Discoveries lie hidden behind the façade of popular assumptions: Q & A

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The latest published work in Cell Reports includes this intriguing paper from Nikolai Slavov and Alexander van Oudenaarden and their colleagues at Harvard and MIT: "Constant Growth Rate Can Be Supported by Decreasing Energy Flux and Increasing Aerobic Glycolysis." Slavov *et al* (2014) show (in yeast batch cultures) that exponential growth at a constant growth rate represents not a single metabolic/physiological state but a continuum of changing states characterized by different oxidative- and heat-stress resistance, protein expression, and metabolic fluxes. We asked Dr. Slavov to tell us more about the work, his ideas, and his experiences.

How did you get into this area? What drew you to this question?

Cells can produce energy (ATP) via fermentation or via respiration. Although respiration has higher ATP yield per glucose molecule, 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 counter–intuitive metabolic strategy of using the less energy-efficient pathway is conserved from yeast to human and has been extensively studied for decades; yet it remains poorly understood. One can come up with very many reasonable trade-offs that theoretically could account for aerobic glycolysis. Such hypotheses make sense and appear plausible but are diametrically opposing each other. For example, aerobic glycolysis could either increase the total rate of ATP production (if the flux of fermented glucose increases enough to overcompensate for the reduction in ATP flux generated by respiration) or decrease the total rate of ATP production (if the flux of fermented glucose does not increase enough to compensate for the reduction in ATP flux generated by respiration). These hypotheses are exactly the opposite of each other, but they both appear plausible and have indeed been suggested and hotly contested in the literature. Yet, in the absence of direct measurements of the absolute rates of respiration and fermentation, these hypotheses cannot be distinguished. Our motivation was to collect direct and

accurate measurements of the absolute rates of respiration and fermentation that can distinguish the trade-offs relevant to cells from the ones that appear plausible and theoretically possible but are not relevant to living cells. Direct measurements were essential. We wanted to directly detect and quantify carbon dioxide and oxygen, not their surrogates, such as changes in pH and fluoresce of oxygen-binding fluorophores.

Any interesting moments/stories from your early life as a scientist?

I did my doctoral research in the Botstein lab, which was a great learning experience. I found Davids opinions to be substantiated by deep insight and compelling data. There was one exception: David claimed that yeast cells do not reach steady-state during the standard batch conditions of cultivation. I did not believe that claim. My disbelief came from assuming that exponentially growing cells are at steady-state and from having convinced myself that the growth of a yeast batch culture can be exponential; I had measured (Slavov, 2010; Slavov and Botstein, 2011) carefully the growth of yeast batch cultures and found that the deviations from exponential growth at low biomass-densities, if any, were smaller than my measurement error (< 0.2 %). I took such exponential growth over several doublings at a constant rate as evidence for steady-state. The data in our Cell Reports paper convinced me that contrary to my assumption exponentially growing cells can represent not a single metabolic/physiological state but a continuum of changing states characterized by different metabolic fluxes. This result reconciles perfectly my measurements of exponential growth in batch cultures with the claim that batch cultures do not reach a steady-state. This reconciliation was not part of my motivation for doing the experiments, but it is nonetheless a particularly gratifying resolution of a long-standing question in my mind.

What were some of the key factors that facilitated the success of your research?

One key factor was collecting quantitative measurements in a well-controlled system. Quantitative data are often essential even for making qualitative observations. For example, I find the observations that aerobic glycolysis increases and the total ATP flux decreases during the first exponential growth phase very interesting even as qualitative observations. However, these qualitative observations depended crucially on collecting and analyzing quantitative data. Another key factor was making direct measurements. I found my data and their implications so surprising that if my mea-

surements were not direct no matter how quantitative I would have ignored the results, at least until I could come up with a direct approach to measuring the relevant fluxes. For example, if I had estimated the carbon dioxide flux by an indirect surrogate such as changes in the pH I would not have had the confidence to overturn long-standing assumptions.

What are the big questions right now in your field? The big challenges? Big changes?

A primary challenge in systems biology, which we also encountered during the work on our Cell Reports paper, is the causal interpretation and conceptual understanding of coincident/correlated events during complex physiological responses. We do not have a general approach, experimental or theoretical, to confidently deconvolve direct causal interactions from the many indirect correlations that we observe. We can easily make computational inferences based on a myriad of algorithms that are likely correct but not inferences that are certainly correct. We can also overexpress and delete individual genes or small groups of genes, which is very helpful. However, even such perturbation experiments fall far short of identifying and understanding the mechanisms of biological dynamics dependent on multiple molecules, as physiological responses often are.

Any interesting stories about this work? Setbacks or unexpected insights? Mistakes, humor, epic experiments, all-nighters?

The surprising trends in the data brought both thrilling excitement and excruciating discomfort from the possibility of artifacts. I had plenty of all-nighters during the long time-courses (over 50 hours) and many early-morning visits to the lab since I would wake up before sunrise wondering how the data from the new experiment running overnight looked and whether they remained consistent with the current model. Initially I was very skeptical of the pervasive dynamics during exponential growth and did a lot of control experiments some of which provided interesting new leads just to convince myself and rule out artifacts.

What would you like non-scientists to know about your work?

In my opinion, the most general lesson is to always be a little skeptical of well-established assumptions, especially those that allow convenient simplifications and have been accepted before precise

quantitative measurements were available. Rather, one should collect the most direct empirical data that one can. We have a lot to learn and understand about even the most widely used and studied scientific model systems if we approach them quantitatively. I strongly believe that much of this understanding is essential to developing effective therapies with minimal unintended consequences. Without understanding, we may engineer desirable results but cannot rule out potential unintended consequences of our assumptions.

What are the next steps for your group and/or this project?

I think that our results raise many questions. One question that I find intriguing, even though we did not discuss it in our report, is that some the measured dynamics might reflect anticipatory cellular responses. Scientific systems are often chosen or assumed to be at steady-state since the steady-state assumption simplifies the analysis. However, cells in the real world often exist in a more dynamic environment. Optimal responses to dynamic environments require sensing environmental changes and hedging the optimal future outcomes. My speculative guess is that sensing the dynamical changes in the growth conditions is among the factors causing the dynamics that we observed during growth at a constant rate. Coming up with clever experiments to characterize such dynamical sensing and responses can add significantly to our understanding of cellular physiology in changing environments.

References

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