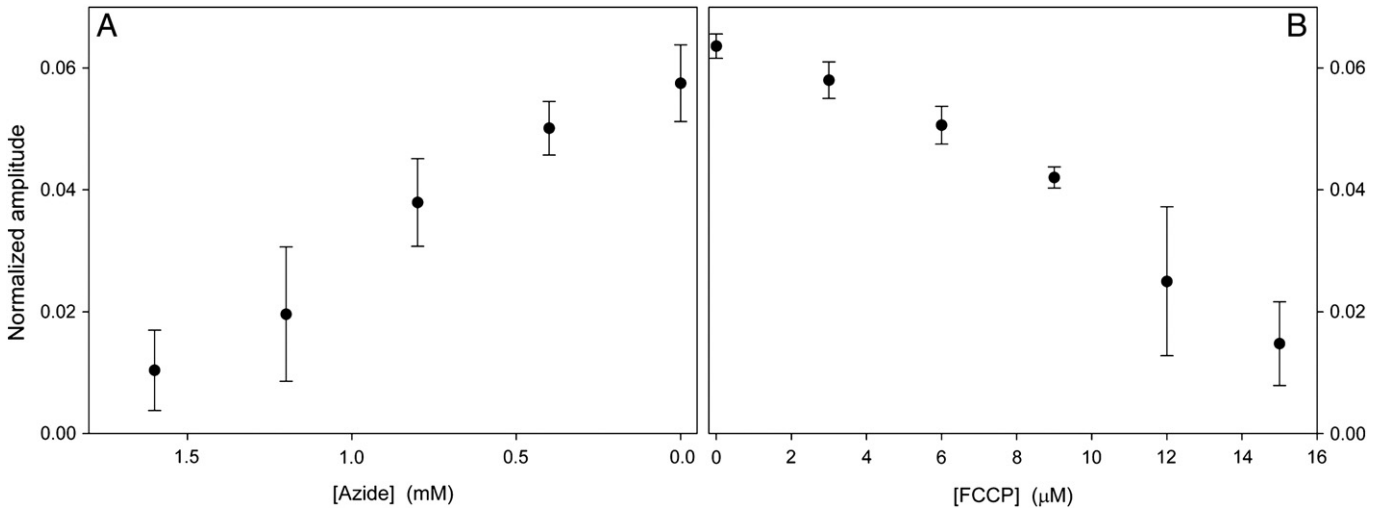

Fig. 3. Effect of inhibiting (A) or stimulating (B) the activity of the mitochondrial F_0F_1 ATPase on the normalized amplitude of oscillations. The ATPase is inhibited by incubating the yeast cells with sodium azide (see text) or stimulated by incubation with FCCP. The normalized amplitudes of NADH oscillations were plotted against the concentrations of azide and FCCP. The axis of azide concentration is inverted such that the direction left to right corresponds to increasing ATPase activity in both plots. Oscillations were induced by addition of 30 mM glucose and 5 mM KCN to the yeast suspension. Temperature 25 °C.

Combining Eqs. (1) and (2) and separating the radial and angular parts of this equation ($z = Re^{i\phi}$) yields the equation:

$$\begin{aligned}\frac{dR}{dt} &= \frac{\alpha c}{\alpha c+1}\left(\lambda_0 + gR^2\right)R - \frac{\tau}{\alpha c+1}R \\ \frac{d\phi}{dt} &= \frac{\alpha c}{\alpha c+1}\omega_0\end{aligned} \tag{3}$$

We solved Eq. (3) using the parameters ω_0, λ_0, c, τ and g proposed by De Monte et al. [14] except for λ_0, which was estimated here as 0.017 s^{-1} instead of the value of 0.015 s^{-1} estimated by De Monte et al. [14]. The normalized amplitude of the resulting oscillations is plotted as the dashed line in Fig. 1. We note that the data in Fig. 1 correspond well to those presented by De Monte et al. [14], although the latter were obtained in a CSTR and not, as here, in a batch reactor. Hynne et al. [23] suggested that under the conditions used here batch and CSTR experiments are compatible because the glucose influx into the cells stays nearly constant even though the extracellular glucose concentration is changing, because the glucose transporter is saturated. In separate experiments using mass spectrometry we verified that the glucose flux was constant and almost independent of the extracellular glucose concentration in the range 5–30 mM (see Fig. S2 in the supplementary material). Fig. S2 also shows a time series of ethanol production and a simulated time series produced using the estimated Michaelis–Menten parameters.

