Supplemental Information

Pyruvate Kinase Triggers a Metabolic Feedback Loop that Controls Redox Metabolism in Respiring Cells

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Inventory of Supplemental Information

- **Figure S1**
  Legend Figure S1

  Figure S1 is connected to Figure 3B. Figure S1 demonstrates an increase of resistance to the oxidant diamide with decreased PYK activity also on SC media containing galactose as carbon source.

- **Table S1**
  Legend Table S1

  Table S1 is connected to Figure 5B and presents absolutes values of PPP metabolites which are shown as ratios in Figure 5B.

- **Figure S2**
  Legend Figure S2

  Figure S2 is connected to the discussion part of the manuscript (Figure 7). The increase in oxygen consumption in PPP and PCK mutants with low PYK activity rules out a regulatory role of these enzymes in respiration activation.

- **Supplemental Experimental Procedures**
  Yeast cultivation
  Plasmids
  Gene deletion
  *Bona fide* ρ0
  Quantitative RT-PCR
  Enzyme activity assays

- **Supplemental References**
Figure S1, related to Figure 3B: Reduced PYK activity and galactose media leads to higher resistance to oxidants. Strains were grown over night, diluted to an OD$_{600}$ of 3.0, and spotted as 1:5 dilution series onto YPD and YPGal containing diamide at the indicated concentration.
Table S1, related to Figure 5B: PPP metabolites are increased in strains with low PYK activity.

Values are given normalized to dhap concentration in a reference measurement (correction factor 36.5, black) and as ratio to metabolite concentration in the BY4741 wild-type strain (grey). g6p (glucose 6-phosphate), f6p (fructose 6-phosphate), 6pg (6-phosphogluconate), r5p (ribose 5-phosphate), x5p (xylulose 5-phosphate), rib5p (ribulose 5-phosphate), s7p (sedoheptulose 7-phosphate), e4p (erythrose 4-phosphate), dhap (dihydroxyacetone phosphate), gly3p (glyceraldehydes 3-phosphate).

|                | g6p / f6p | f6p   | r5p   | x5p / rib5p | s7p   | e4p   | dhap   | gly3p |
|----------------|-----------|-------|-------|-------------|-------|-------|--------|-------|
| BY4741 wt      | 0.885     | 0.075 | 0.117 | 0.033       | 0.660 | 0.056 | 0.370  | 0.011 |
|                | 100.00    | 100.00| 100.00| 100.00      | 100.00| 100.00| 100.00 | 100.00 |
| TEFp-PYK1      | 0.964     | 0.087 | 0.164 | 0.029       | 0.673 | 0.058 | 0.670  | 0.014 |
|                | 129.61    | 132.70| 131.17| 91.73       | 91.67 | 115.69| 128.51 | 130.07 |
| TEFp-PYK2      | 1.328     | 0.107 | 0.233 | 0.029       | 0.299 | 0.086 | 1.252  | 0.027 |
|                | 150.03    | 148.42| 150.32| 116.90      | 116.74| 115.00| 154.75 | 202.24 |
| CYCp-PYK1      | 1.329     | 0.111 | 0.211 | 0.039       | 0.696 | 0.096 | 1.567  | 0.023 |
|                | 194.16    | 190.69| 180.00| 120.46      | 126.01| 190.93| 208.15 | 213.50 |

|                | g6p / f6p | f6p   | r5p   | x5p / rib5p | s7p   | e4p   | dhap   | gly3p |
|----------------|-----------|-------|-------|-------------|-------|-------|--------|-------|
| BY4741 wt      | 3.349     | 0.177 | 0.181 | 0.099       | 0.118 | 0.120 | 1.061  | 0.026 |
|                | 100.00    | 100.00| 100.00| 100.00      | 100.00| 100.00| 100.00 | 100.00 |
| TEFp-PYK1      | 3.973     | 0.148 | 0.236 | 0.065       | 0.141 | 0.096 | 1.266  | 0.006 |
|                | 100.61    | 84.46 | 140.20| 93.20       | 119.77| 81.82 | 117.16 | 100.52 |
| TEFp-PYK2      | 4.280     | 0.177 | 0.234 | 0.074       | 0.151 | 0.109 | 2.296  | 0.048 |
|                | 109.37    | 99.99 | 140.21| 124.65      | 128.22| 80.37 | 212.00 | 161.05 |
| CYCp-PYK1      | 4.399     | 0.259 | 0.199 | 0.117       | 0.186 | 0.115 | 3.135  | 0.054 |
|                | 124.04    | 140.85| 123.37| 157.37      | 158.36| 95.91 | 250.99 | 205.26 |
| CYCp-PYK2      | 4.388     | 0.434 | 0.387 | 0.165       | 0.263 | 0.201 | 6.825  | 0.065 |
|                | 129.31    | 245.81| 236.48| 229.13      | 215.17| 167.25| 839.62 | 360.52 |
Figure S2, related to discussion/Figure 7: Oxygen consumption increases in with low PYK activity in PPP and PCK mutants. PPP enzymes or PCK1 were deleted in Δpyk1Δpyk2 yeast strains containing the different PYK constructs. Oxygen consumption was determined for logarithmically YPD-growing cultures in a closed chamber oxygraph (Oroboros). Tkl2 (encoding transketolase), Tal1 (transaldolase), Rpe1 (ribulose 5-phosphate epimerase), Tk1 (transketolase), Pck (pyruvate carboxykinase), Zwf1 (glucose 6-phosphate dehydrogenase). Low PYK activity increased oxygen consumption in all deletion strains tested.
Supplemental Experimental Procedures

Yeast cultivation
Yeast were grown at 28-30°C either in yeast-extract peptone 2% dextrose (YPD), yeast-extract peptone 2% galactose (YPGal), 3% ethanol/0.1% glucose (YPEtOH) or in synthetic complete (SC) media lacking the indicated amino acids/bases.

