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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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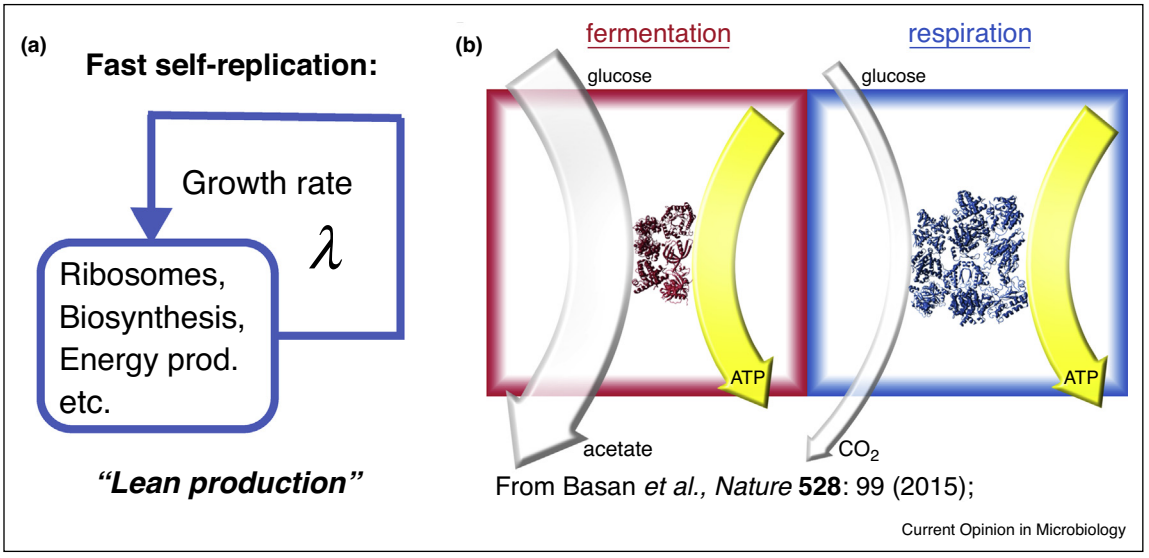
Introduction

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

interpretation of their model, suggesting that the large variation of growth rates that is observed between different carbon sources arises primarily from variations in the protein cost of carbon specific metabolism ('nutritional capacities') is probably oversimplified. For instance, many simple genetic modifications lead to much faster growth rates on supposedly 'slow' carbon sources [20,21••], casting doubt on this hypothesis and suggesting that evolutionary objectives other than maximization of steady-state rate of growth play a dominant role on these carbon sources.

Catabolite repression

The coordination of catabolic and biosynthetic protein sectors was further investigated by You *et al.* [3••], who showed a linear increase in the abundances of a large number of catabolic (defined as cAMP regulated) genes with decreasing growth rates, upon limitation of carbon influx, but a linear decrease under limitation of nitrogen or sulfur influx. Biosynthetic genes exhibited the opposite linear growth rate dependence under these limitations. Importantly, You *et al.* proposed and validated a regulatory mechanism mediating this pattern of catabolite repression, in which metabolic precursors, including oxaloacetate, alpha-ketoglutarate and pyruvate inhibit cAMP signaling [3••]. This mechanism explains how a catabolite repression pattern emerges for diverse carbon substrates, independent of the long-known effect of the phosphotransferase system on cAMP [22]. In a later study, Hermsen *et al.* used the concerted growth rate dependence of the abundance of different catabolic genes characterized by You *et al.* [3••] and combined this with the nutritional capacity concept from Scott *et al.* [17••], to derive a growth rate composition formula that was able to predict the growth rate on a subset of co-utlized carbon substrates from the growth rate on the individual substrate [23•]. Recent proteomics studies have confirmed the gene expression patterns observed by You *et al.* and extended this analysis to other growth limitations [2,24,25].

While these recent works have led to a better understanding of the global metabolic patterns of resource allocation and their regulation, the underlying function and evolutionary benefit of catabolite repression remains much less clear. Proteomics studies reveal that many of the genes that are upregulated with decreasing carbon quality have no immediate benefit in the specific growth conditions, for example, many transporters of carbon sources that are not present in the medium [2]. Instead, the expression of these genes constitutes a substantial burden in terms of protein cost, similar to useless protein expression [17••]. This incomplete repression is illustrated by an observed, 'soft' hierarchy or semi-hierarchical pattern of sugar co-utilization [26]. While specific levels of cAMP are required for optimal growth on different carbon sources, high levels of cAMP are generally detrimental for growth, presumably for this reason. In fact, in a recent work, Towbin *et al.* have shown wild-type cAMP levels on many carbon sources to be sub-optimal for maximizing growth rates [21••]. Curiously, glucose can become one of the worst carbon sources in poor nitrogen conditions [27].

