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Published in:
European Journal of Biochemistry

DOI:
10.1111/j.1432-1033.1996.00238.x

Citation for published version (APA):
Acetaldehyde mediates the synchronization of sustained glycolytic oscillations in populations of yeast cells

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(Received 11 October 1995) — EJB 95 1657/1

In the presence of cyanide, populations of yeast cells can exhibit sustained oscillations in the concentration of glycolytic metabolites, NADH and ATP. This study attempts to answer the long-standing question of whether and how oscillations of individual cells are synchronized. It shows that mixing two cell populations that oscillate 180° out of phase only transiently abolishes the macroscopic oscillation. After a few minutes, NADH fluorescence of the mixed population resumes oscillations up to the original amplitude. At low cell densities, addition of acetaldehyde causes transient oscillations. At higher cell densities, where the oscillations are autonomous, 70 μM acetaldehyde causes phase shifts. Extracellular acetaldehyde is shown to oscillate around the 70 μM level. We conclude that acetaldehyde synchronizes the oscillations of the individual cells.

Keywords: cell-cell communication; signalling; control; dynamics; self-organisation.

It is becoming clear that the function of the living cell extends beyond the steady state [1, 2]. Of general interest are the states that derive from the non-linear properties of intracellular regulation and lead to oscillations in important signalers such as the calcium ion (Ca²⁺) [3, 4]. The oscillations become even more intriguing when they involve the dynamic interaction of individual cells such as in Dictystelium differentiation [5]. Glycolytic oscillations in yeast are an example of the coupling of the dynamics of metabolism across cell boundaries [6, 7].

Transient glycolytic oscillations can be induced by adding glucose followed by cyanide to a suspension of starved yeast [8–11]. The oscillations last longer at higher cell densities [12, 13]. This has been interpreted as being due to a synchronization mechanism which prevents individually oscillating cells from becoming out of phase [14]. Under certain conditions sustained oscillations can be observed with populations of cells [6, 15]. Although various substances have been considered to be the intercellular signaler, conclusive evidence for any one of them has been elusive for 25 years [7, 13, 16, 17]. In this report we identify and circumvent a complication precluding the measurement of acetaldehyde concentrations in the presence of cyanide. This allows us to demonstrate that the extracellular acetaldehyde concentration oscillates at the frequency of the intracellular glycolytic oscillations. The dependence of the phase shift on the acetaldehyde concentration and on the phase of acetaldehyde addition validates acetaldehyde as the elusive synchronizing agent.

MATERIALS AND METHODS

Strain and preparation of cells. The yeast Saccharomyces cerevisiae (X2180 diploid strain) was used. Cells were grown on glucose as described in [6]. At the diauxic shift, i.e. just after glucose 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 3 h at 30°C. The cells were then collected by filtration, resuspended in the same phosphate buffer and placed on ice until use. The protein concentration was determined, as a measure of the cell density, using the method of Lowry et al. [18].

Induction and monitoring of the oscillations. Oscillations were induced at 25°C by adding 20 mM glucose to the starved cells and after 4 min adding potassium cyanide as indicated in the figure legends. The oscillations were monitored by NADH fluorescence (Shimadzu RF-5001 PC spectrophotometer; excitation 352 nm, emission 462 nm, bandwidth 10 nm) in a stirred and thermostatically regulated cuvette.

Measurement of the extracellular acetaldehyde concentration. The extracellular acetaldehyde concentration was measured by extracting aliquots of approximately 0.5 ml of the cell suspension through a Dyna Gard filter (Microgon) into a 2-ml syringe. 200 μl of the filtrate was immediately transferred to a cuvette containing 800 μl 100 mM potassium phosphate, pH 6.8, alcohol dehydrogenase (0.1 mg/ml, Boehringer) and 0.17 mM NADH (disodium salt, Boehringer). The time between filtration and addition of the sample to the cuvette was 4–6 s. The absorbance at 340 nm was measured before and after addition of the filtrate. The acetaldehyde concentration was calculated by reference to acetaldehyde standards.