Plasmids
Plasmids encoding TPI were previously described (Ralser et al., 2006). PYK-encoding plasmids were generated by amplifying PYK1 and PYK2 from yeast genomic DNA by PCR, and ligating the products into centromeric yeast plasmids containing the TEF1 promoter (p413TEF), the CYC1 promoter (p413CYC), or a GPD1 promoter (p416GPD) (Mumberg et al., 1995). All plasmids were verified by sequencing and primer sequences are given in the table below.

|        | Primer Sequence                         |
|--------|----------------------------------------|
| PYK1-fw-BamH1 | 5'-GAGGATCCATGTCTAGATTAGAAAGA-3'       |
| PYK1-as-Sal  | 5'-GAGTCGACTAAACGGTAGAGACTTG-3'       |
| PYK2-fw-BamH1 | 5'-GAGGATCCATGCGAGTCCAGATTG-3'       |
| PYK2-as-Sal1  | 5'-GAGTCGACCTAGAATTCTTGACCAAC-3'     |

underlined DNA sequences indicate introduced restriction sites

Gene deletion
Genes were deleted in BY4741 strains by homologous recombination, by single gene replacement with the nourseothricin (natMX4), kanamycin (kanMX4), or hygromycin (hphMX4) markers. Primer pairs (which overlap with 20 bases of the marker gene and 35-45 bases with the target locus) were used to amplify the marker cassette and then transformed into yeast. Positive transformants were selected on YPD containing antibiotics, and isolated recombinants were verified by PCR. Primer sequences are given in the table below.
Isogenic PYK mutants were generated by plasmid shuffling. \( \Delta pyk2 \) yeast was transformed with an URA3-plasmid encoding for PYK1 (p416GPD-PYK1). Then, endogenous PYK1 was deleted using natMX4, and positive knock-outs were selected by PCR. The \( \Delta pyk1\Delta pyk2 \) pCEN-URA3-PYK1 strain was subsequently transformed with HIS3-marked PYK plasmids (p413TEF-PYK1, p413CYC-PYK1, p413TEF-PYK2, p413CYC-PYK2). Finally, the URA3-plasmid was counter-selected for positive transformants on SC\(^{\text{HIS}}\) containing 0.15% 5'FOA. \( \Delta pyk1\Delta pyk2\Delta zwf1 \) and \( \Delta pyk1\Delta tpi1 \) yeast expressing PYK1, PKY2, and/or TPI from centromeric plasmids were generated in a similar fashion.

_Bona fide_ \( \rho 0 \) strains were generated through repeated treatment with 50 µg/ml ethidium bromide as previously described (Goldring et al., 1970).

**Quantitative RT-PCR**

qRT-PCR was performed as previously described in (Wamelink et al., 2010). Yeast were cultivated overnight in YPD, washed once in water, and grown to log phase (OD\(_{600} \sim 0.8\) ) in YPD or YPGal. For boost experiments, YPD was exchanged with YPGal one hour before harvesting cells. mRNA was extracted and qRT-PCR was performed using an ABI prism 7800HT system. Primer sequences are listed in the Supplemental Information. Expression of
COX1, COX2, COX3 was normalized to the expression of the reference genes ATG27 and TAF10 as by the method of (Pfaffl, 2001).

**Enzyme activity assays**

Pyruvate kinase activity was determined as described by (Bergmeyer et al., 1974). Briefly, a reaction mixture containing 24 mM KH$_2$PO$_4$/K$_2$HPO$_4$ (pH 7.0), 150 µM NADH, 1 mM fructose 1,6 bisphosphate, 2.4 mM ADP, 25 U lactate dehydrogenase (Sigma-Aldrich), 10 mM MgSO$_4$, and 4 µg centrifugation-cleared whole-cell extract was supplemented with 800 µM PEP. OD$_{340}$ was used to detect NADH oxidation in 6- to 10-s intervals using an spectrophotometer (Amersham US 2000). TPI activity was determined as previously described (Ralser et al., 2006). $K_m$ and $K_i$ were determined by saturation curves with gly3p and PEP, respectively, in yeast extracts (BY4741), transgenic yeast expressing human TPI (MR101) (Ralser et al., 2006), or purified rabbit muscle TPI (Sigma-Aldrich).
**Supplemental References**

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Mumberg, D., Muller, R., and Funk, M. (1995). Yeast vectors for the controlled expression of heterologous proteins in different genetic backgrounds. Gene 156, 119-122.

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