

Paper 2 Topics:

The switch from glucose-limited aerobic chemostat growth to full anaerobiosis and glucose excess resulted in rapid acceleration of fermentative metabolism

2 distinct phases in up-regulation of glycolysis upon a switch to fermentative conditions

- I. Initial phase - regulation occurs completely through changes in metabolite levels
- II. Second phase - regulation achieved through a combination of changes in V_{\max} (capacity of enzymes of the metabolic pathway) AND metabolite concentrations

*Above indicates there needs to be a series of types of fermentation equations - possibly one part that becomes limiting or conditional on the other

Paper suggests taking a systems biology approach to understand the “glycolytic system” taking into account multiple levels of regulation

In this paper enzyme concentrations and intracellular metabolite concentrations were considered using kinetic rate equations. Not sure if this is something I will want to include - might need to reconsider for later

The multiple phases also leads me to believe that in terms of glycolysis rates, something that needs to be considered in the rate of glucose consumption is whether aerobic, or phase 1 or phase 2 anaerobic respiration is taking place. This would require including a conditional variable though and I'm not sure if that would work here.

Glycolytic flux was investigated through looking at the specific glucose uptake rate
Concentrations of acetate, lactate, pyruvate, succinate, and acetaldehyde
apparently increased slightly along with glycerol concentration

TABLE 3. Physiological parameters for an aerobic glucose-limited chemostat culture and for 2 h after the change to anaerobic glucose-excess conditions^a

Culture	μ	Y_{rx}	q_{gluc}	q_{O_2}	q_{CO_2}	q_{eth}	q_{glyc}	q_{ac}	Carbon recovery (%)
Chemostat	0.10 ± 0.01	0.50 ± 0.01	1.09 ± 0.03	2.7 ± 0.1	2.8 ± 0.1	NA ^b	NA	NA	102 ± 1
2 h after change	0.14 ± 0.00	0.07 ± 0.04	13.4 ± 1.0	NA	21.6 ± 0.9	19.6 ± 1.4	3.28 ± 0.14	0.19 ± 0.02	93 ± 7

^a The data are the averages and standard deviations for at least three independent cultivations. Abbreviations: μ , specific growth rate determined by measurement of the dry weight; Y_{rx} , biomass yield on glucose (grams of biomass per gram of glucose consumed); q_{gluc} , millimoles of glucose consumed per gram of biomass per hour; q_{O_2} , millimoles of oxygen consumed per gram of biomass per hour; q_{CO_2} , millimoles of carbon dioxide produced per gram of biomass per hour; q_{eth} , millimoles of ethanol produced per gram of biomass per hour; q_{glyc} , millimoles of glycerol produced per gram of biomass per hour; q_{ac} , millimoles of acetate produced per gram of biomass per hour.

^b NA, not applicable.

Above table shows change in parameters between aerobic baseline and 2hrs after anaerobic processes

Intracellular metabolites (V_{\max} of glycolytic enzymes) found to strongly influence regulation of glycolytic flux

It should be noted this section of the paper starts delving into gene regulatory networks it seems like

All Kinetic Equations from Appendix with explanation*

*See following page

All these equations really seem to point towards kinetic equations as being highly important in modeling the process of glycolysis regulation; in model might need to consider the role of the presence of certain enzymes in whether fermentation or respiration is occurring

(regulation of enzyme capacity, primarily via protein synthesis and/or degradation) and metabolic regulation (modulation of enzyme activity, primarily via low-molecular-weight metabolites). Calculation of hierarchical and metabolic coefficients (see File S2 in the supplemental material) confirmed that in the first 45 min after the switch to fermentative conditions, regulation could be completely assigned to metabolic regulation. In the second phase (45 to 120 min), almost completely hierarchical regulation (which covers transcription, translation, and posttranslational modification) was observed for the enzymes PGI, FBA, TPI, GAPDH, GPM, ENO, PYK, and PDC. The remaining four enzymes, HXK, PFK, PGK, and ADH, were completely governed by metabolic regulation during the entire experiment. Recent studies of steady-state cultures have pioneered the use of regulation analysis for dissecting hierarchical regulation into transcriptional, translational, and post-translational regulation (8). Application of such a high-information-density, multilevel, systems biology approach to the dynamic situation described in the present study should contribute to a deeper understanding of the mechanisms of glycolytic regulation in *S. cerevisiae*.

APPENDIX

Kinetic equations. The reversible reaction catalyzed by PGI was described by one-substrate, one-product reversible Michaelis-Menten kinetics (41):

$$v = V_{\max} \frac{\frac{[G6P]}{K_{G6P}} \left(1 - \frac{\Gamma}{K_{eq}}\right)}{1 + \frac{[G6P]}{K_{G6P}} + \frac{[F6P]}{K_{F6P}}} \quad (2)$$

where v is the flux, V_{\max} is the maximal rate, $[G6P]$ is the concentration of glucose-6-phosphate, $[F6P]$ is the concentration of fructose-6-phosphate, Γ is the mass-action ratio ($[F6P]/[G6P]$), K_{eq} is the equilibrium constant, and K_{G6P} and K_{F6P} are the Michaelis-Menten constants for glucose-6-phosphate and fructose-6-phosphate, respectively.

