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On the Coupling of Intracellular K^+ to Glycolytic Oscillations in Yeast

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Abstract

We have investigated the interplay between glycolytic oscillations and intracellular K^+ concentration in the yeast *S. cerevisiae*. Intracellular K^+ concentration was measured using the fluorophore PBFI. We found that K^+ is an essential ion for the occurrence of glycolytic oscillations and that intracellular K^+ concentration oscillates synchronously with other variables such as NADH, intracellular ATP and mitochondrial membrane potential. We also investigated if glycolysis and intracellular K^+ concentration oscillate in a number of yeast strains with mutations in K^+ transporters in the plasma membrane, mitochondrial membrane and in the vacuolar membrane. Most of these strains are still capable of showing glycolytic oscillations, but two strains are not: (i) a strain with a deletion in the mitochondrial Mdm38p K^+/H^+ transporter and (ii) a strain with deletion of the late endosomal Nhx1p K^+/H^+ (Na^+/H^+) transporter. In these two mutant strains intracellular K^+ concentration seems to be low, indicating that the two transporters may be involved in transport of K^+ into the cytosol. In the strain Mdm38pΔ oscillations in glycolysis could be restored by addition of the K^+/H^+ exchange ionophore nigericin. Furthermore, in two non-oscillating mutant strain with a defective V-ATPase and deletion of the Arp1p protein the intracellular K^+ is relatively high, suggesting that the V-ATPase is essential for transport of K^+ out of the cytosol and that the cytoskeleton may be involved in binding K^+ to reduce the concentration of free ion in the cytosol. Analyses of the time series of oscillations of NADH, ATP, mitochondrial membrane potential and potassium concentration using data-driven modeling corroborate the conjecture that K^+ ion is essential for the emergence of oscillations and support the experimental findings using mutant strains.

KEYWORDS

potassium ion, glycolytic oscillations, *Saccharomyces cerevisiae*,

Abbreviations: $DiOC_2(3)$, 3,3′-diethyloxacarbocyanine iodide; PBFI, potassium-binding benzofuranisophthalate

Mdm38p, Nhx1p

Take-away

- Intracellular potassium concentration is essential for glycolytic oscillations in yeast.
- Mutations in potassium transporters or deletions in mitochondrial/vacuolar functions disrupt these oscillations.
- Computational analysis corroborates the critical role of potassium in yeast metabolism

1 | INTRODUCTION

Potassium is the major cation inside eukaryotic cells. In addition to helping to maintain a negative electrical potential across the plama membrane, potassium ion participates in the regulation of a number of important functions in the cell (Clausen and Poulsen, 2013). For example, potassium ion acts as counterion for negatively charged groups on macromolecules and contributes to the regulation of intracellular pH and cell volume (Arino et al., 2010). By contrast, sodium ion is toxic to most organisms at high intracellular concentrations. The high concentration of intracellular potassium in mammalian cells is known to be created by the Na^+–K^+-pump originally discovered by Skou (Skou, 1988). In yeast the Na^+-K^+ pump is absent and yet intracellular potassium concentration may be as high as 300 mM (Yenush, 2016), which is more than twice the concentration in mammalian cells. How is this high intracellular concentration of potassium in yeast formed? Instead of a Na^+-K^+ ATPase the yeast plasma membrane contains an H^+-ATPase, Pma1p, which generates a pH gradient across the plasma membrane (Serrano et al., 1986). This pH gradient helps maintaining a high intracellular K^+ concentration through electrogenic and non-electrogenic K^+ transport across the plasma membrane (Yenush, 2016). Another important H^+-ATPase in *S. cerevisiae* is the V-ATPase in the vacuolar membrane, which collaborates with the plasma membrane ATPase in maintaining cytosolic pH homeostasis (Nishi and Forgac, 2002). The membranes of intracellular organelles also contain a number of ion channels and ion exchangers, which cooperate with ion transporters in the plasma membrane to maintain sodium and potassium homeostasis (Yenush, 2016). However, the concentration of K^+ in yeast is not necessarily evenly distributed within the cell. In a yeast wild type strain a large fraction of intracellular potassium was shown to be located in the vacuole whereas a relatively low concentration was measured in the cytosol (Herrera et al., 2013). Furthermore, certain proteins in the cytoplasm may bind potassium, resulting in a relatively low concentration of free K^+ in the cytosol (Olsen et al., 2020).

To study the regulation of potassium homeostasis in cells it may be advantageous to be able to measure the temporal behavior of potassium ion in the cytosol. Such measurements can be performed using K^+-sensitive probes. One of these probes is the potassium-sensitive fluorescent probe PBFI (short for potassium-binding benzofuranisophthalate) (Minta and Tsien, 1989; Szmacinski and Lakowicz, 1999). PBFI is not absolutely specific for potassium ion, but it has a higher affinity for K^+ compared to other cations (Minta and Tsien, 1989; Szmacinski and Lakowicz, 1999), and, since the intracellular concentration of potassium ion is higher than those of other cations, then PBFI may be successfully used to perform time-resolved measurements of free potassium ion in the cytosol (Olsen et al., 2020). Interpretations and analyses of measurements of intracellular variables are further strengthened when cells are in a transient state. In the yeast *Saccharomyces cerevisiae* one may induce oscillations in glycolysis under anaerobic or semi-anaerobic conditions (Duysens and Amesz, 1957; Goldbeter, 1996; Richard, 2003), e.g. when glucose and potassium cyanide are added to a dense suspension of yeast cells. In such suspensions all cells oscillate in phase (Richard et al., 1996), but glycolytic oscillations have also been observed on a single cell level (Weber et al., 2012, 2020; Hauser, 2022). These oscillations are believed to be caused by feed-back regulations in the glycolytic pathway (Goldbeter

and Lefever, 1972; Goldbeter, 1996; Madsen et al., 2005; Chandra et al., 2011), but they also depend on multiple other intracellular processes not directly involved in the glycolytic pathway, such as mitochondrial and vacuolar ATPase activities (Olsen et al., 2009). Glycolytic oscillations also sem to be intimately connected to the physical state of intracellular water (Thoke et al., 2015, 2017). Glycolytic oscillations represent a multitude of temporal transients and hence the effects of inhibitors and gene knockouts are thought to be relatively easy to interpret and simulate by mathematical models (Goldbeter, 1996; Williamson et al., 2012; Schroder et al., 2013). Oscillations in glycolysis have also been observed in other eukaryotic cells (Amemiya et al., 2022; Merrins et al., 2013; Tornheim et al., 1991; Yang et al., 2008; Ganitkevich et al., 2010) and in prokaryotes on the single cell level (Bi et al., 2023).

