

How Yeast Cells Synchronize their Glycolytic Oscillations: A Perturbation Analytic Treatment

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ABSTRACT Of all the lifeforms that obtain their energy from glycolysis, yeast cells are among the most basic. Under certain conditions the concentrations of the glycolytic intermediates in yeast cells can oscillate. Individual yeast cells in a suspension can synchronize their oscillations to get in phase with each other. Although the glycolytic oscillations originate in the upper part of the glycolytic chain, the signaling agent in this synchronization appears to be acetaldehyde, a membrane-permeating metabolite at the bottom of the anaerobic part of the glycolytic chain. Here we address the issue of how a metabolite remote from the pacemaking origin of the oscillation may nevertheless control the synchronization. We present a quantitative model for glycolytic oscillations and their synchronization in terms of chemical kinetics. We show that, in essence, the common acetaldehyde concentration can be modeled as a small perturbation on the “pacemaker” whose effect on the period of the oscillations of cells in the same suspension is indeed such that a synchronization develops.

INTRODUCTION

Glycolysis, the step-by-step breakdown of glucose and the storing of the released Gibbs energy in the form of ATP, is present in almost all organisms. As a simple and fundamental “glycolysis machine,” the yeast cell is the obvious subject for the study of the glycolysis. Under special conditions the concentrations of the metabolites involved in yeast glycolysis engage in sustained oscillations (Betz and Chance, 1965; Hess and Boiteux, 1968; Pye, 1969). More recently, growth, harvest, and starvation conditions of *Saccharomyces cerevisiae* have been found that reliably and reproducibly give rise to sustained oscillations in nongrowing populations of intact cells. These oscillations, furthermore, have been looked at in terms of NADH fluorescence (Richard et al., 1993), heat production (Teusink et al., 1996a), and concentrations of glycolytic intermediates and coenzymes (Richard et al., 1996). Various models have been developed to describe glycolytic oscillations (Goldbeter and Lefever, 1972; Selkov, 1975). One aim of these models is to identify the “engine” of the oscillations in terms of chemical kinetics. An oscillating chemical reaction can arise from a positive feedback, i.e., a reaction that is stimulated by its own product. Some models pointed to product stimulation of the third enzyme in the pathway (phosphofructokinase) as the source of the oscillation (Goldbeter and Lefever, 1972). Other models have stressed the fact that initially Gibbs energy has to be invested and

two ATPs have to be hydrolyzed before, further down the glycolytic pathway, four ATPs are produced, and a net surplus of ATP results (Selkov, 1972; Cortassa et al., 1991). Numerical analysis and local stability analysis have been used to study these models and relate them to experimental observations (Heinrich et al., 1977).

In a metabolic pathway the fluxes and the concentrations generally depend on the activities and kinetic properties of all of the involved enzymes simultaneously (Kacser and Burns, 1973; Westerhoff and Van Dam, 1987). Because the kinetics of most enzymes are nonlinear, it is often unpractical or even impossible to develop a precise analytical description even of steady-state fluxes and concentrations. Most studies have at that point resorted to numerics. We have recently developed an approach to approximate the glycolytic dynamics with analytic functions (Bier et al., 1996). In this approach we summarize the glycolytic dynamics in a yeast cell by the following dynamical system:

$$\dot{G} = V_{in} - k_1GT \quad (1a)$$

$$\dot{T} = 2k_1GT - k_p \frac{T}{K_m + T} \quad (1b)$$

Here G stands for glucose and T for ATP. V_{in} represents the constant inflow of glucose and k_1 represents the phosphofructokinase activity. Note that the ATP has a positive feedback on its own production. The ATP is also broken down according to Michaelis-Menten kinetics. The positive feedback loop of the ATP together with the saturable breakdown (Michaelis-Menten) leads to limit cycle oscillations (Bier et al., 1996). In this reference the model was studied in the presence of an additional nonsaturable ATPase contribution, i.e., a $-k_9T$ term in the expression for \dot{T} . Because we still obtain limit cycles without this linear term and because quantitative accuracy is not the main focus of this article, we shall here study the simpler system (1).

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System 1 does not allow for an analytic solution, but when the system is in the neighborhood of the Hopf bifurcation, which occurs when $K_m\sqrt{(k_1k_p)} = k_p - 2V_{in}$, the standard linear analysis describes the limit cycle well (Bier et al., 1996). In this approximation we let the fixed point (G_0, T_0) of system 1 be the average concentrations around which the metabolite concentrations are oscillating:

$$(G_0, T_0) = \left(\frac{k_p - 2V_{in}}{2K_m k_1}, \frac{2K_m V_{in}}{k_p - 2V_{in}} \right). \quad (2)$$

For the relevant parameter values this point is an unstable vortex. Linear analysis around this point gives a good estimate for the radial velocity of the limit cycle in the neighborhood of the Hopf bifurcation:

$$\omega = \sqrt{\frac{2k_1 V_{in}(k_p - 2V_{in})}{k_p}}. \quad (3)$$

Many of the parameters of system 1 are involved in the expressions for ω , G_0 , and T_0 , and we thus showed how, even with this “bare bone” model, the control of glycolytic oscillations does not reside in a single step in the glycolytic pathway (Bier et al., 1996; see also Baconnier et al., 1993, and Teusink et al., 1996b).