Fig. 2 shows the effect of the cyanide concentration on the relative amplitude of the NADH oscillations. At low concentration (<2 mM) or at high concentration (>10 mM) of cyanide the amplitude is zero, i.e. the oscillations disappear. The oscillations have maximum amplitude around 5–6 mM cyanide. The role of cyanide is twofold: (i) it serves to arrest respiration, and (ii) it reacts with acetaldehyde released by the cells, maintaining a low concentration of acetaldehyde in the external solution [37]. If the acetaldehyde concentration becomes too high or too low the cells no longer synchronize their metabolism, and the oscillations disappear. At low concentrations cyanide reacts too slowly with acetaldehyde and the concentration of acetaldehyde becomes relatively high. Therefore, the amplitudes of the acetaldehyde oscillations become too small to be able to synchronize the cells' metabolism. At high concentrations of cyanide the acetaldehyde concentration, and hence also the amplitude of acetaldehyde oscillations, becomes too

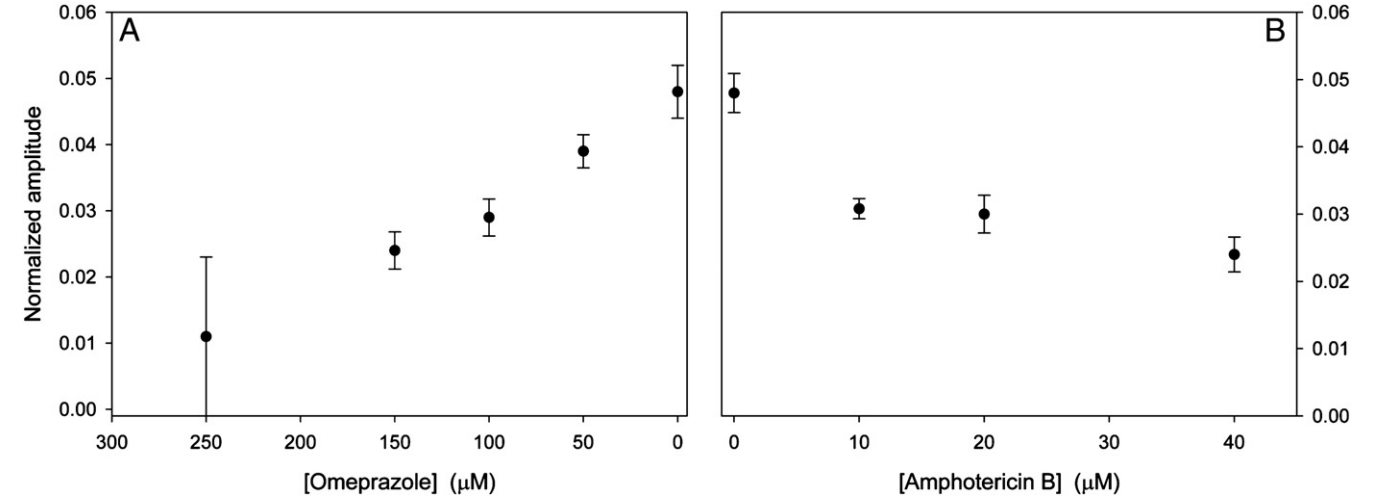

Fig. 4. Effect of inhibiting (A) or stimulating (B) the activity of the plasma membrane ATPase (Pma1p) on the normalized amplitude of oscillations. The ATPase activity is inhibited by incubation of the yeast cells with omeprazole and the activity is stimulated by incubation with Amphotericin B. The normalized amplitudes of NADH oscillations were plotted against the concentrations of omeprazole and Amphotericin B. The axis of omeprazole concentration is inverted such that the direction left to right corresponds to increasing ATPase activity in both plots. Oscillations were induced by addition of 30 mM glucose and 5 mM KCN to the yeast suspension. Temperature 25 °C.