Here we use PBFI to measure the temporal behavior of free intracellular K^+ in the yeast *S. cerevisiae*. Specifically, we study the dynamics of K^+ during glycolytic oscillations. In addition to time-resolved intracellular K^+ concentration we measure NADH, intracellular ATP and mitochondrial membrane potential. Such time resolved measurements can convey a lot of information about the connection between the measured variables, especially when these variables are oscillating. We show that the oscillations in glycolysis in yeast require intracellular potassium and that the concentration of free intracellular potassium is oscillating with the same frequency as glycolytic intermediates and other cellular variables such as mitochondrial membrane potential. Furthermore, using isogenic mutant strains with deletions of different cationic transporters, we show that the mitochondrial K^+/H^+-exchanger Mdm38p seems to make important contributions to the induction of glycolytic oscillations and the corresponding oscillations in intracellular potassium concentration. This suggests that transport of K^+ through the mitochondrial membrane is essential for a normal yeast metabolism. The results obtained using mutant strains are corroborated by analysis of the time series of NADH, ATP, mitochondrial membrane potential and intracellular K^+ using data-driven modeling (Brunton et al., 2016).

2 | MATERIALS AND METHODS

2.1 | Materials

The fluorescent probes 3,3'-diethyloxacarbocyanine iodide ($DiOC_2(3)$) and PBFI (tetraammonium salt) were obtained from ThermoFischer Scientific (Waltham, MA). The aptamer switch probe used to measure intracellular ATP concentration was obtained from VBC Biotech (Vienna). All other chemicals were purchased from Sigma Aldrich (Munich, Germany).

2.2 | Cell growth

Cells of the yeast *Saccharomyces cerevisiae* diploid strain BY4743 wild type and isogenic mutants from the Euroscarf collection were grown and harvested as described previously (Schroder et al., 2013). The starved cells were suspended to a density of 10% (w/v) in 100 mM potassium phosphate buffer, pH 6.8, or in 100 mM sodium phosphate buffer, pH 6.8, and starved for 3-5 h at room temperature before use.

2.3 | Labelling of cells

PBFI was loaded into yeast cells by electroporation using a gene pulser transfection apparatus equipped with a capacitor extender, both from Bio-Rad Laboratories (Hercules, CA) as described previously (Poulsen et al., 2008) in a 1 M sorbitol solution containing 40 μM or 0 μM of the dye. The cells were then washed twice and finally suspended to a density of 10% (w/v) in the measurement buffer (100 mM potassium phosphate or 100 mM sodium phosphate, pH

6.8). $DiOC_2(3)$ dissolved in DMSO was added to a 1 ml of 10% (w/v) cell suspension in 100 mM potassium phosphate buffer, pH 6.8 and incubated for 15 min at 25 ^oC. Thereafter, the cells were centrifuged in an Eppendorf MiniSpin centrifuge at 8900 g and resuspended to the same volume in 100 mM potassium phosphate buffer, pH 6.8 and used immediately for measurement.

2.4 | Time resolved measurement of NADH, $DiOC_2(3)$ and PBFI

Measurements of NADH, $DiOC_2(3)$ and PBFI in cuvette were made in a SPEX Fluorolog spectrofluorometer (Edison, NJ) or in an Edinburgh Instrument FL 920 spectrofluorometer (Edinburgh, U.K.), both fitted with a temperature-controlled cuvette holder (Quantum Northwest, Liberty Lake, WA, USA) . The temperature of the sample was maintained at 25 $^oC \pm 0.01^oÇ$. NADH was excited at 366 nm, and its fluorescence emission was measured at 450 nm. $DiOC_2(3)$ was excited at 488 nm and its fluorescence emission was measured at 600 nm (Andersen et al., 2007). PBFI was excited at 340 nm, and its fluorescence emission was measured at 505 nm (Szmacinski and Lakowicz, 1999). Temporal measurements of PBFI fluorescence were corrected for background fluorescence by subtracting the fluorescence from cells electroporated without the dye. The sampling frequency was 1 Hz. For all excitations and emissions, a slit corresponding to 4.5 nm was used. Changes in cellular volume can also be a potential source for changes in PBFI fluorescence. However, in a previous study (Thoke et al., 2018b) we demonstrated that volume changes measured by light scattering are only noticeable when the cell wall is removed with Zymolyase®, a condition that was not used here. All measurements were performed in triplicate and the results were shown to be reproducible in independent measurements.

2.5 | Measurements of intracellular ATP

Measurements of intracellular ATP using an aptamer-based nanobiosensor were done essentially as described earlier (Ozalp et al., 2010; Ytting et al., 2012). The nanosensor consists of an approximately 30 nm polyacrylamide particle containing the aptamer switch probe BlackHole2-GTAGTAAGAACTAAAGTAAAAAAAAAATTAAAGTAGCCACGCTT-[CH2-CH2-O]36-TTACTAC-TexasRed and with Alexa Fluor 388 dextran as the reference dye. The sensor is inserted into the cells by electroporation as described previously (Ozalp et al., 2010). The sensor uses the ratio of the Texas Red fluorescence and Alexa Fluor 488 to determine the intracellular ATP concentration and can be calibrated in vitro (Ozalp et al., 2010). 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 (Kloster and Olsen, 2012). Temporal measurements of the fluorescence of the ATP nanosensor were made as the ratio of the emission at 605 with an excitation of 580 nm (Texas Red) over the emission at 520 nm with an excitation at 470 nm (Alexa Fluor 488) in an Edinburgh Instrument FL 920 spectrofluorometer (Edinburgh, U.K.) fitted with a temperature-controlled cuvette holder (Quantum Northwest, Liberty Lake, WA, USA). The sampling frequency was 1 Hz.