Calculation of phase of addition and phase shift. The phase was defined according to that of a sinus. Thus, the phase of the NADH oscillation is 0° at the inflection point of rising NADH. The phase of addition was calculated as 90° + 360° · t/T, where t is the time passed between the last NADH maximum and the addition of acetaldehyde, and T is the period of the oscillation. The phase shift was calculated as 360° · (Tₜ – T)/T, where Tₜ is the time elapsed between the last NADH fluorescence maximum before, and the first NADH maximum after, the addition of acetaldehyde.
cells that are sufficiently similar, transient oscillations can be shown to be out of phase in the individual cells [13]. However, for the sustained oscillations observed under some conditions [6, 15], continuous synchronization is required. Fig. 1 presents a more direct indication of intercellular synchronization. Two cell populations, each exhibiting sustained oscillations but approximately 180° out of phase, were mixed at the time point indicated. If after mixing, the cells continue to oscillate independently, the oscillation of the mixed suspension should be macroscopically invisible. If the cells synchronize with each other, then the macroscopically observable oscillations should dampen initially, but subsequently should reappear as the synchronization becomes effective. Fig. 1 shows that reappearance was observed. Because the oscillations in the mixed cell populations were sustained (over 30 cycles; data not shown) and of the same amplitude as before mixing, the re-emergence of oscillations cannot be explained as an interference pattern due to a slight frequency difference between the two subpopulations. This finding and the observation that it takes some time before the oscillations re-emerge after mixing, is in variance with earlier reports concerning transient oscillations in mixed cell populations [7, 17]. This difference with the previous studies may well arise from the rather subtle dependence of the dynamic behaviour of yeast metabolism on the history of the yeast cells [6, 7].

The requirements for any intercellular-signalling mechanism are, (a) the signal has to be sensed by the cells; (b) the signal has to be emitted; and (c) the signal has to pass through the extracellular medium (if there is no physical contact between the cells). Sustained synchronization requires oscillations in the extracellular concentration of the signalling compound. We shall show that extracellular acetaldehyde meets these requirements.

(a) Yeast cells respond to acetaldehyde pulses. When, at low cell concentrations, the macroscopic oscillations had faded, they could be restarted by addition to 100 μM acetaldehyde (Fig. 2). Acetaldehyde added during oscillations induced phase shifts, depending on the phase of addition [16] and on the acetaldehyde concentration [19] (Fig. 3). The threshold concentration above which added acetaldehyde induced a phase shift, was around 30 μM. Pulses of acetate and ethanol up to 1 mM did not shift the phase of oscillations. A pulse of sodium acetate up to 10 mM abolished the oscillations (data not shown).

(b) Acetaldehyde is secreted. The cells were filtered and the filtrate analysed by means of the assay of Shachar-Nishri and Freeman [20] with modifications in order to assay low concentrations. This assay measures the total acetaldehyde concentration, including acetaldehyde which has reacted with cyanide to form lactonitrile. We found a significant accumulation of total acetaldehyde under the conditions described in Figs 1 and 3 [21]. Shortly, after cyanide addition, the total concentration of acetaldehyde became too high for oscillations to be significant [21].

(c) The extracellular free acetaldehyde concentration oscillates. The measurement of free acetaldehyde in the extracellular medium is complicated by the reaction of acetaldehyde with cyanide to form lactonitrile [22], for which we measured a rate constant of 1.5 M⁻¹ s⁻¹. Under our conditions, free acetaldehyde should have had a half-life of approximately 1 min. To measure free acetaldehyde within the time scale of a few seconds, we filtered the cells and transferred the filtrate rapidly to a cuvette containing alcohol dehydrogenase and NADH. In this way the amount of free acetaldehyde is reflected by a decrease in NADH concentration. The lower part of Fig. 4 shows the free acetaldehyde concentration as a function of time, measured after a processing time of less than 7 s. The upper part of the figure shows the corresponding intracellular NADH fluorescence. The concentration of acetaldehyde oscillated between approximately 40 μM and 100 μM at the same frequency as oscillations in the concentration of NADH, and with a phase delay of some 200°. Extracellular acetate and ethanol concentrations oscillated by less than 10% (data not shown).

DISCUSSION

This report presents strong evidence that intercellular acetaldehyde mediates synchronization of yeast glycolytic oscillations. Acetaldehyde exhibits various properties required [23] for a syn-
The extracellular acetaldehyde concentration was at its maximum, the ad-
dition. (R) Different amounts of acetaldehyde were added at 0°. The phase
of addition and the phase shift were calculated as described in Materials
and Methods.

Fig. 3. Phase shifts induced by acetaldehyde addition. The conditions
used were as described in the legend to Fig. 1, except that the protein
concentration was 10 g/l, and 5 mM KCN was used. Acetaldehyde was
added approximately 5 min after the induction of the oscillations. (A)
70 μM acetaldehyde was added at different phases of the NADH oscil-
ation. (B) Different amounts of acetaldehyde were added at 0°. The phase
of addition and the phase shift were calculated as described in Materials
and Methods.

