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Resource allocation and metabolism: the search for governing principles

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Elucidating strategies of resource allocation and metabolism is crucial for a better understanding of microbial phenotypes. In particular, uncovering the governing principles underlying these processes would be a crucial step for achieving a central aim of systems microbiology, which is to quantitatively predict phenotypes of microbial cells or entire populations in diverse conditions. Here, some of the key concepts for understanding cellular resource allocation and metabolism that have been suggested over the past years are reviewed. In particular, recent experimental studies that have shown how phenotypic patterns from orthogonal genetic and environmental perturbations can help to differentiate between competing hypotheses and their respective predictions are discussed. Phenomenological models have proven to be a valuable addition to genome-scale models, capable of making quantitative predictions with only few parameters and having aided the identification of molecular mechanisms.

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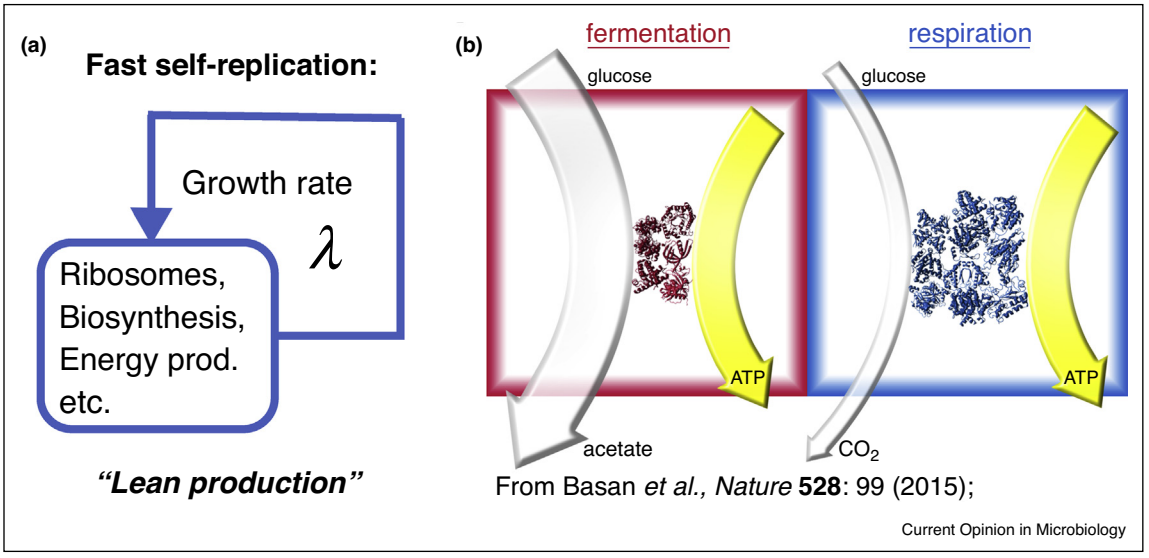
Introduction

A central question of microbiology is what determines growth rates in different environments and more generally what gives rise to the enormous variations of phenotypes including, gene expression and metabolism in different conditions [1,2]. In recent years, a consensus has emerged that resource allocation strategies play a key role in determining microbial phenotypes and a range of governing principles has been proposed to underlie this variation. There have been some remarkable success stories, for example, recent work in which theoretical models have helped elucidate regulatory and molecular mechanisms of catabolite repression [3••]. However, for understanding even simple phenomena like overflow metabolism, there are still many competing hypotheses currently debated, including limited oxygen availability [4], limited membrane space [5], recycling of cofactors [6], molecular crowding [7] and protein cost [8•,9,10••]. Phenotypic patterns resulting from orthogonal genetic and environmental perturbations can help to differentiate between these ideas and their respective predictions. [By orthogonal perturbations, we mean perturbations that affect growth rate, but elicit distinct and sometimes complementary regulatory responses (e.g. carbon limitation versus nitrogen limitation)]. This review summarizes important works and conceptual advances in the search for governing principles underlying patterns of resource allocation and metabolism. Unfortunately, because of the long history and broad scope of these questions, it is not possible to provide a comprehensive review here. Instead, the primary focus is on physics-inspired models that have recently been proposed and tested experimentally. Invariably, important and interesting works that would deserve to be included in this review have been omitted (Figure 1).