Intact yeast cells can synchronize their oscillations with each other. When two equally large populations of yeast cells that oscillate with opposite phase are mixed, they at first seem to almost extinguish each other. However, in the course of 5 to 15 periods the amplitude grows again and eventually the autonomous oscillation with constant amplitude is reestablished (see Fig. 1) (Ghosh et al., 1971; Richard et al., 1995). This suggests that some oscillating metabolite in the glycolytic chain is able to permeate cellular membranes and affect the glycolytic kinetics of neighboring cells. Our experiments have shown that this metabolite is acetaldehyde (Richard et al., 1995). Somehow acetaldehyde is able to affect the dynamics of the autonomous oscillations such that eventually all cells oscillate in phase again on the same limit cycle. By comparing the relative amplitudes of the various oscillating concentrations, we were able to propose a route along which the oscillations transfer through the metabolic pathway of individual cells and then, via acetaldehyde, to other cells (Richard et al., 1996).

The main focus of this article is to understand how, on the level of chemical kinetics with as simple a model as possible, acetaldehyde can bring about the synchronization. One theoretical study that demonstrated synchronization of metabolic pathways in two communicating cells used a kinetic model that was only loosely based on experimental observations (Wolf and Heinrich, 1997). That demonstration was, moreover, based on a stability analysis of a complicated nonlinear system describing the two coupled pathways.

Here we take a different approach. We conceive the two glycolyzing cells as autonomous oscillators and we describe the effect of the acetaldehyde coupling with a perturbation term. After reducing the chemical kinetics to a core model, perturbation analysis yields a description of the synchronization in terms of system parameters.

MATERIALS AND METHODS

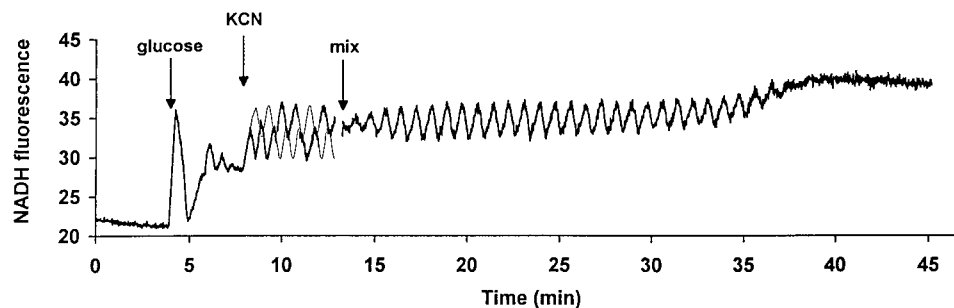
Strain and preparation of cells

The yeast *S. cerevisiae* (X2180 diploid strain) was used. Cells were grown as described previously (Richard et al., 1993). At the diauxic shift, i.e., just after the glucose in the medium had been depleted, the cells were harvested by filtration, washed with 100 mM potassium phosphate (pH 6.8), resuspended, and starved in the same buffer for 2 h at 30°C. The cells were then collected by filtration, resuspended in the same phosphate buffer to a final cell density of 5.2 ± 0.1 mg protein/ml, and placed on ice until use. The protein concentration was determined according to Lowry et al. (1951).

Induction and monitoring of the oscillations and their synchronization

Oscillations were induced at 21°C by adding 30 mM glucose to the starved cells and 3 mM KCN after 4 min. The oscillations were monitored by NADH fluorescence (Shimadzu RF-5001PC spectrofluorimeter; excitation 352 nm, emission 462, bandwidth 10 nm) in a stirred and thermostatically regulated cuvette. When assaying synchronization, oscillations were induced in two cuvettes 24 s after each other, so that the cell populations oscillated 180° out of phase. Only one cuvette could be followed at a time, so the cuvettes were alternately put in the light beam (*thick lines* in Fig. 1), while the other cuvette was stirred and thermostatted. The signals were interpolated with a sine function. Subsequently, equal amounts of the two cell suspensions were mixed and the NADH fluorescence of the mixed suspension was followed. Initially the amplitude of oscillation was low as expected, since the oscillations in the two cell populations should cancel each other out. Within a few cycles, however, the oscillation was observed

FIGURE 1 Two suspensions of yeast cells that oscillate with equal amplitude and opposite phase are mixed. We again observe that macroscopic oscillations develop and that the amplitude increases until a limit is reached asymptotically. This behavior is indicative of limit cycle dynamics.



to increase its amplitude to a stationary level. After mixing the oscillation was sustained until glucose was depleted at $t = 36$ min.

The proposed mechanism

The glycolytic oscillations discussed here are transient under anaerobic conditions, but sustained in the presence of cyanide. Cyanide inhibits respiration and also removes excess acetaldehyde from the system. The latter process is essential for keeping the acetaldehyde concentration in a range in which it can affect the glycolytic dynamics (Richard et al., 1995). Acetaldehyde is only one step, the ethanol dehydrogenase step, removed from ethanol, which is the final product. At an earlier stage in glycolysis, at the glyceraldehyde-phosphate dehydrogenase (GAPdeh) reaction, NADH is formed from NAD. The production of one NAD toward the very end of the glycolytic pathway thus closes a moiety conserved cycle (see Fig. 2). At the GAPdeh reaction the dynamics of the NADH/NAD ratio is coupled with the dynamics of the ATP/ADP ratio. The latter ratio is engaged in the presumed pacemaking of the oscillations in our model.

Experiments are conducted in a well-stirred cell suspension and acetaldehyde readily permeates cell membranes. For a high enough concentration of cells, a sufficiently permeable membrane, and a long enough period of oscillation, we can assume that the acetaldehyde concentration is completely homogeneous, i.e., that at all times intracellular and extracellular concentrations of acetaldehyde are identical and every cell has the same intracellular concentration of it.