Fig. 4. Oscillations of NADH fluorescence and extracellular acetalde-
hyde concentration. The upper part of the figure shows the NADH
fluorescence as a function of time, starting 5 min after cyanide addition.
The conditions used were as in Fig. 3, except that the temperature was
20°C. The extracellular acetaldehyde concentration was measured as de-
scribed in Materials and Methods.

chronising signaller. First, it permeates biological membranes
[24]. Second, the phase shift that it induces in oscillating cells
depends on the phase of addition (Fig. 3A) and on the amount
added (Fig. 3B). The former dependence is such that the in-
duced phase shift appears largest when the NADH fluorescence
increases most rapidly [16] (Fig. 3A). At 270°, when the extra-
cellular acetaldehyde concentration was at its maximum, the ad-
tion of extra acetaldehyde prolonged the subsequent period.
The extracellular acetaldehyde concentration oscillated between
40 μM and 100 μM, which is the range in which the cells are
phase shifted by the addition of acetaldehyde.

Fig. 1 shows that two cell populations that were out of phase
became synchronized upon mixing. The data concerning acet-
aldehyde allow one to address the question of how a single oscilla-
tory behaviour may arise from the mixing of out-of-phase cells.
Fig. 4 shows that as the intracellular NADH of the front-running
subpopulation was being oxidized, the latter increased the extra-
cellular acetaldehyde concentration. This should shift the phase
of all runners-up (i.e., the other sub-population, or more gen-
erally cells becoming out of phase), increasing the oxidation
state of their NADH and synchronizing them with the front run-
ers. Subsequently, the endogenous oscillations in the front-run-
cing cells reduced the NAD of the latter, causing a decrease
in acetaldehyde concentration. Again, the runners-up should be
accelerated because the decrease in intercellular acetaldehyde
concentrations helps to increase their concentrations of NADH.

Acetaldehyde had been proposed [7] but then dismissed [17]
as the synchronizing agent by Pye and colleagues. The reason
for the dismissal was that alcohol dehydrogenase plus NADH
(added to the extracellular medium to serve as a trap for extra-
cellular acetaldehyde) enhanced rather than eliminated the oscil-
lations after mixing cell populations. These authors concluded
that acetaldehyde desynchronized the oscillations [17]. The ad-
dition of acetaldehyde did not eliminate oscillations however
[13]. Our recent finding [21] that there is an optimum concentra-
tion at which cyanide induces oscillations, may rationalize these
observations: if the acetaldehyde secreted by the cells is trapped
at an intermediate rate, then the extracellular acetaldehyde con-
centration may oscillate in a range in which it could affect the
synchronization of individual cells. When too much cyanide is
added, the extracellular acetaldehyde concentration may drop
below the threshold level for signalling (Fig. 3B). Acetaldehyde
should be removed by reaction with cyanide, hence only tran-
siently affecting the oscillations. When azide rather than cyanide
is added, an extra acetaldehyde trap (e.g. NADH plus alcohol
der hy genase [17]) may enhance the oscillations. In the ab-

The question of what couples the metabolic oscillators of
individual yeast cells during glycolytic oscillations has been
quite persistent. At the pH used in this study (pH 6.8), ethanol
and acetaldehyde are the only feasible compounds that could
meet the requirements stated above. The ethanol concentration
however, increases continuously to such high levels that the rela-
tive amplitude of any oscillation in extracellular ethanol concentra-
tion was too low (less than 10%, data not shown) for the
ethanol to mediate the synchronization of the cells [25]. An im-
provement of the acetaldehyde assay has allowed us to provide
the evidence that acetaldehyde synchronizes the cells, i.e., the
extracellular acetaldehyde concentration oscillates. Our findings
also suggest that for such coupling to occur, a subtle balance should exist between secretion of acetaldehyde by the cells and acetaldehyde trapping. In the present study, trapping was due to reaction with cyanide. In other experiments various other Schiff bases enhanced glycolytic oscillations [17, 21]. At higher cell densities [15] intracellular alcohol dehydrogenase may suffice. It remains to be seen whether, under physiological conditions, aldehyde evaporation, trapping by chemical substances, disturbance of the redox balance [26] or absorption by other organisms causes synchronization of metabolic dynamics of cells in a population, and what the implications for cell physiology may be [1, 27]. Our findings suggest that aldehydes should be added to the list of intercellular signalers.

We thank Prof. Dr B. Hess for stimulating discussions. This study was supported by the Netherlands Organisation for Scientific Research (NWO).

REFERENCES