Self-replication and growth laws

A major step forward for understanding microbial metabolism, going beyond flux balance analysis, was the realization that the cost of producing enzymatic machinery itself is an important factor determining growth rates [10••,11]. The cell can be considered a self-replicating system [12–14] that needs to duplicate all of its components within the doubling time. To accomplish this, the cell must on the one hand, polymerize all cellular macromolecules like proteins, RNA and the cell envelope and on the other hand, using metabolic pathways break down substrates and produce the biomass precursors and additional energy to fuel these polymerization processes (for more information on the constraints of self-replication see [15]). Growth rate is determined by a balance of fluxes from metabolic precursors and polymerization. Polymerization of macromolecules requires an investment in machinery, consisting primarily of ribosomes. Recently, it was shown that the optimization for autocatalytic production explains many features of the ribosome like that a few large RNA molecules dominate ribosomal mass and that their protein content is divided into small, similarly sized units [16••]. Similarly, metabolic reactions are catalyzed by enzymatic machinery. Therefore, to achieve optimal growth rates, the cell must balance the fluxes from metabolic reactions and polymerization, while minimizing the investment in enzymatic machinery and

Figure 1



Self-replication and 'lean production' illustrated. **(a)** Illustration of the cell as a self-replicating system. Key components of this system are ribosomes for polymerization of proteins, biosynthetic pathways that provide the precursors for macromolecules and energy production pathways that supply the energetic requirements for polymerization. During stead-state growth, the cell must duplicate all of its components before a cell division, but at the same time, the replication process itself is catalyzed by these components. Therefore, any alternative pathway or mechanism that provides the same flux of biomass precursors or energy, but requires a smaller investment in cellular machinery (primarily proteins), will enable faster replication. To draw and analogy to economics, lean production pathways maximize growth rate by maximizing the return on investment of finite cellular resources. **(b)** Illustration of the lean production hypothesis for the example of energy metabolism and acetate excretion taken from Basan *et al.* [37•]. For the same ATP production flux (yellow arrow), fermentation consumes a much larger carbon flux (gray arrow) as compared to respiration. However, fermentation requires a smaller absolute investment in enzymatic machinery (red and blue proteins) to catalyze this flux and therefore enables faster growth.

investing the 'right' proteome fractions in these processes. For example, if the cell were to invest too many resources into catabolic processes, this would result in the production of more precursors and energy than could be processed by the ribosomes (and some of the investment in the catabolic processes would be effectively wasted). On the other hand, too much investment in ribosomes and biosynthetic machinery would result in a situation, where biosynthesis could not be adequately supplied with precursors and energy (part of the investment in ribosomes and biosynthetic machinery would be effectively wasted). Moreover, because the cell has finite proteomic resources (fractions of total proteome), any increase in investment in a one process must coincide with a corresponding decrease in investment in another. Maximum growth rate is therefore achieved when the cell invests optimal proteome fractions in different cellular processes, such that it balances fluxes from different processes while minimizing the resource investment in each of these processes.

The simple linear growth rate dependences of the abundances of ribosomes and metabolic pathways that become particularly apparent when proteins are clustered together in proteome fractions, can be understood from these argument, as realized by Scott *et al.* and translated into growth laws [17••,18,19]. While abundances of ribosomes and biosynthetic pathways exhibit a proportional increase with growth rate for different carbon sources, the abundances of many metabolic pathways show the opposite dependence and decrease with increasing growth rate and 'better' carbon quality [2], referred to as a higher nutritional capacity in Scott *et al.* [17••] or lower investment of gathering carbon in Molenaar *et al.* [10••]. Scott *et al.* tested this picture experimentally by adding sublethal doses of the translation inhibiting antibiotic chloramphenicol to the growth medium and by overexpressing different quantities of useless, but otherwise harmless protein [17••]. Chloramphenicol resulted in a higher allocation of proteomic resources to ribosomes (working at a lower speed), while useless protein expression resulted in an additional proteomic burden constraining available proteomic resources. Scott *et al.* were able to successfully recapitulate these finding with their growth laws and realized that remarkably, a large fraction of the proteome is unaffected by these perturbations and remains at a constant proteome fraction. This work convincingly demonstrated the importance of allocation of proteomic resources in determining growth rates and how simple, thermodynamics-inspired models can be used to make quantitative predictions on the cellular scale. In hindsight, as discussed in the following sections, an