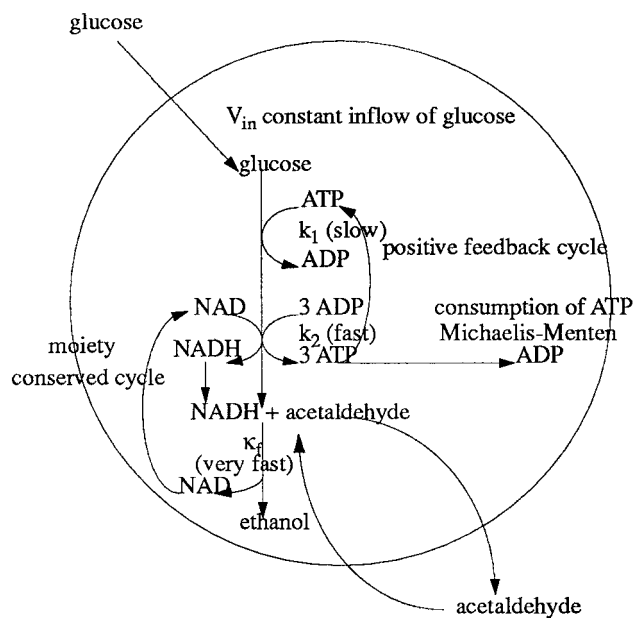


FIGURE 2 A schematic view of the glycolysis and the proposed mechanism for the synchronization. Two ATPs are bound in the beginning. The slowest binding step is also taken to be the rate-limiting process for all of the glycolysis and is determining the value of k_1 in system 1. Four ATPs are produced further down in the cycle, and at the same time ATP is consumed in other processes in the cell. The ATP-ADP cycle is the engine of the autonomous oscillations. An NAD molecule is consumed and produced again at the bottom of the anaerobic glycolysis. Whereas the ADP-ATP loop is a positive feedback cycle, the NAD-NADH loop is a moiety-conserved cycle ($NAD + NADH = \text{constant}$). Because acetaldehyde freely permeates the cell membrane, the NAD-NADH cycle is effectively shared by all cells in the suspension. The NAD-NADH cycle and the ATP-ADP cycle share a few steps, and this is how acetaldehyde can indirectly influence the oscillatory dynamics.

The essence of the synchronization mechanism that we propose is that acetaldehyde is involved in the above-mentioned moiety conserved NAD-NADH cycle. It can affect the speed of this cycle and hence, indirectly, perturb the speed of the “main” ATP-ADP cycle that powers the glycolytic oscillation in each individual cell (Fig. 2). So via the shared acetaldehyde concentration cells can influence each other’s glycolytic kinetics. The influence should be such that when a “dissident” cell is oscillating out of phase with the other cells in the suspension it will be forced back into phase.

Acetaldehyde is at the bottom of the glycolytic chain. In that part of the chain the oscillations in the carbon metabolites (such as pyruvate) have damped out (Richard et al., 1996). Consequently, the production of acetaldehyde from pyruvate is approximately constant. Suppose that there were no acetaldehyde flow through the membrane. In that case the change in time of the acetaldehyde concentration should obey the following equation:

$$\frac{d[Ac]}{dt} = J_{Ac} - \kappa_f(N - [NAD])[Ac] + \kappa_b[NAD]. \quad (4)$$

Here $[Ac]$ is the concentration of acetaldehyde, J_{Ac} is the production of acetaldehyde by pyruvate decarboxylase, κ_b is the reverse reaction of κ_f (cf. Fig. 2), and we have used the moiety conservation relation $[NAD] + [NADH] = N$, where N is constant. The rate κ_b is proportional to the concentration of ethanol, which is virtually constant in relative terms during the timespan covered by the synchronization experiments. The enzyme that catalyzes the reduction of acetaldehyde to ethanol is present at high activities, so κ_f and κ_b are high and the κ_f and κ_b reactions are fast. This means that the relaxation dynamics of Eq. 4 is faster than the oscillating glycolysis, and that we can study Eq. 4 taking $[NAD]$ to be constant. The homogeneous part of the solution is $[Ac]_H = A_0 \exp\{-\kappa_f(N - [NAD])t\}$, where A_0 is a constant. The particular solution is $[Ac]_P = (J_{Ac} + \kappa_b[NAD]) / (\kappa_f(N - [NAD]))$. With large κ_f (Fig. 2) the homogeneous solution damps out very fast and the actual acetaldehyde concentration will be given by the particular solution. If J_{Ac} is small relative to $\kappa_b[NAD]$, i.e., if the relaxation to equilibrium almost instantaneously absorbs the steady production rate of acetaldehyde, then we have $[Ac] = \gamma[NAD] / (N - [NAD])$ and $[NAD] = N[Ac] / (\gamma + [Ac])$, where $\gamma = \kappa_f / \kappa_b$. The important conclusion is that $[Ac]$ and $[NAD]$ vary proportionally and that the acetaldehyde oscillation follows the NAD oscillation. If $[Ac]$ is a collective variable, i.e., the same for all cells and homogeneous over the whole bath, then $[NAD]$ is also collective, provided that κ_f and κ_b are fast enough.

We summarize the part of the glycolysis that leads to acetaldehyde as $\text{glucose} + \text{ATP} \rightarrow 3 \text{ATP}$. In actuality, two ATPs are bound at the top and four ATPs are produced at the end, but of the two binding steps at the beginning, one is significantly slower than the other. The flux control effectively rests with the slowest step and we take this step to have a rate k_1 . The simplified expression for the rate of the $\text{glucose} + \text{ATP} \rightarrow 3 \text{ATP}$ reaction is $k_{\text{eff}} = (1/k_1 + 1/k_2)^{-1}$. The rate k_2 here represents the rate of the ATP producing part of the glycolytic chain. The k_2 part is fast relative to the k_1 part and exerts less control over the eventual flux (i.e., $1/k_1 \gg 1/k_2$, see Fig. 2). If the κ_f reaction is faster than the k_2 reaction, then the acetaldehyde concentration can, via the NAD concentration, speed up or slow down the production of ATP. Because of the positive feedback of the ATP concentration on the production of ATP, the acetaldehyde concentration will then affect the speed of the glycolytic oscillation.

