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Constant Growth Rate Can Be Supported by Decreasing Energy Flux and Increasing Aerobic Glycolysis

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SUMMARY

Fermenting glucose in the presence of enough oxygen to support respiration, known as aerobic glycolysis, is believed to maximize growth rate. We observed increasing aerobic glycolysis during exponential growth, suggesting additional physiological roles for aerobic glycolysis. We investigated such roles in yeast batch cultures by quantifying O_2 consumption, CO_2 production, amino acids, mRNAs, proteins, posttranslational modifications, and stress sensitivity in the course of nine doublings at constant rate. During this course, the cells support a constant biomass-production rate with decreasing rates of respiration and ATP production but also decrease their stress resistance. As the respiration rate decreases, so do the levels of enzymes catalyzing rate-determining reactions of the tricarboxylic-acid cycle (providing NADH for respiration) and of mitochondrial folate-mediated NADPH production (required for oxidative defense). The findings demonstrate that exponential growth can represent not a single metabolic/physiological state but a continuum of changing states and that aerobic glycolysis can reduce the energy demands associated with respiratory metabolism and stress survival.

INTRODUCTION

Understanding cell growth is essential to both basic science and treating diseases associated with deregulated cell growth, such as cancer. Accordingly, cell growth has been studied extensively, from the pioneering work of Krebs and Eggleston (1940) and Monod (1949) to recent discoveries (DeBerardinis et al., 2007; Slavov et al., 2011; Scott et al., 2010; Youk and van Oudenaarden, 2009; Clasquin et al., 2011; Chang et al., 2013). Although the major biochemical networks were identified by the 1950s (Krebs and Eggleston, 1940; Monod, 1949), understanding the coordination between these pathways in time remains a major challenge and opportunity to systems biology (Hartwell et al., 1999; McKnight, 2010; Slavov and Botstein, 2011). The challenge stems from the fact that both cell growth and metabolism may take alternative parallel paths. For example, cells can produce energy either by fermenting glucose to lactate/ethanol to generate two ATP molecules per glucose molecule or by respiration (oxidative phosphorylation) to generate substantially more (between 16 and 36 depending on the estimate) ATP molecules per glucose molecule. The estimates for the in vivo efficiency of oxidative phosphorylation vary widely depending on the measurement method and the underlying assumptions. These estimates come from (1) measuring ATP production during fermentative growth and assuming it equals the ATP production and demand during respiratory growth (von Meyenburg, 1969; Verduyn et al., 1991; Famili et al., 2003), (2) in vivo ^{31}P NMR flux measurements (Campbell et al., 1985; Gyulai et al., 1985; Portman, 1994; Sheldon et al., 1996), and (3) in vitro measurements with isolated mitochondria (Rich, 2003).

Although respiration has higher ATP yield per glucose, cancer/yeast cells tend to ferment most glucose into lactate/ethanol even in the presence of sufficient oxygen to support respiration, a phenomenon known as aerobic glycolysis. This apparently counterintuitive metabolic strategy of using the less efficient pathway is conserved from yeast to human and has been recognized as a hallmark of cancer (Vander Heiden et al., 2009; Hanahan and Weinberg, 2011). Numerous competing models have been proposed to explain aerobic glycolysis both in yeast and in human (Warburg, 1956; Newsholme et al., 1985; De Deken, 1966; Pfeiffer et al., 2001; Gatenby and Gillies, 2004; Molenaar et al., 2009; Vander Heiden et al., 2009; Vazquez et al., 2010; Shlomi et al., 2011; Lunt and Vander Heiden, 2011; Ward and Thompson, 2012).

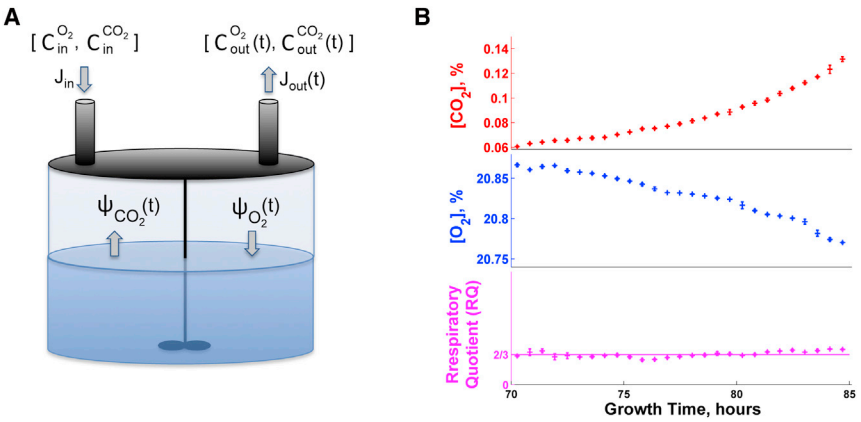

Figure 1. Experimental Design for Precision Measurements of O_2 Uptake and CO_2 Production in Time

(A) A conceptual schematic of the method used for precision measurements of O_2 and CO_2 fluxes in low-density yeast cultures; C_{in} are the concentrations of gases in the air entering the reactor at rate J_{in}, and the C_{out} are the concentrations of gases existing the reactor at rate J_{out}.