2.6 | Induction of glycolytic oscillations in yeast cells

A suspension of yeast cells at a density of 10% (w/v) in 100 mM potassium phosphate buffer, pH 6.8, were added to a 1 mL stirred cuvette mounted in the spectrofluorometer. Oscillations were induced by adding first 30 mM glucose and 60 s later 5 mM KCN to the suspension. Temperature was 25 ^oC in all experiments.

2.7 | Data-driven analysis of experimental data using SINDy

Data-driven modeling is a relatively new modeling concept where models use one or more measured variables of a system as input (Zhang et al., 2011; Mangiarotti et al., 2012). Data-driven modeling can provide important results about an experimental system, and may be used for predicting the future of the system. Some methods in data-driven modeling rely on neural networks and this approach usually does not lead to any further understanding of the system studied (black-box models), while other methods can provide knowledge about an unknown system and in some cases the output is a set of ordinary differential equations which are directly estimated from the data without any constraint on the model structure (Mangiarotti et al., 2012, 2018; Thenon et al., 2022). Data-driven models are especially useful when the underlying model structure and mechanistic interactions between variables are not known. In some cases, it is possible to retrieve the original governing equations of a dynamical system from the data (Brunton et al., 2016; Mangiarotti and Huc, 2019). Experimental time series of NADH, ATP, mitochondrial membrane potential (measured as fluorescence of $DiOC_2(3)$) and intracellular K^+ concentration (measured as fluorescence of PBFI) were analyzed using the SINDy (sparse identification of nonlinear dynamics) algorithm (Brunton et al., 2016). In order to do so linear and second order trends were first removed and the resulting data were then rescaled on the interval [0 1] (Prokop and Gelens, 2023). A brief description of the SINDy application is given in the Supporting Information.

3 | RESULTS

3.1 | Intracellular potassium is essential for glycolytic oscillations

Glycolytic oscillations are often measured as oscillations in NADH (Duysens and Amesz, 1957; Goldbeter, 1996). Fig. 1 shows time-resolved measurements of NADH in cells of *S. cerevisiae* strain BY4743 following addition of glucose and KCN. The cells in Fig. 1A were suspended in 100 mM potassium phosphate buffer, while the cells in Fig. 1B were suspended in 100 mM sodium phosphate. We note that only the cells suspended in potassium buffer display oscillations in NADH. Apart from the oscillations the two time series display essentially the same temporal form: A rapid rise in NADH following addition of glucose and KCN followed by a decline to an almost constant plateau with or without oscillations superimposed, and, finally, around 2000 second a slow rise to an almost fully reduced state. At the inflexion point of this rise in NADH all glucose is used up (Hald and Sørensen, 2010). Other experiments (see Fig. S1 in the Supporting Information) show that the amplitude of oscillations in NADH decrease with decreasing concentration of potassium in the extracellular medium and that oscillations may be induced in cells suspended in buffers containing as little as 0.5 mM potassium ion. Hence, the presence of potassium in the medium is essential for oscillations to be observed. The question is now: why is potassium ion essential for obtaining oscillations in glycolysis.

3.2 | Deletions of mitochondrial and vacuolar H^+-ATPase activity abolish glycolytic oscillations and K^+ transport

Previously, we have shown (Olsen et al., 2020) that free intracellular potassium ion is oscillating in synchrony with NADH and ATP. By contrast, extracellular potassium ion, even in media with a low potassium concentration, but still supporting glycolytic oscillations, does not show any oscillations (Olsen et al., 2020). To get a better understanding of the link between oscillations in NADH and in free intracellular potassium ion it would be useful to measure other essential variables in yeast metabolism such as intracellular ATP and mitochondrial membrane potential. Both these

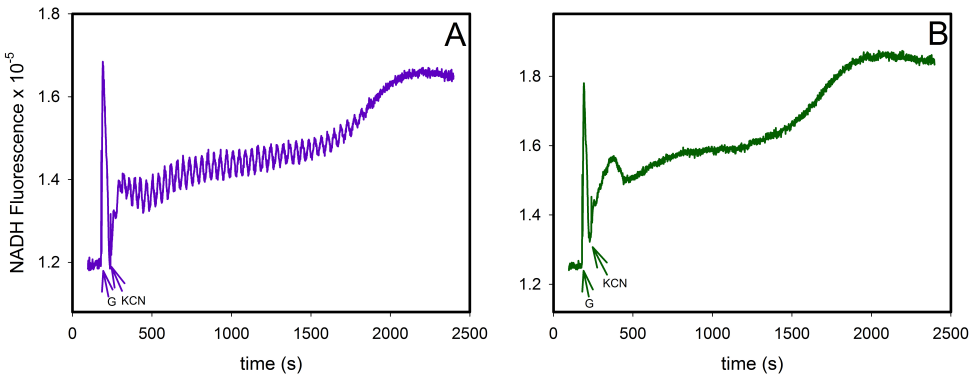

FIGURE 1 Observations of oscillations in glycolysis require that the cells are suspended in media containing potassium ion. Cells of of the *S. cerevisia* strain BY4743 (10% w/v) were starved for three hours in either (A) 100 mM potassium phosphate buffer, pH 6.8, or (B) in 100 mM sodium phosphate buffer. Then the cells were transferred to a cuvette and 30 mM glucose (G) followed by 5 mM KCN were added to the suspension at time points indicated by the arrows. Temperature 25 °C.

variables together with intracellular pH have previously been shown to oscillate in synchrony with NADH (Andersen et al., 2007; Olsen et al., 2009; Ozalp et al., 2010). Furthermore, since membranes may be involved in the transport of potassium ion into the cell and from the cytosol to the organelles, it may be useful to measure the properties of at least one intracellular membrane system. In an earlier study we showed that intracellular ATP oscillates in phase with PBFI fluorescence (Olsen et al., 2020), and while all measurements presented below were accompanied by measurements of intracellular ATP, the latter do not provide additional information to the interpretations of the experiments, so these measurements are not included in most of the graphs, but their behavior will be described in the text. Fig. 2 shows measurements of NADH, intracellular ATP, mitochondrial membrane potential, and intracellular potassium concentration in a typical experiment where glucose and KCN have been added to a dense suspension of yeast cells to induce oscillations in glycolysis. Following the addition of glucose and KCN the fluorescence of NADH first rises sharply and then settles on an oscillation around an almost constant level for about 1000 s after which the oscillations quickly dampen as the glucose is used up. The concentration of ATP also shows an initial rise, but simultaneously with oscillations the average ATP concentration slowly decreases until it reaches a minimum after which it starts to increase