These kinetic effects also operate in the reverse direction. When the ATP concentration in a cell goes up, the balance in the k_2 reaction ($NAD + ADP \leftrightarrow NADH + ATP$) will be pushed toward the NAD side. In terms of the system in Fig. 2 this means that the production of NADH goes down. This will decelerate the alcohol dehydrogenase reaction (κ_f) such that a higher $[Ac]$ is attained.

Suppose that we have a bath with two cells that have identical parameters V_{in} , k_1 , k_p , and K_m . Let both cells oscillate out of phase with one another. In each of the two cells the ATP oscillation is carried over into an

NAD oscillation, which in turn is carried over into an oscillation of the acetaldehyde concentration. Because of the permeability of the membrane to acetaldehyde, the acetaldehyde concentration in one cell is “felt” in the other cell. Via the reverse pathway ($\text{Ac} \rightarrow \text{NAD} \rightarrow \text{ATP}$) the acetaldehyde concentration can speed up or slow down the oscillating glycolysis.

If the $[\text{NADH}][\text{Ac}]/[\text{NAD}]$ equilibration, the $[\text{NAD}][\text{ADP}]/([\text{NADH}][\text{ATP}])$ equilibration, and the permeation of the membrane by acetaldehyde were fast relative to the glycolytic oscillation, then the two cells would immediately synchronize their ATP concentrations and, consequently, synchronize their oscillations. These processes, however, are not immediate and, eventually, account for a continuous “pressure” toward equalizing the ATP concentrations.

The perturbation method and a numerical solution

Next we will develop an analytical method to describe this “pressure” and its effect. In the simplest approximation we can incorporate this “pressure” as a linear perturbation term $\varepsilon(T_2 - T_1)$ in the expression for dT/dt (cf. Eq. (1)). The two-cell model is then described by the following set of coupled equations:

$$\begin{aligned} \dot{G}_1 &= V_{\text{in}} - k_1 G_1 T_1 \\ \dot{T}_1 &= 2k_1 G_1 T_1 - k_p \frac{T_1}{K_m + T_1} + \varepsilon(T_2 - T_1) \\ \dot{G}_2 &= V_{\text{in}} - k_1 G_2 T_2 \\ \dot{T}_2 &= 2k_1 G_2 T_2 - k_p \frac{T_2}{K_m + T_2} - \varepsilon(T_2 - T_1). \end{aligned} \quad (5)$$

So we have two second-order nonlinear systems that are linearly coupled via a small ε term. When $\varepsilon = 0$ the two systems are uncoupled and each follows its own limit cycle. In the proposed mechanism ε should be positive, so that when $T_2 > T_1$, the T_1 production is speeded up and the T_2 production slowed down. Limit cycle oscillations are structurally stable, i.e., a small perturbation to the system can affect the shape and frequency, but the cycle itself persists (Glansdorff and Prigogine, 1971). The $\varepsilon(T_2 - T_1)$ -form reflects the fact that there is a diffusive mass transfer between the two systems. Such a form of the perturbation is commonly found in setups where two systems, each with its own dynamics, interact through a small diffusion term. In our case it is not the actual ATP that is transferred, but the acetaldehyde transfer and its subsequent effects on the chemical kinetics have an equivalent result. A thorough and general treatment of systems like (5) is found in the textbook by Murray (1993). Fig. 3 shows a simulation of system 5 using STELLA. The initial conditions were picked such that at $t = 0$ the identical cells 1 and 2 both oscillate very close to the limit cycle, but at very different phases. We see that the perturbation has the expected synchronizing effect. Parameter values in the simulation were set such that the period and the time to get to a new limit cycle were those of the actual experiment, i.e., 50 s for the period and ~ 10 periods for complete synchronization.

Fig. 3 *b* shows how the quantity $(T_2 - T_1)$ decreases and asymptotically approaches zero as time evolves. For small phase differences the rate of decrease of the amplitude of $(T_2 - T_1)$ is the same as the rate of synchronization. This can be proven analytically as follows. When T_2 and T_1 oscillate harmonically and have a small phase difference δ , we have $d(T_2 - T_1)/dt = A\{\cos(\omega t + \delta) - \cos(\omega t)\}$. The right-hand side quantity equals $-2A \sin(\delta/2) \sin(\omega t + \delta/2)$, which for small δ reduces to $A\delta \sin(\omega t)$ (since $\sin(\alpha) \approx \alpha$ for small α). An exponential decrease of δ should thus, for small δ , be visible as an equally fast exponential decrease of $(T_2 - T_1)$.