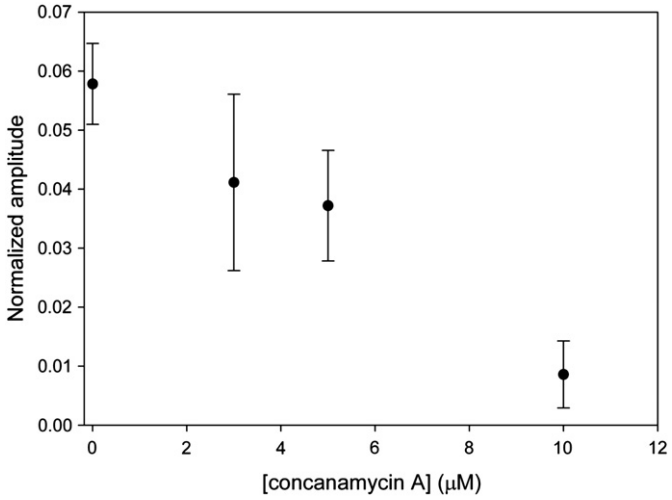

Fig. 5. Effect of inhibition of the activity of the V-ATPase on the normlized amplitude of oscillations. The V-ATPase activity is inhibited by incubation of the yeast suspension with concanamycin A and the normalized amplitude of NADH oscillation was plotted against the concentration of concanamycin A. Oscillations were induced by addition of 30 mM glucose and 5 mM KCN to the yeast suspension. Temperature 25 °C.

low to efficiently synchronize the cells' metabolism. A similar effect of cyanide on the oscillation amplitude was observed previously in a batch reactor [37] and also for yeast cells in a continuous-flow-stirred-tank reactor (CSTR) [23].

In previous works [18,20,21] we provided evidence that intracellular ATPase activity is important for the occurrence of glycolytic oscillations. Predominantly F_0F_1-ATPase, hydrolyzing ATP and hence pumping protons, and Pma1p were shown to be responsible for this ATPase activity. In most models of oscillating glycolysis unspecific ATPase activity is modeled as a single first order reaction (e.g. refs. [1,23,25]). However, so far no modeling study has been made to demonstrate how this ATPase activity is expected to affect the oscillations. In the following we shall present experimental data where we have gradually changed the intracellular ATPase activity, either by the use of inhibitors of ATPase activity or by stimulating the ATPase activity by addition of ionophores which increase the proton permeability of mitochondrial and plasma membranes. Later we will attempt to simulate these data by a simple mathematical model. Fig. 3 shows the effect of increasing concentrations of azide and FCCP on the amplitude of NADH oscillations. Azide is generally known as an inhibitor of cytochrome aa_3, but it also acts as a strong inhibitor of the ATP hydrolyzing activity of F_0F_1-ATPase [38]. FCCP acts as a fast carrier of protons across the mitochondrial,

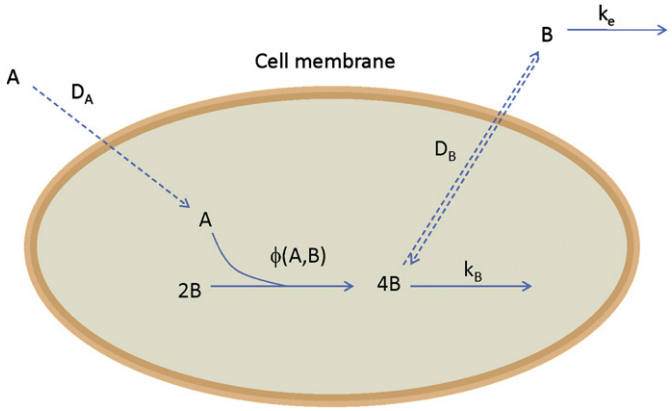

Fig. 6. Illustration of the autocatalytic model with diffusion. In the model glycolysis is reduced to a single autocatalytic reaction with two reactants, A and B, where the latter is the autocatalytic species. Species B is also serving as a synchronizing agent by rapid diffusion across the cell membrane. See the text for more details.

Table 1
Parameters used in the simulations of the simple autocatalytic model of glycolysis.

Parameter	Value
$[A]_e$	1
D_A	0.01
ϕ_{max}	40
K_A	1
K_B	0.1
L	372
D_B	2
k_B	0.57
k_e	0.28
α	0.2

and possibly also other cellular membranes. The increased diffusion of protons across the inner mitochondrial membrane accelerates the hydrolysis of ATP. In Fig. 3A the axis of azide concentration has been inverted such that the direction left to right corresponds to increasing ATPase activity in both Fig. 3A and B. We note that the amplitude of oscillation has its optimum at intermediate ATPase activity and declines both at high and low ATPase activity.