TABLE 1 Glycolytic oscillations in null mutants in potassium transporters in *S. cerevisia*

Orf	Protein affected	Ion(s) transported	Location	Glycolytic oscillation
YJL129c	Trk21.	K^+	plasma membrane	yes
YKR050w	Trk2p.	K^+	plasma membrane	yes
YLR138w	Nha1p	K^+/H^+ (Na^+/H^+)	plasma membrane	yes
YNL321w	Vnx1p.	K^+/H^+ (Na^+/H^+)	vacuolar membrane	yes
YDR456w	Nhx1p.	K^+/H^+ (Na^+/H^+)	endosomal memrane	no
YOL027c	Mdm38pp.	K^+/H^+	mitochondrial membrane	no

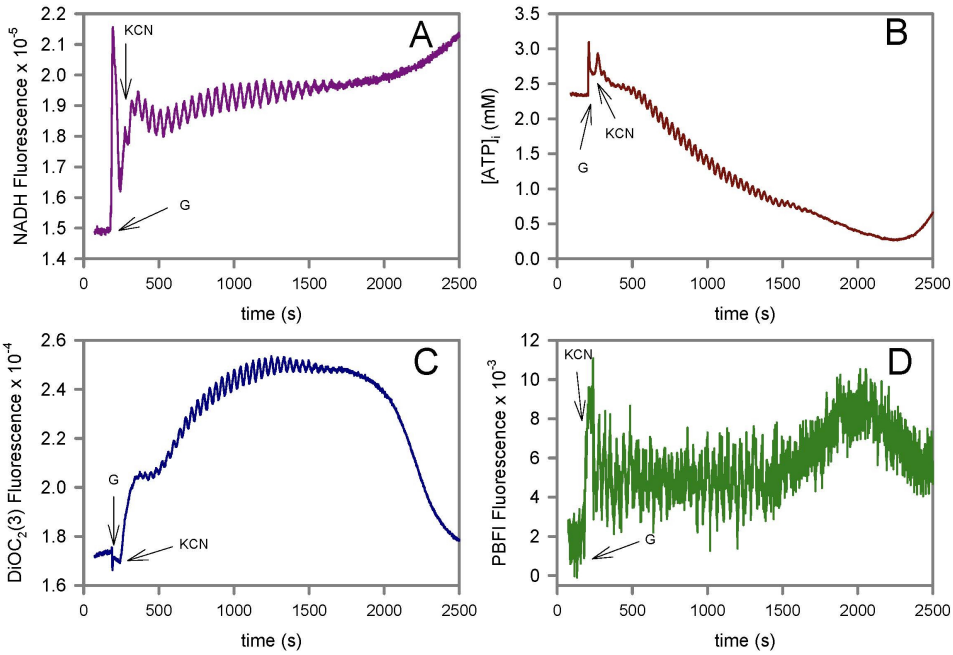

FIGURE 2 Intracellular K^+ oscillates in yeast cells with an oscillating glycolysis. Time series of (A) NADH, (B) Intracellular ATP concentration, (C) fluorescence of $DiOC_2(3)$ and (D) fluorescence of PBFI. 30 mM glucose (G) and 5 mM KCN were added to a dense suspension (10% (w/v)) of cells of the *S. cerevisia* strain BY4743 in 100 mM potassium phosphate, pH 6.8. Temperature 25 °C.

again to its initial level (Ozalp et al., 2010). The minimum ATP concentration coincides with the exhaustion of glucose. The fluorescent dye $DiOC_2(3)$ has been shown to be a good indicator of the mitochondrial membrane potential in yeast (Andersen et al., 2007; Olsen et al., 2009). Following addition of glucose and KCN the fluorescence of $DiOC_2(3)$ first rises rapidly and then more slowly until it reaches a maximum. Oscillations take place on top of this rise in membrane potential. When glucose is fully used up (at around 2000 s) the membrane potential returns to its resting level. The generation of a mitochondrial membrane potential in yeast performing glycolysis in the presence of CN^- is due to the mitochondrial F_1F_0-synthase operating as an ATPase and hence pumping protons out of the mitochondrial matrix (García-Trejo and Morales-Ríos, 2008; Yang et al., 2008). As for the fluorescence of PBFI it rises rapidly after addition of glucose and KCN and then settles on an oscillation around a constant level. This constant level is maintained for a long time, but eventually returns to a resting state.

The question is now: what is driving the oscillations of intracellular K^+? Potassium may enter the cytosol from a number of sinks: (i) the cell exterior, which is rich in potassium, (ii) the mitochondria and (iii) the intracellular vacuoles. We have previously shown that knockouts of potassium transporters in the plasma membrane have no effect on the oscillations of potassium (Olsen et al., 2020). To test if mitochondria are involved in the oscillations of intracellular potassium we have repeated the experiment shown in Fig. 2 with an isogenic strain Atp2pΔ where the beta subunit of the F_1 segment of the mitochondrial F_1F_0 ATPase has been deleted. The result is shown in Fig. S2 in the Supporting