Fig. 3, *a* and *b* show that the actual synchronization takes place during the first five periods after the cell suspensions have been mixed. During

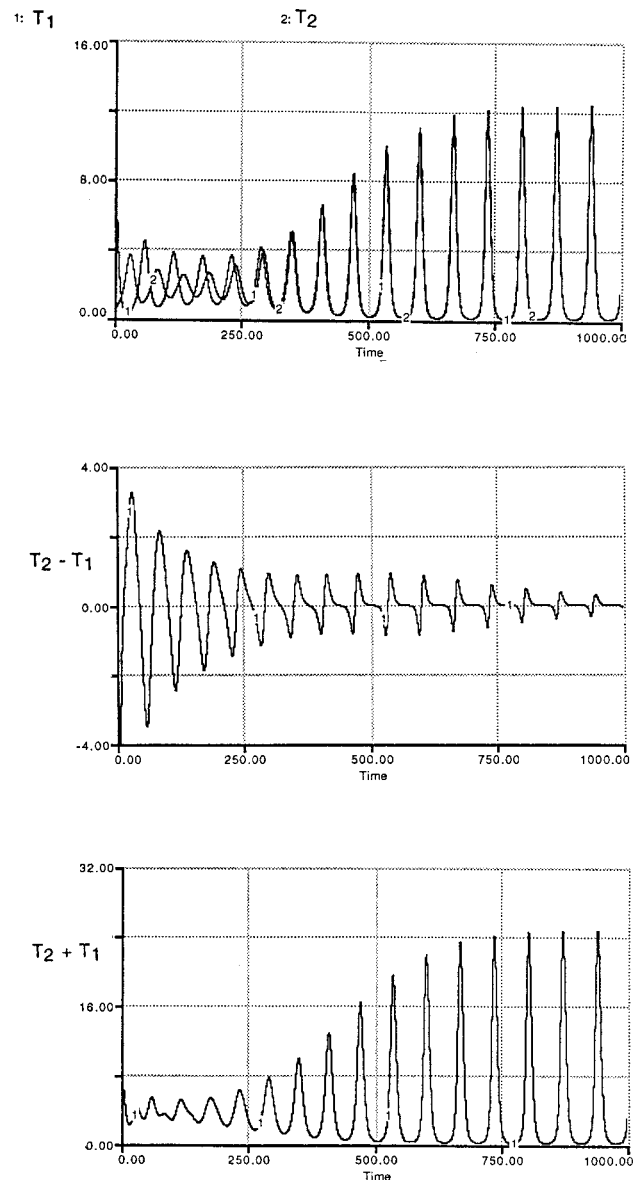


FIGURE 3 A simulation of system 5 for the following parameter values: $V_{\text{in}} = 0.36$, $k_1 = 0.02$, $k_p = 6.0$, $K_m = 13.0$, and $\varepsilon = 0.01$. G denotes glucose and T denotes ATP. The initial conditions were such that both cells oscillate close to the limit cycle, but are substantially out of phase: $G_1 = 6.6$, $T_1 = 7.6$, $G_2 = 10.3$, and $T_2 = 0.41$. (a) T_1 and T_2 ; (b) the difference $T_2 - T_1$; (c) the sum $T_2 + T_1$. In the course of five periods an almost complete synchronization is attained. Within ~ 10 periods the ATP concentration is back at the limit cycle.

these five periods the perturbation has also driven each oscillation away from the limit cycle toward a smaller amplitude. After the synchronization is almost completed and, consequently, the perturbation term almost zero, it takes another five periods for both system 1 and system 2 to converge back to the limit cycle.

Proof of synchronization

Finally we will see how system 5 predicts synchronization and we will derive an expression for the speed of the synchronization in terms of

system parameters. As we mentioned before, the limit cycle structure itself will not be affected by the perturbation. What we want to know is how the cycling will be speeded up or slowed down by the perturbation. Let either one of the two systems in (5) in its full generality look like:

$$\dot{G} = f_1(G, T) + \xi_1, \quad \dot{T} = f_2(G, T) + \xi_2. \quad (6)$$

What matters for the cycling speed is “the amount of perturbation” in the direction of the vector (\dot{G}) (which is the direction of motion), i.e., the dot product of the perturbation vector (ξ_1) and the unit tangent vector to the limit cycle. This dot product can be expressed as $(f_1\xi_1 + f_2\xi_2)/\sqrt{(f_1^2 + f_2^2)}$. In the unperturbed system we have for the changes ΔG and ΔT in a small time interval Δt : $\Delta G = f_1(G, T)\Delta t$ and $\Delta T = f_2(G, T)\Delta t$. With the perturbation one obtains:

$$\Delta G = f_1\Delta t \left(1 + \frac{f_1\xi_1 + f_2\xi_2}{f_1^2 + f_2^2} \right), \quad (7)$$

$$\Delta T = f_2\Delta t \left(1 + \frac{f_1\xi_1 + f_2\xi_2}{f_1^2 + f_2^2} \right).$$

So the net effect of the perturbation is a modification of Δt ; the oscillation is speeded up (if $(f_1\xi_1 + f_2\xi_2)/(f_1^2 + f_2^2) > 0$) or slowed down (if $(f_1\xi_1 + f_2\xi_2)/(f_1^2 + f_2^2) < 0$). For the phase interval that (6) covers in the course of Δt we have $\Delta\varphi = \omega\Delta t$, where ω is the radial velocity of the unperturbed system (cf. Eq. 3). In the course of one period of the unperturbed system the phase shift due to the perturbation is:

$$\delta\varphi = \omega \int_0^{2\pi/\omega} \frac{f_1\xi_1 + f_2\xi_2}{f_1^2 + f_2^2} dt. \quad (8)$$