We also tested if inhibition or stimulation of the Pma1p activity would affect the oscillations. Fig. 4 shows the effect of omeprazole and amphotericin B on the normalized amplitude of NADH oscillations. Omeprazole is a strong inhibitor of the H^+-K^+-ATPase of the gastric mucosa [39]. Omeprazole has also been shown to inhibit other P-ATPases including the *S. cerevisiae* Pma1p [40]. Amphotericin B, on the other hand, is stimulating the permeability of the plasma membrane to protons. Amphotericin B induces leakage of K^+ out of the cell and influx of protons from the environment [41]. This is expected to stimulate the Pma1p ATPase activity. We note that increasing concentrations of both omeprazole and amphotericin B induce a decrease in the normalized amplitude. Thus, inhibition as well as stimulation of Pma1p activity will affect the glycolytic oscillations.

Having confirmed that intact ATPase activity in both the plasma membrane and the mitochondrial membrane [18,20] are important for the glycolytic oscillations we also tested if other ATPases could have an effect on the oscillations. Concanamycin A is an inhibitor of the vacuolar ATPase (V-ATPase) [42]. The effect of concanamycin A on the oscillations is shown in Fig. 5. We note that the amplitude of oscillations decreases with increasing concentration of concanamycin A and conclude that also V-ATPase activity is important for the oscillations to occur. A similar result to that shown in Fig. 5 was obtained with bafilomycin A1, which is also an inhibitor of Pmap1 activity [43].

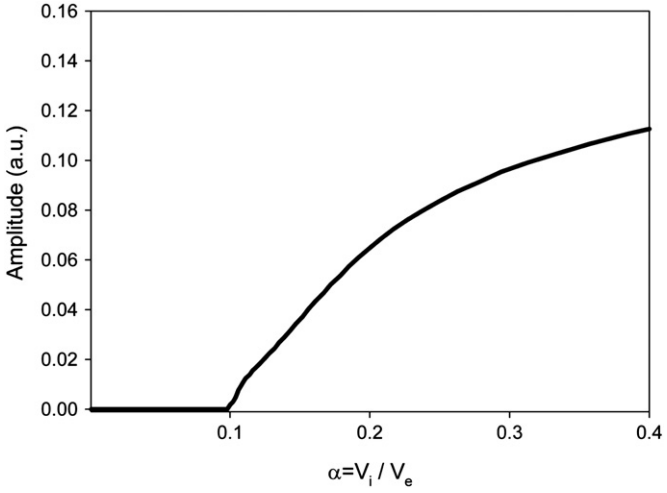

Fig. 7. Effect of changing the dilution factor α on the amplitude of oscillations of species B in the simple autocatalytic model (Eqs. (4)–(6)). Other parameters as in Table 1.

Finally, we measured time-resolved intracellular concentrations of ATP and glucose 6-phosphate to compare with the NADH oscillations. These are presented in Fig. S4 in the supplementary material. The oscillations in ATP and glucose 6-phosphate are almost in phase and about 180° out of phase with NADH.

2.5. Modeling the glycolytic oscillations

In most cases models of metabolic networks can be divided into simple and detailed models. In the latter details about the individual reaction steps in the metabolic network are included in the reaction scheme. The advantage of this type of modeling is that the models become more realistic even though certain features such as molecular crowding are usually not considered. The disadvantage of these models is that a deep understanding of the dynamic behavior of the model cannot always be obtained. In the simple models only the essential features of the metabolic pathway are included. Thus, it is usually possible to get a fairly good understanding of the model properties which are responsible for the model's dynamic behavior. At the same time we lose the ability to model most of the intermediates in the reaction network.

Here we have simulated glycolytic oscillations using both detailed and simple models in order to reproduce and understand the experimental results obtained in the previous section. Ideally, any credible model of glycolysis in yeast should be able to reproduce at least the following features;

1. The experimentally determined concentrations of metabolites (see below).
2. The sinusoidal waveform of oscillations in NADH and other metabolites (see below).
3. The dependency of the amplitude on cell density (Fig. 1).
4. The dependency of amplitude on the removal rate of acetaldehyde (Fig. 2).
5. The dependency of oscillations on intracellular ATPase (F_0F_1, Pma1p and V-ATPase) activity (Figs. 3–5).