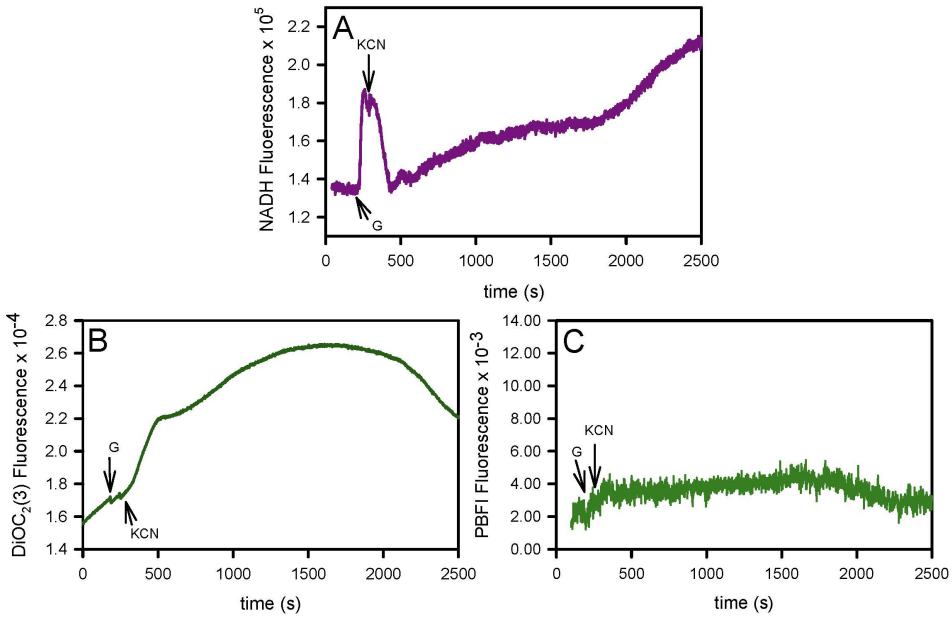

FIGURE 3 Glycolytic oscillations are absent in the mutant strain Mdm38pΔ lacking mitochondrial K^+/H^+ exhange activity. Time series of (A) NADH, (B) fluorescence of $DiOC_2(3)$ and (C) fluorescence of PBFI. 30 mM glucose (G) and 5 mM KCN were added to a suspension (10% (w/v)) of cells of the *S. cerevisia* strain YOL027c in 100 mM potassium phosphate, pH 6.8. Temperature 25 °C.

Information. In addition to the loss of glycolytic oscillations there is no generation of a mitochondrial membrane potential and the intracellular potassium concentration remains at its resting state. Measurements of intracellular ATP show that the slow decline of intracellular ATP has become even slower (Ytting et al., 2012), The same results as shown in Fig. S2 was obtained with several other strains with deletions of subunits of the F_1F_0-ATPase.

Repeating the experiments shown in Figs. 2 and S2 using a mutant strain with a deletion of subunit A (Vma1) of the V1 periferal domain of the vacuolar V-ATPase also showed a loss of glycolytic oscillations as shown in Fig. S3 in the Supporting Information. However, as opposed to the strain with a deletion of F_1F_0 ATPase activity, a mitochondrial membrane potential is generated and, surprisingly, the intracellular K^+ concentration is relatively high, both before and after addition of glucose and KCN. The same result was obtained with other mutant strains with deletions of subunits of the V-ATPase. This confirms that vacuolar V-ATPase is essential in maintaining K^+ homeostasis (Yenush, 2016).

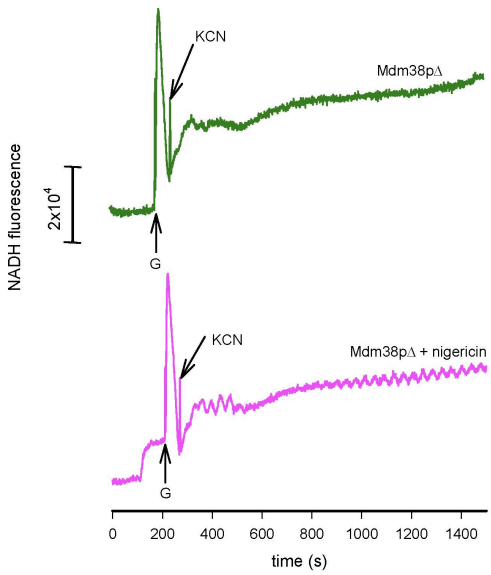

FIGURE 4 Nigericin restores glycolytic oscillations in mutant strain Mdm38pΔ. Measurements of NADH fluorescence in a suspension (10% (w/v)) of cells of the *S. cerevisia* strain YOL027c in 100 mM potassium phosphate, pH 6.8, following addition of first 30 mM glucose (G) and then 5 mM KCN and in the absence (top) or the presence (bottom) of 10 μM nigericin. Temperature was 25 ^{o}C.

3.3 | Deletions of mitochondrial and endosomal K^+/H^+ exhange activity also abolish glycolytic oscillations

The yeast plasma membrane and the membranes of the mitochondria, the vacuole, late endosome and golgi apparatus contain a number of transport proteins with K^+/H^+ exchange activity (Yenush, 2016). An example is the Mdm38 protein in the inner mitochondrial membrane (Nowikovsky et al., 2004, 2007). Deletion of the gene encoding this protein may lead to reduced content of respiratory chain complexes, altered mitochondrial morphology and loss of mitochondrial K^+/H^+ exchange activity resulting in osmotic swelling (Nowikovsky et al., 2007). Fig. 3 shows measurements of NADH, mitochondrial membrane potential and intracellular potassium ion in the Mdm38pΔ mutant strain. We note that glycolytic oscillations are completely absent in this mutant. The decline in intracellular ATP (not shown in figure) is a little slower and the formation of a mitochondrial membrane potential is slightly delayed compared to the wild type BY4743 strain (Fig. 2). The latter is consistent with previous observations using submitochondrial particles (Froschauer et al., 2005). Furthermore, as in the Atp2pΔ mutant the concentration of free intracellular K^+ is low. The fact that a mitochondrial membrane potential can still be formed in this mutant strain suggests that the F_1F_0-ATPase has retained almost normal activity. It was previously shown that addition of nigericin, an electroneutral K^+/H^+ exchanger, may restore mitochondrial function (Nowikovsky et al., 2004, 2007). Therefore, we tested if nigericn could also restore glycolytic oscillations in the Mdm38pΔ mutant strain. The result is shown in Fig. 4. We observe that the presence of nigericin does indeed restore the cells' ability to exhibit glycolytic oscillations. By contrast, deleting the plasma membrane Trk1p and Trk2p K^+ uniport transporters and the electrogenic Nha1p proton antiporter with similar affinity to K^+ and Na^+ did not have any effect on the glycolytic oscillations; neither did deletion of the Vnx1p antiport protein of the vacuolar membrane with K^+/H^+ and Na^+/H^+ transport activity. Surprisingly, deletion of the Nhx1 protein of the late endosomal membrane, which also has both K^+/H^+ and Na^+/H^+ transport activity (Brett et al., 2005) also leads to a loss of glycolytic oscillations. However, for this strain addition of nigericin to a Nhx1pΔ strain did not lead to the restoration of glycolytic oscillations. Why is the concentration of free K^+ low in the resting state of