When the parameters are close to where the Hopf bifurcation is, the oscillations have small amplitudes and can be well-approximated to be harmonic. We describe the oscillation as $G(t) = G_0 + g_1 \cos(\omega t + \Psi)$ and $T(t) = T_0 + \theta_1 \cos(\omega t)$, where Ψ is the phase difference between the G and the T oscillation. G_0 and T_0 are given by Eq. 2. This implies

$$\dot{G}(t) = f_1(G, T) = -\omega g_1 \sin(\omega t + \Psi)$$

and

$$\dot{T}(t) = f_2(G, T) = -\omega \theta_1 \sin(\omega t).$$

We furthermore have for the first oscillator in system 5: $\xi_1 = 0$ and $\xi_2 = \varepsilon(T_2 - T_1) = \varepsilon\theta_1 \{\cos(\omega t + \Delta\varphi) - \cos(\omega t)\} \approx \varepsilon\theta_1 \Delta\varphi \sin(\omega t)$, where $\Delta\varphi$ is the phase difference between the two oscillations in system 5. This phase difference is assumed to be small. So for our case the integral 8 becomes:

$$\begin{aligned} \delta\varphi &= -\varepsilon\Delta\varphi \int_0^{2\pi/\omega} \frac{\sin^2\omega t}{\eta \sin^2(\omega t + \Psi) + \sin^2\omega t} dt \\ &= -\frac{2\pi\varepsilon\Delta\varphi}{\omega} \frac{\eta^2 \cos(2\Psi) + 1 + \eta(\eta^2 - 1)|\sin\Psi|}{\eta^4 + 2\eta^2 \cos(2\Psi) + 1} \\ &= -\frac{2\pi\varepsilon\Delta\varphi}{\omega} \Gamma, \end{aligned} \quad (9)$$

where η represents the ratio of the amplitudes, i.e., $\eta = g_1/\theta_1$; Γ is a ratio that is independent of the phase difference or coupling strength of the two limit cycles. Linear analysis around the fixed point (G_0, T_0) leads to the

following expressions for η and Ψ :

$$\eta = \frac{1}{2} \sqrt{\frac{k_p^2}{2k_1 V_{in}} - \frac{k_p}{k_1} + 1}, \quad (10)$$

$$\Psi = \pi - \arctan \frac{(k_p - 2V_{in})^2}{2K_m k_p \omega}.$$

We can now work out how fast the synchronization proceeds. Suppose that in the course of one period of the unperturbed system, in the setup of Eq. 5, the (G_1, T_1) system oscillates $\Delta\varphi$ ahead of (G_2, T_2) and is pulled back $\delta\varphi$ by the (G_2, T_2) system. Then simple reciprocity demands that (G_2, T_2) is pulled forward by (G_1, T_1) by the same amount $\delta\varphi$. We then have for the rate at which $\Delta\varphi$ is changing:

$$\frac{d}{dt}(\Delta\varphi) = \frac{2\delta\varphi}{(2\pi/\omega)} = -2\varepsilon\Gamma\Delta\varphi, \quad (11)$$

where the constant Γ is given by Eq. 9. It can be easily proven that Γ is positive for all values of Ψ and η , so the differential Eq. 11 predicts an exponential decrease for $\Delta\varphi$:

$$\Delta\varphi(t) = \Delta\varphi(0)e^{-2\varepsilon\Gamma t}. \quad (12)$$

Fig. 3 shows the result of a simulation of system 5 for $V_{in} = 0.36$, $k_1 = 0.02$, $k_p = 6.0$, $K_m = 13.0$, and $\varepsilon = 0.01$. Substituting these values in Eqs. 9, 10, and 12 leads to a predicted half-time for $\Delta\varphi$ of 93 s. Fig. 3 b shows how the amplitude of $(T_2 - T_1)$, which should change at the same rate as $\Delta\varphi$, decreases exponentially with a half-time of ~ 100 units. For a first-order approximation the above theory gives a very good estimate.

That, after the mixing at $t = 0$, system 1 and system 2 are driven away from the limit cycle is a consequence of the component of the perturbation (ξ_2) that is perpendicular to the direction of the vector of motion (\dot{G}) , i.e., of the cross product of (\dot{G}) and (ξ_2) . The total time to get back to the limit cycle is roughly equal to the sum of the synchronization time and the relaxation time back to the amplitude of the limit cycle. For the parameter values that we choose these times appear to be about the same.

RESULTS AND DISCUSSION

The experimental conditions under which yeast cells exhibit sustained glycolytic oscillations are nonphysiological. Starved cells in an anaerobic environment are suddenly fed glucose and subsequently inhibited with cyanide. Cyanide is not normally present in yeast habitats. However, in our experiments cyanide serves the dual purpose of inhibiting respiration, i.e. simulating anaerobic conditions, and steadily removing acetaldehyde (Richard et al., 1994). The experimental condition is close to an actual condition experienced by *S. cerevisiae* in dough where anaerobiosis may occur and acetaldehyde is removed by evaporation.

This paper explains how, in a population of yeast cells, sustained macroscopic glycolytic oscillations can occur, even though it is expected that the oscillations in the individual cells are independent and would have no way to synchronize. That active synchronization of the individual cellular oscillations is required to attain macroscopically observable oscillations has been concluded after earlier experiments (cf. Teusink et al., 1996a; Perez et al., 1996), but the mechanism of synchronization has long been elusive.

Recently, oscillating intracellular concentrations of acetaldehyde, a small molecule that can permeate the cell membrane, were shown to be crucially involved in the synchronization (Richard et al., 1994). This finding appeared to be in conflict with the prevailing idea that glycolytic oscillations were a feature of the top of the glycolytic pathway (Hess and Boiteux, 1968; Goldbeter and Lefever, 1972).