Our primary goal is to understand the role of ATPase activity on the oscillatory dynamics rather than to simulate in detail the dynamics of all metabolites in the glycolytic pathways. Therefore in our first attempt we have refrained from simulating the experimental data with a detailed model of glycolysis as such a model may mask the behavior of these ATPases, and , furthermore, our knowledge about the kinetics is insufficient to set up detailed kinetic expressions for these enzymes. However, glycolysis is known to be autocatalytic and we therefore attempted to simulate the experimental data of Figs. 1–5 using a simple autocatalytic model in order to obtain a better understanding of the phenomena responsible for the change in dynamics induced by the various inhibitors. If this model can reproduce and explain the experimental data presented here then a detailed model similar to those proposed by Hynne et al. [23] and Hald and Sørensen [25] should also be capable of simulating these data in addition to simulating the temporal changes of glycolytic intermediates.

2.6. A simple autocatalytic model

Basically, two properties are important for the concerted glycolytic oscillations in intact yeast cells: (i) an oscillophore and a diffusing species. As for the former, the complex regulation of phosphofructokinase has often been considered as the main source of the oscillations. Concerning the latter, most evidence point to acetaldehyde as the diffusing species. However, a simpler and equally realistic mechanism that could explain the oscillations is that the production of ATP in glycolysis is autocatalytic. Two molecules of ATP are used to phosphorylate hexose in the upper part of glycolysis, but four molecules of ATP are formed in the lower part of the pathway. It is well known that autocatalytic reactions may lead to oscillatory behavior [27]. The current model of yeast glycolysis is based on the autocatalytic production of ATP in the cell, and hence ATP should be the primary metabolite in the model. However, in order to reduce the complexity of our model as much as possible and at the same time make the model generally applicable to other similar systems the model is expressed in general terms using A and B rather than specific names for the involved chemical species. Species A represents pooled species upstream of phosphofructokinase, while species B represents ATP and other species downstream of phosphofructokinase. Central to the cellular system is an autocatalytic reaction transforming the substrate *A* into the product *B* (last term in Eq. (4) and middle term in Eq. (5)). The product *B* can be converted into downstream metabolites. In order not to have to introduce more reactions than are necessary we further assume that the autocatalytic species B is also diffusing rapidly across the cell membrane and hence serves as the synchronizing metabolite. This situation could be translated into yeast glycolysis by assuming that the intracellular concentration of acetaldehyde is somehow proportional to the concentration of ATP. To test this we simulated the detailed model of Hald and Sørensen [25] and found that ATP and acetaldehyde are oscillating in phase, which lends some support to our assumption. Thus, our simplifying assumption does not change the general behavior of the model. The model is implemented as a population of cells with autocatalytic kinetics coupled by diffusion to a homogeneous extracellular suspension.

The basic mechanism of the model is shown in Fig. 6. The rate equations for the changes of A and B concentrations in a single cell and the concentration of B in the external solution are the following:

$$\frac{d[A]_i}{dt} = D_A([A]_e - [A]_i) - \phi(\mathrm{A},\mathrm{B}) \tag{4}$$

$$\frac{d[B]_i}{dt} = D_B([B]_e - [B]_i) + 2\phi(\mathrm{A},\mathrm{B}) - k_B[B]_i \tag{5}$$

$$\frac{d[B]_e}{dt} = \frac{\alpha}{n}\sum_{i=1}^{n} D_B([B]_i - [B]_e) - k_e[B]_e \tag{6}$$

$$\phi(A,B) = \frac{\phi_{\max}\frac{[A]_i}{K_A}\frac{[B]_i}{K_B}\left(1+\frac{[B]_i}{K_B}\right)}{L + \frac{[A]_i}{K_A} + \left(1+\frac{[B]_i}{K_B}\right)^2}$$

Here $[A]_i$ and $[B]_i$ represent intracellular concentrations of species A and B in cell no *i*, while $[A]_e$ and $[B]_e$ represent the concentrations of species A and B in the external solution. The first term in the rate expression for $[A]_i$ (Eq. (4)) represents the diffusion of species A into the cell, which is assumed to be simple Fick's diffusion with the constant