the wild type BY4743 strain and in most mutants? Fig. S4 in the Supporting Information shows time series of NADH, mitochondrial membrane potential and intracellular K^+ concentration from the Arp1pΔ mutant. This mutant lacks the actin-related protein of the dynactin complex. While ARP1 is a nonessential gene, the Arp1pΔ mutant shows a decrease in growth rate in rich media; moreover, it is temperature-sensitive and, important in this context, has abnormal spindle morphology and nuclear migration. Apart from oscillations the overall form of the NADH fluorescence $DiOC_2(3)$ time series are similar to those obtained with the BY4743 wild type. However, the PBFI signal in the resting state is elevated as for the Vma1pΔ mutant and remains so after addition of glucose and KCN.

3.4 | Analysis of glycolytic oscillations using SINDy

Data-driven modeling is becoming increasingly important in analyses of experimental data, e.g., time series. Data-driven models do not require any *a priori* assumptions about model structure and may be used to predict the future of a system (Gouesbet and Letellier, 1994; Mangiarotti et al., 2012; Brunton et al., 2016). Furthermore, some data-driven model applications may unravel unknown couplings between experimental variables (Thenon et al., 2022; Prokop and Gelens, 2023). Here we use the SINDy machine learning application (Brunton et al., 2016) to analyze the time series of NADH fluorescence, intracellular ATP concentration, $DiOC_2(3)$ fluorescence ($\Delta\psi_m$) and PBFI fluorescence (K^+). A brief description of the SINDy method and an example of its application to artificial data is given in the Supporting information. Following the suggestion by Prokop and Gelens (2023) we first removed linear and nonlinear trends of the four time series (NADH, ATP, $\Delta\psi_m$ and K^+) and rescaled the data on the intervale [0 1]. The resulting time series are shown in Fig. S6 in the Supporting Information. Then the SINDy algorithm was applied to these data with a maximum polynomial order of 2 and a threshold λ= 0.062. Surprisingly, this yielded a sparse coefficient matrix with 13 terms (see Table S1 in the Supporting Information), that could be translated into the following model:

$$\dot{w} = 0.2329x - 0.2195z - 0.1981x^2 + 0.183z^2 \tag{1}$$

$$\dot{x} = 0.1497x - 0.2895xz \tag{2}$$

$$\dot{y} = 0 \tag{3}$$

$$\dot{z} = -0.1109x - 0.1341w^2 + 0.1379wy + 0.0955wz + 0.2195x^2 - 0.1831yz + 0.0633z^2 \tag{4}$$

Here, w indicates the rescaled NADH data, x indicates the rescaled ATP data, y indicates the rescaled $\Delta\psi_m$ ($DiOC_2(3)$) data and z indicates the rescaled K^+ (PBFI) data. 3D phase plots of three of the four variables of the original data and the model described by equations (1) to (4) are shown in Fig. 5A and Fig. 5B, respectively. The model described by eqs. (1) - (4) should not be considered as a true model of the oscillations of NADH, ATP, $\Delta\psi_m$ and K^+, but the equations seem to reveal important information about the coupling of the four variables. For example, the equations show that ATP is important for the oscillations of NADH (and not the other way around) and that autocatalysis seems to a be driving force for the oscillations of ATP (Kloster and Olsen, 2012), as evidenced by the positive term $0.1497x$ in eq. (2). This is in accordance with current knowledge about the control of glycolysis (Goldbeter, 1996; Kloster and Olsen, 2012). However, the SINDy model also reveals that K^+ concentration seems to be important for oscillations of both NADH and ATP. For the latter the negative term $0.2895xz$ in eq. (2) suggests that K^+ is coupled to ATPase activity. Furthermore, it is surprising that oscillations of $\Delta\psi_m$ apparently do not strongly depend on neither NADH nor ATP. This is most likely because the coefficients of linear and nonlinear terms in eq. (3) are below the threshold $\lambda = 0.062$. Finally, oscillations of intracellular K^+ seem to depend on all the other three variables investigated here. It is worth mentioning that the positive quadratic term $0.2195x^2$ in eq. (4) signals a positive coupling between ATP

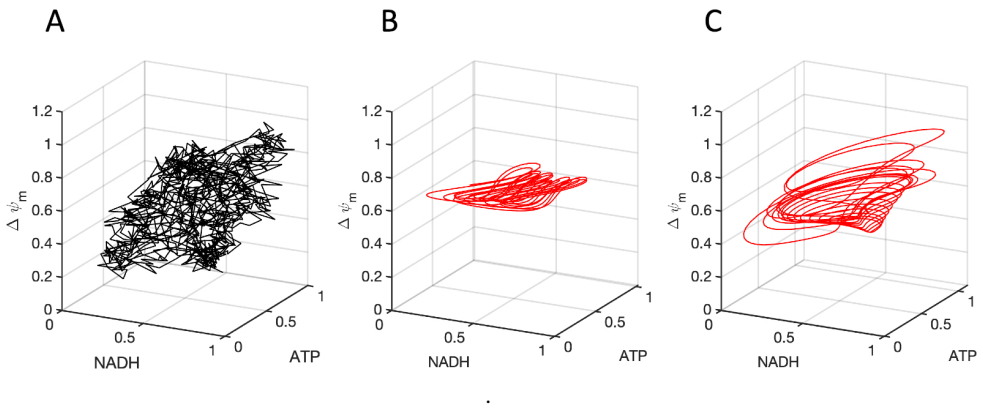

FIGURE 5 3D phase plots of original data and the SINDy models of the time series of NADH, ATP, mitochondrial membrane potential and intracellular K^+ concentration. The plot in A irepresents the rescaled experimental data, while the plot in B represents data from a SINDy model with 13 non-zero elements in the coefficient matrix (λ= 0.062, see eqs. (1) - (4)), and the plot in C represents data from a model with 36 non-zero elements in the coefficient matrix (λ=0.04). 870 data points. Maximum polynomial order is two.