In this paper we examined how the implementation of a small amount of control from the bottom of the glycolytic pathway can account for the synchronization by acetaldehyde of the oscillations of the individual cells. We confined the analysis to one of the simplest models for yeast glycolytic oscillations (Bier et al., 1996). For a single cell this model consists of two reaction steps and involves a positive feedback (Fig. 2). The predicted oscillations in the glycolytic flux lead, via oscillations in the NAD concentration, to an oscillation in the production of acetaldehyde. Acetaldehyde readily permeates the membrane and its concentration was treated as a pool common to all cells. It is via this pool that the cells "communicate." Indirectly, the common acetaldehyde concentration affects the rates of the two reactions at the top of the glycolytic chain that drive the oscillation. The control of the acetaldehyde concentration on the glycolytic engine is small and a treatment in terms of perturbation theory is warranted. The ensuing model does indeed exhibit the synchronization, and parameters can be found such that the synchronization takes place within about ten cycles. The essence is the concept that oscillations of individual cells may synchronize through the relatively minor cross-influence that is constituted by a common metabolite. Our perturbation analysis aims to be the simplest possible method to demonstrate this phenomenon.

These results form a subtle example of entrainment of oscillations. Entrainment of oscillations commonly arises when a macroscopic external oscillation (like an electromagnetic field) affects an oscillating system that is sensitive to such external forces (like a chemical reaction involving displacement of charges) (Vance and Ross, 1995; Westerhoff et al., 1986; Astumian et al., 1989). The system we studied consists of two identical cells that interact symmetrically. This bilateral entrainment (cf. Perez et al. (1996), Martinez De La Fuente et al. (1995), and Kamp et al. (1988) for a case with implications for free-energy transduction) is an example of self-organizing dynamics of a biological system (Glansdorff and Prigogine, 1971; Hess and Mikhailov, 1994). Understanding of cell-cell interaction may more generally be important in the analysis of cases where a population of actual cells behaves nonchaotically, whereas the dynamics of an individual cell allow chaos (Movileanu and Flonta, 1992). Implications for multicellular organisms may even go beyond that.

With "the control of the glycolytic oscillations" we refer to the control of the frequency, the amplitude, the average, and the waveform of the oscillations of the concentrations and fluxes (Kholodenko et al., 1997; Larter et al., 1984;

Termonia and Ross, 1981). Recent studies suggest that the control of glycolytic oscillations may well be distributed over several steps along the glycolytic pathway (Teusink et al., 1996b). Although this paper may demonstrate the essence of the synchronization of the glycolytic oscillations of the individual yeast cells in a population, the detailed validation will require a more detailed model.

In preparation for such a detailed model we have experimentally determined the concentrations of various glycolytic intermediates during the sustained glycolytic oscillations (Richard et al., 1996). Preliminary results indicate that oscillations are indeed generated at the top of the glycolytic chain and involve ATP and ADP. Through the ATP/ADP ratio these oscillations induce oscillations at the level of glyceraldehyde phosphate dehydrogenase. This, in turn, leads to oscillations in the NADH/NAD ratio. The latter oscillation (rather than oscillations in the rate of pyruvate decarboxylase (Richter et al., 1975)) leads to oscillations in the rate of the ethanol dehydrogenase reaction and consequent oscillations in the acetaldehyde concentration.

The oscillations in yeast cells described here differ from oscillations in yeast cells growing in a chemostat (Chen et al., 1990; Keulers et al., 1996). These appear to involve respiration and have periods that are close to an hour, but they also exhibit synchronization and, although the details will be different, methods similar to our perturbation treatment may apply. The kind of perturbation treatment we developed here may also prove useful for other biological systems that synchronize in the course of a few periods (Goldbeter, 1996).

What we have described in this paper is a very primitive form of communication between cells. A cell observes what surrounding cells in the suspension are doing and adjusts its behavior accordingly. The model we have presented describes this process in terms of elementary chemical kinetics and a perturbation analysis. The biochemistry eventually leads to Eqs. 5. System 5 describes two limit cycles with a linear coupling that can be treated as a perturbation, and this coupling is ultimately responsible for the synchronization of the glycolytic oscillations.

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REFERENCES

- Astumian, R. D., P. B. Chock, T. Y. Tsong, and H. V. Westerhoff. 1989. Effects of oscillations and energy driven fluctuations on the dynamics of enzyme catalysis and free energy transduction. *Phys. Rev. A*. 39: 6416–6435.
- Baconnier, P. F., P. Pachot, and J. Démonget. 1993. An attempt to generalize the control coefficient concept. *J. Biol. Syst.* 1:335–347.
- Betz, A., and B. Chance. 1965. Phase relationship of glycolytic intermediates in yeast cells with oscillatory metabolic control. *Arch. Biochem. Biophys.* 109:585–594.

