Metabolism Drives Distinct Cellular Growth Phases

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***Short Abstract* — Growing cells maintain functioning metabolism in the face of extrinsic uncertainty and potentially toxic side-effects of metabolic activity. For example, the primary cellular fitness cost of *lac* operon expression in *E. coli* arises from imbalances between substrate import and catalysis. We find that even a small metabolic fitness cost creates an inescapable fate of cells entering a non-growing phase, or even undergoing lysis. The physiological configuration conferring the fastest growth rate often exists adjacent to the irreversible critical point. We have therefore found that fit bacterial populations can be maintained metastably. Time-lapse microscopy of bacterial microcolonies supports our interpretation.**

***Keywords* — fitness, metabolism, metastability, criticality**

I. Population Fitness and Cellular Growth

GLOBAL phenotypes in living matter are often driven by subtle critical points at small scales that have major implications for overall fitness [1, 2, 3]. For example, fluctuations may drive metabolite concentrations across enzyme saturation points, creating, paradoxically, efficient metabolic pathway utilization at the flux ratio where intermediates are most prone to building up in large concentration excesses [4, 5]. The phenomenon of slow-growing persister cells observed in bacteria [6] further suggest the importance of growth rate regulation in overall cellular growth dynamics. We hypothesized that the combination of enzyme gene expression noise and saturation creates a cost-benefit tradeoff driving population fitness in growing bacteria.

II. Growth Phases in Metabolism

Using a timescale separation assumption, we derived a single-variable model for metabolite concentration that predicts the emergent growth rate as a function of metabolite. The model strikingly reveals three growth regimes: (*i*) a classical substrate-limited phase where single-cell growth is relatively slow; (*ii*) a phase where cell growth is not highly dependent on the pathway; (*iii*) a phase with essentially no growth. For regimes where the metabolite is in high demand, such as in minimal medium with a single sugar, transitions between the phases can coincide with fast growth rates.

A population-level model predicts that, in these phase regions, a significant fraction of growing cells can irreversibly enter the non-growing phase while still providing the population with the fastest overall growth rate.

III. Fitness and Lactose Utilization in Bacteria

The lactose utilization system in *E. coli* is known to carry a fitness cost from imbalances of substrate import and utilization [7]. We measured population growth rates of various *E. coli* strains in minimal media with a range of lactose concentrations and found a trade-off between cost and benefit of lactose. Very high lactose concentrations result in lower overall growth rates than intermediate concentrations.

In a microfluidic perfusion device, we grew *E. coli* microcolonies exposed to low and high lactose concentrations with similarly suboptimal growth rates. The populations show distinct morphological differences: lactose-poor colonies grow slowly while the lactose-rich colonies grow quickly, but with many cells eventually bursting, dramatically leaking cellular contents.

IV. Conclusion

Growth dynamics are predicted to depend on the balance between the rate of critical transitions and the growth rate. Our experiments strongly support the conclusion. We suggest that such critical points may be a major driver of evolution in metabolism, and that the concept of a steady state of biochemical networks may not be applicable for populations undergoing this growth process, including growing microbes and, potentially, cancer.

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