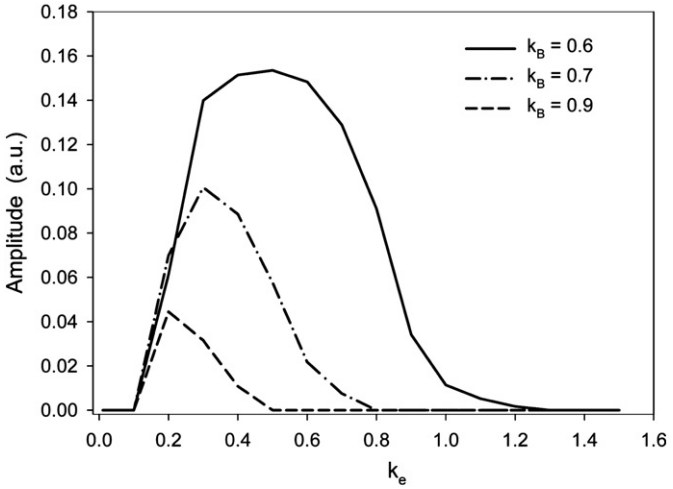

Fig. 8. Effect of changing the rate constant k_e on the amplitude of oscillations of species B in the simle autocatalytic model (Eqs. (4)–(6)). Other parameters as in Table 1.

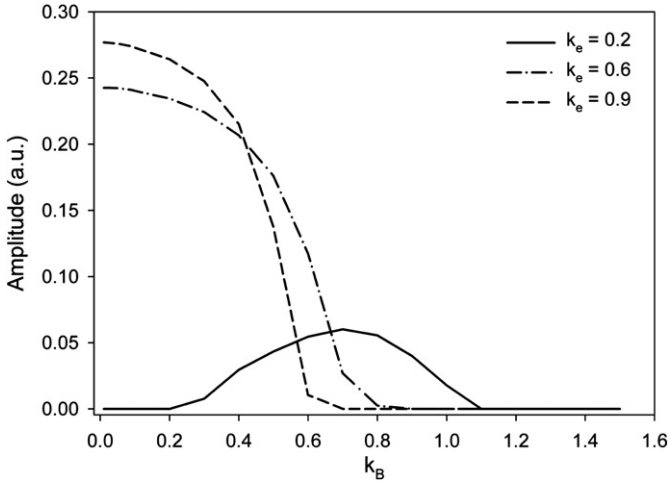

Fig. 9. Effect of changing the rate constant k_B on the amplitude of oscillations of species B in the simle autocatalytic model (Eqs. (4)–(6)). Other parameters as in Table 1.

diffusion coefficient D_A. The concentration of species A in the extracellular solution ($[A]_e$) is assumed to be constant. The second term represents the lumping of all the enzymatic steps in glycolysis into one single enzyme rate expression. The expression displays simple Michaelis–Menten kinetics with respect to species A and cooperative kinetics with respect to species B. ϕ_{max} is the maximum rate and K_A and K_B represent the dissociation constants for the binding of A and B to the enzyme. The constant L has the usual meaning for an enzyme with positive cooperativity. The rate expression for $[B]_i$ (Eq. (5)) consists of three terms. The first term represents diffusion of B into (and out of) the cell, which again is modeled as a simple Fick's diffusion with a constant diffusion coefficient D_B. The second term is the enzyme-catalyzed production (and consumption) of B which is the same term as in the rate expression for species A (Eq. (4)). In this lumped reaction two molecules of B are consumed, but four molecules are subsequently formed and hence the contribution is positive and the expression is multiplied by a factor of 2. The last term represents the intracellular breakdown of B which is modeled as a simple first order reaction with a rate constant k_B. The rate expression for species B in the extracellular solution (Eq. (6)) includes the sum of diffusion terms of B multiplied by the dilution factor α, again representing the ratio of the intracellular volume to the extracellular volume (V_i/V_e) and divided by the number of cells. In addition Eq. (6) includes a first order term ($k_e[B]_e$) representing the irreversible removal of B from the external medium to simulate the equivalent removal of extracellular acetaldehyde by evaporation [34] or by reaction with cyanide [37].

The model presented here is not conceptually different from the normal form equations studied by De Monte et al. [14] (Eq. (2)), but since it includes terms which are compatible with the biochemistry of the cell it allows us to investigate other experimental observations in addition to the sensitivity of the oscillations to cell density. Thus, our model represents an intermediate between the abstract normal form presented by Demonte et al. [14] and more detailed mechanistic models [22,23,25]. The model was simulated using 4, 10 and 20 cells. The simulation results were identical, irrespective of the number of cells. Simulations were performed using Berkeley Madonna (University of California at Berkeley, CA) or COPASI (Virginia Bioinformatics Institute, VA; University of Heidelberg, Germany; University of Manchester, U.K.). The parameters used in the simulations are listed in Table 1.