- Bier, M., B. Teusink, B. N. Kholodenko, and H. V. Westerhoff. 1996. Control analysis of glycolytic oscillations. *Biophys. Chem.* 62:15–24.
- Chen, C.-I., K. A. McDonald, and L. Bisson. 1990. Oscillatory behavior of *Saccharomyces cerevisiae* in continuous culture. I. Effects of pH and nitrogen levels. *Biotechnol. Bioeng.* 36:19–27.
- Cortassa, S., M. A. Aon, and H. V. Westerhoff. 1991. Linear nonequilibrium thermodynamics describes the dynamics of an autocatalytic system. *Biophys. J.* 60:794–803.
- Ghosh, A. K., B. Chance, and E. K. Pye. 1971. Metabolic coupling and synchronization of NADH oscillations in yeast cell populations. *Arch. Biochem. Biophys.* 145:319–331.
- Glandsdorff, P., and I. Prigogine. 1971. *Thermodynamic Theory of Structure. Stability and Fluctuations.* Pitman Press, London.
- Goldbeter, A. 1996. *Biochemical Oscillations and Cellular Rhythms.* Cambridge University Press, Cambridge.
- Goldbeter, A., and R. Lefever. 1972. Dissipative structures from an allosteric model—application to glycolytic oscillations. *Biophys. J.* 12:1302–1315.
- Heinrich, R., S. M. Rapoport, and T. A. Rapoport. 1977. Metabolic regulation and mathematical models. *Prog. Biophys. Mol. Biol.* 32:1–83.
- Hess, B., and A. Boiteux. 1968. Mechanism of glycolytic oscillation in yeast. I. Aerobic and anaerobic growth conditions for obtaining glycolytic oscillation. *Hoppe-Seyler's Physiol. Chem. Bd.* 349:1567–1574.
- Hess, B., and A. Mikhailov. 1994. Self-organization in living cells. *Science.* 264:223–224.
- Kacser, H., and J. A. Burns. 1973. The control of flux. *Symp. Soc. Exp. Biol.* 27:65–104.
- Kamp, F., R. D. Astumian, and H. V. Westerhoff. 1988. Coupling of vectorial proton flow to a biochemical reaction by local electric interactions. *Proc. Natl. Acad. Sci. USA.* 85:3792–3796.
- Keulers, M., T. Suzuki, A. D. Satroudinov, and H. Kuriyama. 1996. Autonomous metabolic oscillation in continuous culture of *Saccharomyces cerevisiae* grown on ethanol. *FEMS Microbiol. Lett.* 142:253–258.
- Kholodenko, B. N., O. V. Demin, and H. V. Westerhoff. 1997. Control analysis of periodic phenomena in biological systems. *J. Phys. Chem. B.* 101:2070–2081.
- Larter, R., H. Rabitz, and M. Kramer. 1984. Sensitivity analysis of limit cycles with application to the Brusselator. *Biophys. J.* 80:4120–4128.
- Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. *J. Biol. Chem.* 193:265–275.
- Martinez De La Fuente, I., L. Martinez, and J. Veiguillas. 1995. Dynamic behavior in glycolytic oscillations with phase shifts. *Biosystems.* 35:1–13.
- Movileanu, L., and M. L. Flonta. 1992. Low dimensional chaotic states in biochemical systems. *Revue Roumaine de Biochimie.* 29:207–214.
- Murray, J. D. 1993. *Mathematical Biology.* Springer-Verlag, Berlin.
- Perez, C. J., A. Corral, A. Diazguilera, K. Christensen, and A. Arenas. 1996. On self-organized criticality and synchronization in lattice models of coupled dynamical systems. *Int. J. Modern Phys. B.* 10:1111–1151.
- Pye, E. K. 1969. Biochemical mechanisms underlying the metabolic oscillations in yeast. *Can. J. Botany.* 47:271–285.
- Richard, P., B. M. Bakker, B. Teusink, K. Van Dam, and H. V. Westerhoff. 1995. Acetaldehyde mediates the synchronisation of sustained glycolytic oscillations in populations of yeast cells. *Eur. J. Biochem.* 235:238–241.
- Richard, P., J. A. Diderich, B. M. Bakker, B. Teusink, K. Van Dam, and H. V. Westerhoff. 1994. Yeast cells with a specific cellular make-up and environment that removes acetaldehyde are prone to sustained glycolytic oscillations. *FEBS Lett.* 341:223–226.
- Richard, P., B. Teusink, M. B. Hemker, K. Van Dam, and H. V. Westerhoff. 1996. Sustained oscillations in free-energy state and hexose phosphate in yeast. *Yeast.* 12:731–740.
- Richards, P., B. Teusink, H. V. Westerhoff, and K. Van Dam. 1993. Around the growth phase transition *S. cerevisiae's* make-up favors sustained oscillations of intracellular metabolites. *FEBS Lett.* 318:80–82.
- Richter, O., A. Betz, and C. Giersch. 1975. The response of oscillating glycolysis to perturbations in the NADH/NAD system: a comparison between experiments and a computer model. *BioSystems.* 7:137–146.
- Selkov, E. E. 1975. Stabilization of energy charge, generation of oscillation and multiple steady states in energy metabolism as a result of purely stoichiometric regulation. *Eur. J. Biochem.* 59:151–157.
- Termonia, Y., and J. Ross. 1981. Oscillations and control features in glycolysis: numerical analysis of a comprehensive model. *Proc. Natl. Acad. Sci. USA* 78:2952–2956.
- Teusink, B., B. M. Bakker, and H. V. Westerhoff. 1996b. Control of frequency and amplitudes is shared by all enzymes in three models for yeast glycolytic oscillations. *Biochim. Biophys. Acta.* 1275:204–212.
- Teusink, B., C. Larsson, J. Diderich, P. Richard, K. Van Dam, L. Gustafsson, and H. V. Westerhoff. 1996a. Synchronized heat flux oscillations in yeast cell populations. *J. Biol. Chem.* 271:24442–24448.
- Vance, W., and J. Ross. 1995. Entrainment, phase resetting and quenching of chemical oscillations. *J. Chem. Phys.* 103:2472–2481.
- Westerhoff, H. V., T. Y. Tsong, P. B. Chock, Y. Chen, and R. D. Astumian. 1986. How enzymes can capture and transmit free energy from an oscillating electric field. *Proc. Natl. Acad. Sci. USA.* 83:4734–4738.
- Westerhoff, H. V., and K. Van Dam. 1987. *Thermodynamics and Control of Biological Free Energy Transduction.* Elsevier, Amsterdam.
- Wolf, J., and R. Heinrich. 1997. Dynamics of two-component biochemical systems in interacting cells: synchronization and desynchronization of oscillations and multiple steady states. *BioSystems.* 43:1–24.