The strength of the activation of carbon specific operons by cAMP determines how much their expression will be accompanied by the expression of other catabolic genes at a substantial expression cost. Most of these catabolic genes offer no obvious benefit for maximizing growth rates in the present conditions and this phenomenon does not appear to be restricted to carbon co-utilization, because it occurs when cells are grown on single carbon sources [3••]. Hence, it is possible that the 'quality' of different carbon sources, as quantified by their steady-state growth rate, may well be largely determined by their relative position in the hierarchy of catabolic activation, rather than carbon-specific properties of their transport and metabolic pathways. Consistent with this notion, relatively simple genetic modifications, like a knockout of the global transcriptional regulator Cra, responsible for the activation of gluconeogenesis [28,29,30•], or aerobic overexpression of the global transcriptional regulator ArcA, involved in regulation of anaerobic growth [31–33], result in substantially improved growth rates as compared to wild-type strains on many carbon sources [34•]. Perhaps these observations indicate limitations of cellular regulatory capabilities. On the other hand, it is possible that the activation of catabolic genes with decreasing growth rate may constitute a preparatory response for other conditions [35]. The 'quality' of a carbon source would then reflect, to a substantial degree, the evolutionary and ecological need to prepare for and to seek other growth conditions. This would result in lower growth rates as a side effect, rather than being a result of some intrinsic properties of the metabolic pathway of a particular substrate. It would be interesting to compare bacteria from more and less constant natural environments to see if these differences are reflected in their patterns of catabolite repression.

Overflow metabolism

A key test of our understanding of resource allocation and metabolism is predicting the use of distinct metabolic strategies in different environments. Overflow metabolism is a puzzling phenomenon that refers to the excretion of fermentation products like acetate or lactate, which occurs even in aerobic conditions for fast growing cells. Overflow metabolism appears wasteful, because a large amount of carbon is lost in the form of fermentation products instead being used for the production of energy or biomass building blocks. Despite being known for almost a century, the origin of overflow metabolism remains controversial, illustrating our limited understanding of metabolic resource allocation, even for simple model organisms like *Escherichia coli*. Standard metabolic models like flux balance analysis do not naturally result in

overflow metabolism and the excretion flux is typically imposed. Numerous hypotheses and theoretical models have been proposed to explain the existence overflow metabolism, including limited oxygen availability [4], limited membrane space [5], recycling of cofactors [6], molecular crowding [7] and protein cost [8•,9,10••]. Potentially related to the protein cost hypothesis, it has been pointed out that metabolic networks resemble a minimal biochemical walk and that metabolic pathways tend to take the shortest possible routes in terms of reaction steps [36]. However, despite these concepts and observations, experiments capable of discerning between these different hypotheses had been largely lacking.

In a recent work, we quantified overflow metabolism and energy metabolism for a set of orthogonal perturbations in order to distinguish between these different hypotheses [37•]. We discovered a distinct response under carbon limitation, proteome limitation and energy limitation for decreasing growth rates. For example, by overexpressing large quantities of useless protein, we were able to decrease the threshold growth rate for acetate excretion and by knocking out flagella proteins we were able to increase this threshold. The patterns that we found are consistent with and can be predicted quantitatively using the protein cost hypothesis, which states that fermentation requires a lower protein investment per ATP produced than respiration. Hence, fermentation constitutes a 'leaner' pathway that allows faster growth. In our work, we also directly quantified the protein cost of energy production via fermentation and respiration by combining flux measurements and proteomics. Indeed, we found that fermentation is roughly twice as efficient as respiration in terms of protein cost per ATP flux. The protein cost or 'lean production' argument for preferential utilization of alternative metabolic pathways has also been applied to the Entner–Doudoroff pathway, which constitutes an alternative glycolytic pathway used by certain microbes [38••]. The importance of metabolite concentrations for thermodynamic driving forces and enzyme saturation in determining protein cost has been recently implemented [39].

The patterns characterized in our recent work [37•], are difficult to reconcile with alternative explanations. For instance, as characterized in our work, the volume of cells overexpressing useless protein increases as the inverse of their growth rate [40]. On the other hand, energy demand per cell scales like cell size multiplied by growth rate and should therefore be roughly constant, while the size of the cells and their surface and membrane area increases with increasing protein overexpression. Hence, for slow growing cells, expressing large quantities of useless protein, both membrane space and oxygen should be abundant, but these cells nevertheless continue to excrete acetate, even at growth rates far below the acetate excretion threshold for carbon limitation. It is important to note that one exception is the molecular crowding hypothesis [41], which is mathematically indistinguishable from the protein cost hypothesis in our formulation, but makes additional predictions regarding changes in dry mass density under carbon limitation. We did not observe such changes in our own measurements [40], but we cannot exclude that a more precise quantification would be required to resolve these effects.