and intracellular K^+ while the negative term $0.1341w^2$ signifies a negative coupling between K^+ and NADH. Finally, the positive term $0.0633z^2$ evidence a positive feedback from K^+ itself. Decreasing the threshold λ to 0.04 results in an increase in the number of terms in the coefficient matrix to 36 (see Table S2 in the Supporting Information), i.e. the matrix is no longer sparse. However, many of the terms from the coefficient matrix for λ=0.062 seem to be preserved in this new matrix. Furthermore, the derivative $\dot{y}$ is no longer zero, The 3D phase plot corresponding to this new (non-sparse) model is shown in Fig. 5C. 3D phase plots of NADH versus ATP versus K^+ of the original data and the two SINDy models are shown in Fig S7 in the Supporting Information. Inspecting the time series of the four rescaled variables in Fig. S6 one might expect that the phase plots in Fig. 5 and Fig S7 would form simple closed loops, corresponding to a simple limit cycle oscillations. However, the form of these plots reflect the rise and fall of the rescaled time series of NADH, ATP, $\Delta\psi_m$ and K^+. Reducing λ even further (below $\lambda = 0.04$) results in coefficient matrices with even more non-zero coefficients (between 50 and 60), but with 3D phase plots similar to those in Fig. 5C and Fig. S7C in the Supporting information.

4 | DISCUSSION

In the current study we have shown that K^+ ions are essential for oscillations in glycolysis in intact yeast cells, and that intracellular K^+ concentration oscillates synchronously with other intracellular variables such as NADH, ATP and mitochondrial membrane potential. This supports the results of a previous study (Olsen et al., 2020) that there is a strong coupling between glycolysis and intracellular potassium ion. Furthermore, we observe here that mutant strains affecting release/adsorption and transport of K^+ between intracellular compartments (mainly K^+/H^+ exchange activity in mitochondrial and endosomal membranes) no longer have the ability to show glycolytic oscillations. By contrast, mutant strains that lack plasma membrane transport proteins for K^+ still show glycolytic oscillations, indicating that influx of potassium from the external medium is not important for the formation of glycolytic oscillations. It was previously found that intracellular (cytosolic) pH oscillates in synchrony with NADH and other variables (Olsen et al., 2009). However, it has yet to be determined how these oscillations in intracellular proton concentration are linked to

the oscillations of intracellular K^+. The mechanism behind the coupling of oscillations in intracellular K^+ to oscillations in intracellular pH is not easy to establish as several potential mechanisms come into play: (i) Oscillations in intracellular pH were originally believed to originate from oscillations in activities of the plasma membrane ATPase Pma1 and the F_1F_0 and V-ATPases in mitochondrial and vacuolar membranes, respectively (Olsen et al., 2009; Ozalp et al., 2010), driven by oscillations in intracellular ATP concentration. These oscillations in pH in turn could drive oscillations in intracellular K^+ concentration through activity of K^+/H^+-antiporters. (ii) Alternatively, it might be oscillations in intracellular K^+ concentration, and not oscillating ATPase activities, that drive the oscillations in intracellular pH. Analyses of data from glycolytic oscillations that include data on intracellular pH using SINDy might help to distinguish between these alternative hypotheses. Unfortunately, experimental data on oscillations in intracellular pH have a fairly low signal-to-noise ratio, which precludes their use in the SINDy analysis performed above.

We note that in strains lacking F_1F_0-ATPase activity potassium concentration in the cytosol is low. This is also the case for the strain with a deletion of Mdm38p K^+/H^+ exhange activity. By contrast in strains lacking V-ATPase activity free cytosolic K^+ concentration is relatively high. These observations suggest that exchange of intracellular K^+ between mitochondria and the cytosol and between the cytosol and the vacuole are essential for a well-functioning metabolism. The loss of glycolytic oscillations associated with deceleration or acceleration of intracellular ATPase activity has previously been explained in terms of the existence of narrow region of ATPase activity outside of which oscillations do not occur (Olsen et al., 2009), but this cannot explain the effect of the loss of K^+/H^+ exchange activity in the mutant strains Mdm38pΔ and Nhx1pΔ, which do seem to have the same ATPase activity as the wild type strain. While the oscillations in glycolysis can be restored by addition of nigericin to the mutant strain Mdm38pΔ this is not the case for the strain Nhx1pΔ. It is possible that for the Nhx1pΔ mutant strain the loss of oscillatory activity may be a secondary effect not directly related to loss of Nhx1p protein. For example, loss of Nhx1p activity may result in growth-sensitivity to low pH and high salt (Mukherjee et al., 2006). If this were the case addition of nigecirin should not restore glycolytic oscillations. It is surprising that deletion of K^+ transport through the Vnx1p K^+/H^+ (Na^+/H^+) antiporter does not affect glycolytic oscillations as opposed to deletions of the Nhx1p K^+/H^+ antiporter. A possible explanation for the lack of effect of Vnx1pΔ is that other monovalent cation antiporters ensure the exchange of potassium between the vacuole and the cytosol.