2.7. Simulation results

Fig. 7 shows the effect of changing the ratio of the internal volume to the external volume ($\alpha = V_i/V_e$) on the amplitude of oscillations of the intracellular species B. At low cell densities ($\alpha < 0.1$) there are no oscillations of this species, i.e. in all cells the concentration of species A and B converge to a steady state. As α increases towards 0.1 damped oscillations are obtained, and here all cells oscillate in phase. As α becomes greater than 0.1 sustained oscillations, again with all cells oscillating in phase, are obtained. The change in amplitude is not quite the the same as that obtained with the experiments, which is probably due to the fact that the transition from steady state to oscillation does not follow a supercritical Hopf bifurcation as proposed by De Monte et al. [14] (data not shown) in the sense that if μ is any parameter in the model and μ_0 is at the bifurcation point then the amplitude *does not* grow linearly with $\sqrt{\mu-\mu_0}$ as is the case for a supercritical Hopf bifurcation and as observed in the experiments ([4,23]). This also means that the oscillations in B, unlike the experimental measurements of metabolites, are not always sinusoidal. Nevertheless, the simulations demonstrate that a relative decrease in extracellular volume, corresponding to an increase in cell density can both induce oscillations in the individual cells and maintain synchrony between the metabolisms in the individual cells.

Fig. 8 shows the effect on the amplitude of oscillation of species B of changing the rate constant k_e, which controls the breakdown of extracellular species B. This process is analogous to the extracellular breakdown of acetaldehyde through reaction with cyanide. We note that we obtain a similar bell-shaped form to that of Fig. 2, in accordance with the suggestion that the role of cyanide is to trap acetaldehyde in the extracellular medium. These simulations therefore support the hypothesis that the removal of acetaldehyde is necessary in order to maintain synchronization between the cells.

Another critical test of the model is to determine if it can reproduce the experimental observations of the dependence of the oscillation amplitude on intracellular ATPase activity (Figs. 3 and 4). Here we found that oscillations require a certain ATPase activity. If the activity is too high or too low the oscillations disappear [20,21]. In our simple autocatalytic model ATPase activity is represented by the rate constant k_B. Fig. 9 shows a plots of the oscillation amplitude against k_B for different values of the rate constant k_e. We note that for $k_e = 0.2$ the amplitude has a similar dependence on k_B as that shown in Figs. 3 and 4 for the dependence of oscillation amplitude on F_0F_1 ATPase and Pma1p activity.

2.8. Results with other model of glycolysis

We also attempted to simulate the experimental data using a detailed model of glycolysis [25]. The model was first simulated with the parameter set used in the original publication [25]. As seen in Fig. S5 in the supplementary material this model generates data for NADH, ATP and glucose 6-phosphate that essentially match those presented in Fig S4 of the supplementary material, both concerning the phase shift in relation to oscillations in NADH and the mean concentration of metabolite.

We have not yet investigated in detail synchronization between cells with this model and the effect of cell density on the oscillation amplitude. We did not observe synchronization between cells with the set of parameters proposed by Hald and Sørensen [25], since the simulated amplitude of acetaldehyde oscillation is relatively small (10–20% of the mean concentration) and hence probably not sufficient to mediate synchronization of the cells' metabolism. However, with a different set of parameters we did obtain synchronization through acetaldehyde. Under these conditions the amplitudes of oscillations in external acetaldehyde were much larger. The simulations revealed a critical cell density below which no oscillations were seen. However, this critical cell density is very low and the increase in oscillation amplitude beyond the critical cell density was sigmoidal as opposed to the form observed in the experiments (Fig. 2). The model also reproduces the effect of cyanide (Fig. 3) reasonably well, but it fails to reproduce the effect of ATPase activity on the oscillation amplitude (Figs. 4 and 5). In this model we observe a decrease in oscillation amplitude when ATPase activity is increased, but there is no decrease in amplitude

when the ATPase activity is decreased (data not shown). It is not clear if it would be possible to reproduce the experimental data on ATPase activity with yet another set of parameters. In addition, with the new set of parameters the variables of the model exhibited relaxation oscillations instead of the sinusoidal oscillations obtained in the experiments.