Supporting the idea that fermentation directly offers benefits for maximizing growth rates, we recently showed that aerobic overexpression of the global transcriptional regulator ArcA, normally involved in regulation of anaerobic growth [31–33], induced acetate excretion, downregulated respiratory pathways and resulted in substantially improved growth rates, as compared to wild-type strains on slow glycolytic carbon sources [34•]. A beneficial effect of fermentation on growth rates would not be expected from models based on limitations in oxygen or membrane space, unless growth rates were always limited by these factors in diverse conditions and at slow growth rates. ArcA overexpressing strains also have higher carbon uptake rates, which shows that the shift from fermentation to respiration on slow carbon sources does not result from limitations or constraints on carbon uptake rates, as implemented in some models [10••]. Instead, these findings raise the question why *E. coli* relies on respiration for energy production, even in many growth conditions where the carbon source is highly abundant. Mathematically, the protein cost model constitutes a tradeoff between growth rate and biomass yield [37•]. But while a higher biomass yield is clearly advantageous, resulting in faster growth, when substrate concentrations are low and diffusion of the substrate is limiting, it is questionable if a high-yield strain could directly outcompete a fast growing strain in any environment with high concentrations of the carbon source [42–44]. Hence, it is not clear why *E. coli* and other bacteria do not ferment many glycolytic substrates and heavily use respiratory pathways, even when these carbon sources are highly abundant in their environment.

Tradeoffs and optimality

In the past, changes in gene expression and metabolic strategies across growth conditions have often been attributed to the optimization of steady-state growth rates [10••,17••,18,45]. However, mounting evidence suggests that cells are capable of significantly faster growth rates in many conditions, including supposedly 'poor' carbon sources [21••,20]. Based on these observations, it is clear that objectives other than optimization of steady-state growth rates must be considered to explain these phenotypes.

In recent years, there has been an increasing interest in the role of conflicting objectives in shaping phenotypes.

Shoval *et al.* argued and illustrated that when facing competing objectives, phenotypes of biological systems fall on surfaces of Pareto optimality, where any objective can only be improved at the detriment of another [46••,47]. Schuetz *et al.* observed that ^{13}C-measured fluxes of nine different bacteria fell close to a surface of Pareto-optimality, defined by biomass yield, ATP-yield and the minimization of the sum of absolute fluxes [48••]. On the genome scale, constraints have been combined with optimality for different objectives to predict genome-wide patterns of metabolism and gene expression [8•,49,50] and it was shown that feasible optimal flux routes can be derived from elementary flux modes [50]. Beyond bacterial growth in constant environments, autocatalytic, constraint-based models have also recently been applied to the time-dependent resource allocation of cyanobacteria during the day–night cycle [51•].

However, despite these advances, difficulties remain in identifying objectives of evolutionary importance, as well as the right experiments to quantify these objectives and their relevance. There is also a very limited understanding of underlying principles and molecular mechanisms that result in tradeoffs of evolutionary importance. While minimizing protein cost for maximizing growth rates or available proteomic resources is well appreciated and most likely constitutes an important evolutionary advantage for microorganisms, it is much less clear how proposed objectives like maximizing the yields of various metabolic processes affect fitness in specific environments. As a result, many of the changes in gene expression and metabolic pathways, observed with decreasing growth rates [2], are only poorly understood today. These changes include the upregulation of hundreds of genes, some of which are known to be involved in stress response [52,53], as well as novel metabolic routes that are not utilized at higher growth rates like the (P-enolpyruvate)-glyoxylate cycle [1,54•].

It is likely that diverse tradeoffs shape the regulatory programs of metabolism in microorganisms [55]. A better understanding of these tradeoffs would be essential to help us understand the variation of phenotypes between growth conditions, as well as the variation between strains [56]. Moreover, tradeoffs and optimality could give rise to complex phenomena like bistability, oscillations and memory effects [57].

Conclusion

In recent years, many different ideas have been proposed to understand microbial resource allocation and metabolic strategies. These ideas have already led to a better understanding of some counterintuitive phenotypes, as well as important regulatory mechanism like catabolite repression. Nevertheless, more work needs to be done to differentiate between competing hypotheses and orthogonal perturbations can be useful for that. Coarse-grained, models have proven to be a valuable addition to genome-scale models, capable of making quantitative predictions of cellular phenotypes with only a few phenomenological parameters. In some cases, these models have also aided identification of molecular mechanisms like the inhibition of cAMP signaling by specific metabolites [3••]. Ultimately, the usefulness of various proposed governing principles should be measured by their ability to make correct and quantitative predictions for diverse experiments, including novel observations and experimental perturbations.

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