We have previously obtained evidence that oscillations in yeast glycolysis in intact cells also depend on the physical state of intracellular water (Thoke et al., 2015, 2017, 2018a). in this context it may be relevant to include a discussion of biomolecular condensates, which are a class of membrane-less cellular organelles that form through phase separation of participating biomolecules. It has been shown that under hypoxia many glycolytic enzymes in yeast are compartmentalized into messenger ribonucleoprotein structures, termed glycolytic (G) bodies, that carry all the signatures of biomolecular condensates (Fuller et al., 2020; Fuller and Kim, 2021). It is interesting that in a mutant strain defective in Ira2, glycolytic oscillations are absent (Williamson et al., 2012). Ira2 is a negative regulator of the pro-growth yeast RAS/cAMP pathway and is required for the formation of G bodies. Furthermoe, it was found in yeast that G bodies appear to form gels. This could explain our previous observations (Thoke et al., 2015, 2017, 2018a) that glycolysis in yeast seem to take place in an environment with restricted dynamics of intracellular water. Note that also multienzymatic complexes of glycolytic enzymes to actin have been found (Araiza-Olivera et al., 2013) and that such complexes seem to optimize glycolytis. It has yet to be determined if such glycolytic enzyme complexes are biomolecular condensates (Fuller and Kim, 2021). Nevertheless, it is interesting that a mutant strain lacking Arp1p also shows lack of glycolytic oscillations. Furthermore, in this mutant strain free cytosolic K^+ is elevated compared to the BY4743 wild type strain.. This suggests that also the cytoskeleton contributes to K^+ homeostasis in the cytosol (Olsen et al., 2020).

The analysis of rescaled time series of NADH, ATP, $\Delta\psi_m$ and K^+ using data-driven modeling (SINDy) suggests that

ATP oscillations involve autocatalysis (Kloster and Olsen, 2012), but that the dynamics of ATP also strongly depend on intracellular K^+ concentration. This is in accordance with the observation that in mutant strains affecting intracellular K^+ dynamics glycolytic oscillations do no occur. By contrast, NADH oscillations seem to be passively coupled to oscillations of ATP and K^+. This is not surprising since, according to most models of glycolysis, the concentration of NADH simply follow the rise and fall of concentration of ATP (Goldbeter, 1996; Hald and Sørensen, 2010). With respect to the oscillations of $\Delta\psi_m$ it must be emphasized that lack of non-zero coefficients in eq. (3) does not imply that $\Delta\psi_m$ is weakly coupled to the other three variables. Non-zero coefficients would most likely appear in experimental data that had a higher signal-to noise ratio compared to the current data. Nevertheless, as for the test example in the Supporting Information, SINDy reveals a non-sparse model that also involves $\Delta\psi_m$ of the experimental glycolysis data for a lower value of λ. As for oscillations of intracellular K^+, they seem to be strongly coupled to the other three variables, NADH, ATP and $\Delta\psi_m$. In the case of NADH its role in oscillations of K^+ could be to interact with components in the mitochondrial respiratory chain. It was shown previously that strains with mutations in respiratory enzymes such as cytochrome c oxidase are unable to show glycolytic oscillations (Schroder et al., 2013; Thoke et al., 2017). This suggests that even under anaerobic or semi-anaerobic conditions the mitochondrial respiratory chain is active in metabolism. The presence of terms for ATP in eq. (4) fits the observation that in mutant strains, where ATP turnover is affected, the dynamics of intracellular K^+ is also affected. However, note that ATP has also been shown to play a role in the dynamics of intracellular water (Thoke et al., 2015, 2017, 2018a), which again may influence intracellular K^+ concentration (Olsen et al., 2020). The presence of terms involving $\Delta\psi_m$ in equation (4) is compatible with the results showing that mutant strains defective in generation of a mitochondrial membrane potential also affect the dynamics of intracellular K^+. Consequently, there is a fine correspondence between the SINDy analysis results and the results obtained with the mutant strains.

It has been argued that glycolytic oscillations arise as a consequence of tight feed-back regulations and therefore have no physiological function (Chandra et al., 2011). However, it is known that in pancreatic $\beta-$cells there is a coupling between oscillating glycolysis and pulsatile insulin secretion (Bertram et al., 2007; Xiong and Garfinkel, 2023) and that this pulsative behavior is lost in individuals with type 2 diabetes (Satin et al., 2015). In $\beta-$cells there is a tight coupling of glycolysis to a number of other metabolic processes involving plasma- and intracellular membranes. Hence, in the case of $\beta-$cells oscillating glycolysis has a clear physiological function (Xiong and Garfinkel, 2023). It is interesting that the results presented here and in previous work (Andersen et al., 2007; Olsen et al., 2009; Williamson et al., 2012; Olsen et al., 2020) evidence an increasing similarity of glycolytic oscillations in yeast to similar oscillations in $\beta-$cells (Bertram et al., 2007; Merrins et al., 2013, 2016), and therefore one might speculate that these oscillations have evolved over millions of years to become part of an important physiological process in mammals, namely the regulation of blood sugar levels.

To summarize, the current study, together with previous studies (Andersen et al., 2007; Olsen et al., 2009; Ozalp et al., 2010; Ytting et al., 2012; Thoke et al., 2015, 2017; Olsen et al., 2020), challenges the classical view that oscillations in glycolysis in intact yeast cells, except for unspecified ATPase activity, only involve enzymes in the glycolytic pathway (Goldbeter, 1996; Hald and Sørensen, 2010). In addition these oscillations seem to be closely linked to glycolytic oscillations in pancreatic $\beta-$cells (Bertram et al., 2007; Xiong and Garfinkel, 2023) and other mammalian cell types such as cardiomycytes (Yang et al., 2008; Ganitkevich et al., 2010).

AUTHOR CONTRIBUTIONS

Lars Folke Olsen conceptualized and designed the experiments. Anita Lunding took care of growth and maintenance of yeast strains and performed measurements of NADH, ATP, $DiOC_2(3)$ and PBFI. Lars Folke Olsen performed data

analysis and wrote the paper.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY

The data that support the findings in this study are available upon reasonable request from the corresponding author

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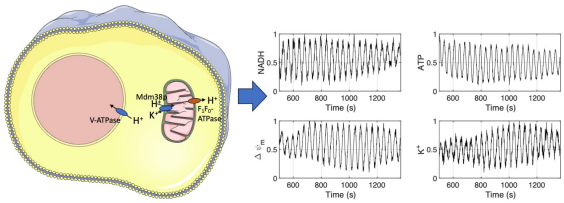
GRAPHICAL ABSTRACT



Glycolytic oscillations in intact yeast cells are coupled to oscillations in intracellular K^+. The coupling involves transport proteins in the membranes of several organelles such as mitochondria and the vacuole as well as cytoskeletal proteins.