The kinetic model used for PFK was a function of the concentrations of fructose-6-phosphate, ATP ($[ATP]$), AMP ($[AMP]$), fructose-2,6-bisphosphate ($[F26bP]$), and fructose-1,6-bisphosphate ($[F16bP]$). All the effects were captured in one rate equation, as described by Teusink et al. (41):

$$v = V_{\max} \frac{g_R \lambda_1 \lambda_2 R}{R^2 + L T^2} \quad (3)$$

with

$$\lambda_1 = [F6P]/K_{R,F6P}, \quad \lambda_2 = [ATP]/K_{R,ATP} \quad (4a,b)$$

$$R = 1 + \lambda_1 \lambda_2 + g_R \lambda_1 \lambda_2, \quad T = 1 + c_{ATP} \lambda_2 \quad (5a,b)$$

$$L = L_0 \left(\frac{1 + C_{L,ATP} [ATP]/K_{ATP}}{1 + [ATP]/K_{ATP}} \right)^2 \cdot \left(\frac{1 + C_{L,AMP} [AMP]/K_{AMP}}{1 + [AMP]/K_{AMP}} \right)^2 \cdot \left(\frac{1 + C_{L,F26bP} [F26bP]/K_{F26bP} + C_{L,F16bP} [F16bP]/K_{F16bP}}{1 + [F26bP]/K_{F26bP} + [F16bP]/K_{F16bP}} \right) \quad (6)$$

where L_0 is the allosteric constant, K_{ATP} , K_{AMP} , K_{F26bP} , and

K_{F16bP} are the constants for ATP, AMP, fructose-2,6-bisphosphate, and fructose-1,6-bisphosphate, respectively, and $C_{L,ATP}$, $C_{L,AMP}$, $C_{L,F26bP}$, and $C_{L,F16bP}$ are the inhibition concentrations of ATP, AMP, fructose-2,6-bisphosphate, and fructose-1,6-bisphosphate, respectively.

For the enzyme PYK two different kinetic equations were used, with and without activation by fructose-1,6-bisphosphate. The kinetics without an activator were described by Michaelis-Menten kinetics for two noncompeting substrate-product couples (41):

$$v = V_{\max} \frac{\frac{[PEP][ADP]}{K_{PEP} K_{ADP}} \left(1 - \frac{\Gamma}{K_{eq}}\right)}{\left(1 + \frac{[PEP]}{K_{PEP}} + \frac{[Pyr]}{K_{Pyr}}\right) \left(1 + \frac{[ADP]}{K_{ADP}} + \frac{[ATP]}{K_{ATP}}\right)} \quad (7)$$

where $[PEP]$ and $[ADP]$ are the concentrations of phosphoenolpyruvate (PEP) and ADP, respectively, $[Pyr]$ and $[ATP]$ are the concentrations of the products pyruvate and ATP, respectively, Γ is the mass-action ratio, and K_{PEP} , K_{ADP} , K_{Pyr} , and K_{ATP} are the constants for phosphoenolpyruvate, ADP, pyruvate, and ATP. The equation of Rizzi et al. (32) included the activation by fructose-1,6-bisphosphate:

$$v = V_{\max} \frac{\frac{[PEP]}{K_{PEP}} \left(\frac{[PEP]}{K_{PEP}} + 1 \right)^{n-1}}{L_0 \left(\frac{[ATP]}{K_{ATP}} + 1 \right) \left(\frac{[FbP]}{K_{FbP}} + 1 \right)^n \left(1 + \frac{[PEP]}{K_{PEP}} \right)^n} \cdot \frac{[ADP]}{[ADP] + K_{ADP}} \quad (8)$$

where $[FbP]$ is the concentration of fructose-1,6-bisphosphate, K_{FbP} is the Michaelis-Menten constant for fructose-1,6-bisphosphate, L_0 is the allosteric constant, and n is the interaction factor for subunits.

The reaction catalyzed by PDC was described by irreversible Hill kinetics, as described by Teusink et al. (41):

$$v = V_{\max} \frac{\left(\frac{[Pyr]}{K_{Pyr}} \right)^{n_H}}{1 + \left(\frac{[Pyr]}{K_{Pyr}} \right)^{n_H}} \quad (9)$$

where $[Pyr]$ is the pyruvate concentration, K_{Pyr} is the constant for pyruvate, and n_H is the Hill coefficient. The parameters used in all kinetic equations are shown in Tables 1 and 2.

ACKNOWLEDGMENTS

We thank Johan Knoll, Jan van Dam, Reza Maleki Seifar, and Angela ten Pierick for technical assistance with measuring the intracellular metabolites.

This project was financially supported by the IOP Genomics Program of Senter Novem, The Netherlands.

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