We have also investigated extensions of our simple model where the diffusing species is not the autocatalytic species, but a new species downstream of phosphofructokinase. An example of such a model is shown in Fig. S6 in the supplementary material. Simulations of this model produce results that are essentially identical to those shown in Figs. 7–9. Our simulations, which will be presented in a forthcoming publication, show that the precise mechanism of interaction of the diffusing species with the core oscillator is not important. Thus, all that is needed to establish synchronization between individual cells in a dense suspension of yeast cells is a diffusing species that interacts with the core oscillator of intracellular dynamics, in accordance with the results obtained by De Monte et al. [14]. Acetadehyde is the diffusing species in the yeast cells. The intracellular acetaldehyde interacts with the upper part of glycolysis through its effect on NADH, which again is coupled to ATP via the glyceraldehydes 3-phosphate dehydrogenase and phosphoglycerate kinase catalyzed reactions . This may be the reason why ATP and acetaldehyde oscillate in phase.

3. Discussion

The current experimental data and computer simulations support and extend our earlier findings that an optimal intracellular ATPase activity is essential for an oscillating glycolysis in the yeast *S. cerevisiae*. If the ATPase activity is too high or too low the oscillations stop. At least three intracellular ATPases are involved in regulating the oscillations: F_0F_1 ATPase, Pmap1 and V-ATPase. Simulations of the simple autocatalytic model suggest that it is the overall ATPase activity that controls the oscillatory behavior rather than specific roles of the individual ATPases.

Furthermore, our simple autocatalytic model can simulate many of the overall behaviors such as synchronization and the effect of cell density and ATPase activity on oscillation amplitude. The model is not capable of simulating details such as oscillations in the concentrations of individual glycolytic intermediates. However, it is worth noting that autocatalysis by ATP invariably exists also in the detailed models of glycolysis as a consequence of the stoichiometry of ATP consumption and formation in the pathway. The waveform of the oscillations obtained with our simple autocatalytic model is not always sinusoidal and the frequency of the oscillations does not remain essentially constant as observed in the experiments and in many detailed models, e.g. [23,25]. In intact yeast cells the period of glycolytic oscillations changes by less than 20% as long as the temperature is constant [44]. Also, the transition from steady-state behavior to oscillations does not go through a supercritical Hopf bifurcation, which is why the dependence of amplitude on the ratio of intracellular to extracellular volume is different in the model and in the experiments. Therefore, we speculate that the details of reaction kinetics and regulatory properties are perhaps more important for the sinusoidal waveform and for the stability of oscillation period.

The results obtained with our simple autocatalytic model are not specific to the current model, but should apply to most other models where glycolysis is modeled as an autocatalytic process. We have also performed simulations similar to those presented in Figs. 7–9 using the model proposed by Cortassa et al. [30] and obtained essentially the same results (data not shown). Furthermore we studied extensions of the current model to explore if the assumption, that the autocatalytic species in the model is also the species responsible for the synchronization, is acceptable. Adding an extra species to the lower part of the model, which then serves as the synchronizing species, did not change the models behavior as long as this new species is connected to the core oscillator responsible for the dynamics in the same way as acetaldehyde is connected to the core oscillator of glycolysis. Thus, our simple model seems to be a reasonable approximation to the overall behavior of glycolysis.

Our results do not provide much information on the question if cells in suspension below the critical limit where oscillations are observed exhibit glycolytic oscillations that are not synchronized or if all the cells stop oscillating below this critical limit in density. Previously we have shown that single cells in isolation do not exhibit glycolytic oscillations unless they are perturbed by pulses of acetaldehyde that are given with the same frequency as that of the autonomous oscillations [15]. However, the possibility exists that in cell suspensions of low cell density individual cells may still show sustained oscillations that are not synchronized with other cells. Simulations of our simple autocatalytic model can accommodate both scenarios.

The detailed model of Hald and Sørensen [25] reproduces reasonably well the oscillations in glycolytic intermediates as seen by comparing Figs S4 and S5. One exception is glucose, which we previously determined to be around 0.5 mM and non-oscillating [45]. In the model by Hald and Sørensen [25] glucose concentration is less than the experimentally determined value of around 0.5 mM, and it is oscillating. As all detailed models of glycolysis automatically include autocatalysis by ATP we conclude that, since it is possible to simulate all the experimental behaviors presented in Figs. 2–5 with simple autocatalytic models of varying complexity, it should be possible to find parameter sets that will show these behaviors in detailed models like the one of Hald and Sørensen.

In conclusion, when it comes to simulating concentration profiles the detailed models are of course superior to the simple model, where all details are omitted. On the other hand, when it comes to simulating overall behaviors of the experimental system such as cell synchronization and dependence of oscillation amplitude on cell density and ATPase activity the simple model performs better than the detailed model. We conclude from this that a good detailed model of yeast glycolysis has yet to be developed.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.bpc.2012.03.003.

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