(B) The respiratory quotient (RQ) estimated from O_2 and CO_2 concentrations measured in a low-density yeast culture growing on ethanol as a sole source of carbon and energy equals the RQ estimate from mass conservation (2/3); the culture was inoculated at a density of 1,000 cells/ml, and measurements began 70 hr after inoculation, when the culture had reached a density of about 10^5 cells/ml.

Error bars denote SDs. See Figure S1 and the Supplemental Information for more control experiments and details.

Although the models propose different and often conflicting mechanisms, they aim to explain aerobic glycolysis as a metabolic strategy for maximizing the cellular growth rate. However, in some cases slowly growing or even quiescent cells exhibit aerobic glycolysis (Boer et al., 2008; Lemons et al., 2010; Slavov and Botstein, 2013). Theoretical models of aerobic glycolysis are limited by the many incompletely characterized tradeoffs of respiration and fermentation, such as the effects of aerobic glycolysis on signaling mechanisms (Chang et al., 2013). Further limitations stem from missing estimates for key metabolic fluxes. For example, depending on whether the increase in the flux of fermented glucose compensates for the low efficiency of fermentation, aerobic glycolysis may either increase or decrease the rate of energy (ATP) production. To better understand the role of aerobic glycolysis for cell growth, we sought to measure directly and precisely the fluxes of O_2 consumption and CO_2 production, and gene regulation (including levels of mRNAs, proteins, and posttranslational modifications) in the conditions of aerobic glycolysis and exponential growth.

RESULTS

Rates of O_2 consumption and CO_2 production have been measured (von Meyenburg, 1969; Verduyn et al., 1991; Van Hoek et al., 1998, 2000; Jouhten et al., 2008; Wiebe et al., 2008) in high-density yeast cultures growing in chemostats at steady state. The most commonly used laboratory growth condition, low-density cultures growing in a batch, however, is a challenging condition for measuring O_2 consumption and CO_2 production because the small number of rapidly growing cells results in small and rapidly changing fluxes. To overcome this challenge and quantify the relative importance of respiration and fermentation during batch cell growth, we developed a bioreactor in which we can accurately measure the absolute rates of O_2 uptake (Ψ_{O_2}) and CO_2 synthesis (Ψ_{CO_2}). In this setup (Figure 1A), a constant flow of air at rate J_{in} containing 20.9% O_2 and 0.04% CO_2 is fed into the bioreactor. By accurately measuring, every second, the O_2 and CO_2 concentrations in the gas leaving the reactor and applying mass conservation (Equations 1 and 2), we can estimate Ψ_{O_2} and Ψ_{CO_2}:

$$\psi_{O_2}(t) = \frac{1}{V_m}\left[J_{in}C_{in}^{O_2} - J_{out}(t)C_{out}^{O_2}(t)\right] \quad \text{(Equation 1)}$$

$$\psi_{CO_2}(t) = \frac{1}{V_m}\left[J_{in}C_{in}^{CO_2} - J_{out}(t)C_{out}^{CO_2}(t)\right] \quad \text{(Equation 2)}$$

The fluxes are normalized to moles per hour by the molar volume V_m of air at $25^{\circ}C$ and 1 atmosphere. The outflow rate (J_{out}) is determined from mass balance analysis and is typically very similar to J_{in}; see Supplemental Information.

To evaluate the accuracy of the O_2 and CO_2 fluxes, we measured these fluxes in control growth conditions for which the molar ratio of CO_2 to O_2, known as respiratory quotient (RQ), is known. First, we grew cells in media containing 100 mM ethanol as a sole source of carbon and energy. The complete oxidation of an ethanol molecule requires three O_2 molecules and produces two CO_2 molecules; thus, chemical stoichiometry and mass conservation require that the RQ for ethanol oxidation equals 2/3, providing a strong benchmark for evaluating the accuracy of our measurements. The measured RQ (Figure 1B) matches the expected value of 2/3. Importantly, the sensitivity of our sensors to very small changes both in O_2 consumption (0.005%) and CO_2 production (0.002%) allows measuring fluxes from low-density yeast cultures (10^5 cells/ml); see Supplemental Information and Figure S1 for details and more control experiments.

Growth Rate and Doubling Time Remain Constant for Nine Doublings

Having established a system for accurate quantification of O_2 and CO_2 fluxes, we applied it to a batch culture of budding yeast; 2 l of well-aerated and well-stirred minimal medium containing 11.11 mM glucose as a sole source of carbon and energy were inoculated to a cell density of 1,000 cells/ml; see Supplemental Information. After 10 hr, the culture reached a cell density allowing bulk measurements and was continuously sampled (Figures 2A and S2). During the first 15 hr of sampling (nine doubling