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Public goods and metabolic strategies

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Microbial growth can be characterized by a limited set of macroscopic parameters such as growth rate, biomass yield and substrate affinity. Different culturing protocols for laboratory evolution have been developed to select mutant strains that have one specific macroscopic growth parameter improved. Some of those mutant strains display tradeoffs between growth parameters and changed metabolic strategies, for example, a shift from respiration to fermentation. Here we discuss recent studies suggesting that metabolic strategies and growth parameter tradeoffs originate from a common set of physicochemical and cellular constraints, associated with the allocation of intracellular resources over biosynthetic processes, mostly protein synthesis. This knowledge will give insight in ecological and biological concepts and can be used for metabolic and evolutionary engineering strategies.

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Bacterial growth laws

One of the major achievements of microbiology has been the identification of species-independent relations between macroscopic parameters of microbial growth, such as growth rate, cell yield, nutrient affinity and maintenance requirements (for definitions see Box 1). Monod's equation relates the growth rate of a microorganism to the concentration of the growth-limiting nutrient by two such parameters: its maximal growth rate on the specific nutrient and its affinity for it [1]. Similarly the Herbert–Pirt equation relates the specific uptake rate of a nutrient to the rates of the formation of new cells, excretion of products and maintenance processes [2]. The Monod and Herbert–Pirt equation can also be combined to relate nutrient concentrations to the formation rate of new cells and cellular products. Alternatively, stoichiometric analysis of metabolism allows for yield predictions [3,4].

The Monod and Herbert–Pirt relations contain macroscopic, phenomenological parameters that 'emerge' from molecular properties. Physics would consider these relations as 'laws'. The ideal gas law, for example, is phenomenological and its mechanistic explanation is given by statistical mechanics in terms of kinetic energies and collisions of (idealized) gas molecules. What statistical mechanics did for physics is similar to what molecular systems biology is aiming to do for microbiology, which is to explain phenomenological macroscopic parameters in terms of molecular properties.

Systems biology uses mathematical models of molecular networks in cells to achieve this aim. Recently, models of microbial growth have been introduced that explain changes in growth rate and nutrient yield, in terms of re-allocation of intracellular resources, such as cellular building blocks, energy, and biosynthetic machinery [5,6,7,8•].

One important aspect of understanding microbial growth, which seems to have no analogy in physics, relates to the evolutionary origin of biological systems: how do macroscopic growth parameters (co-)evolve under different selective forces? How can we explain their values in terms of the underlying molecular circuits, their constraints and the environmental selective pressures? This can be addressed with laboratory evolution-experiments, where the major control variable is the availability of the growth substrate (Box 2; Figure 1). Such experiments can then be followed up by studies of intracellular constraints, and how they limit macroscopic growth parameters, for example, by the analysis of the fixed mutations.

In this review, we will combine recent findings on growth and partitioning of intracellular resources by microorganisms, with those of laboratory evolution experiments, in which either growth rate, cell yield, or nutrient affinity were selected for. Such a synthesis creates a framework to understand microbial growth properties that emerge under various selective forces.

Box 1 Definitions

Biomass: is defined as the total of materials synthesized by a living organism. In microbiology, due to the methods of measurement (optical density, cell counts, dry weight), material that is not an integral part of an organism, like excreted material, is excluded.

Specific growth rate: is defined as the instantaneous relative rate of increase of the number of organisms (rate of increase of the number per number of the organism), or mathematically:

$$\mu(t) = \frac{1}{N} \cdot \frac{dN}{dt}$$

where $\mu(t)$ is the growth rate at time t, and N is the number of organisms. When this rate is constant in time $\mu(t) = \mu$, we speak of balanced growth [50]. During balanced growth, metabolism is at steady state and all extrinsic cellular properties increase exponentially over time, including total cell volume, cell mass and cell number in the culture.

Public and private goods: We speak of private goods when in a population of cells, due to limited diffusion or barriers, each individual has a fixed quantity of substrate available for growth of itself and its immediate offspring. Under conditions of unlimited diffusion, for example, shaking or stirring, all cells in a population share, and compete for, the same substrate pool — we speak of public goods.

Resource allocation: When internal resources, like substrate internalized by transport, total amount of protein *etc.*, are limited, an allocation problem exists for an organism. That is, it can synthesize more of one protein only at the cost of making less of other proteins, or it can use substrate for the synthesis of one cellular component only at the cost of other components. The regulation of gene expression is one way by which an organism can intervene in the allocation of its resources.

Tradeoff: When internal resources are limited, certain functions can only be performed at the expense of other functions. In addition to limitations in amounts of resources in the form of molecules, physical and biochemical limitations, like a restricted amount of volume, maximal rates of molecular diffusion or transport, a maximal amount of protein that can be dissolved in a membrane *etc.*, are forms of limited resources, and lead to resource allocation problems.

Affinity: A measure of the concentration of particular substrate needed to let an organism grow at a certain rate, when other substrates are supplied in excess. Often defined as the inverse of the concentration at which half the maximal growth rate is reached.

Fitness: A relative measure of the success of replication of organisms that compete for the same external resources. Measured as a consistent change of the ratio of these organisms over a period of several generations.

Serial batch propagations in liquid medium, at low cell densities, in which a fraction of the population is used to seed a subsequent one, tends to select for fast growing populations provided that the population is harvested during mid-exponential growth phase. Otherwise, selection pressure may be a myriad of factors besides growth rate, such as resistance to nutrient starvation, varying pH, or oxygen limitation, amongst others.

Serial propagations in emulsion, begin each round with individual cells being isolated in droplets and allowed to grow on the set of nutrients made available to them without having to compete with neighboring cells. Cells with an increased number of viable offspring will grow to higher cell numbers in their respective droplet and increase in overall frequency upon serial propagation (Figures 1 and 2) [37••].

Continuous cultivation experiments provide several advantages despite being more laborious and difficult to maintain for prolonged periods (typically weeks to months by contrast to months to years as in serial propagations). For instance, first, populations can be made to grow at specific growth rates by setting the dilution rate [51]; and second, evolutionary bottlenecks are kept to a minimum, enabling the sequence landscape to be explored in greater depth, that is, more combinations of mutations are given a chance to emerge and compete for taking over the population. Although there exist several types of continuous cultivation methods, by far the most used one, also for laboratory evolution experiments, is the chemostat. In this case the selective pressure is acting on the lowest concentration of limiting nutrient that can still support growth at the fixed dilution rate [52]. In practice, this results in evolved populations with, either transporters with higher affinity (lower K_S), and/or increased transporting ability (higher V_{max}). Note that the affinity K_S is a whole-cell property and is therefore not the same as the K_M of the transporter; K_S tends to be lower because transport activity changes with growth rate [6].

Resource allocation to maximize fitness

The generation of new cellular material, like protein, relies on the synthesis and allocation of intracellular resources, such as free energy and building blocks, over biosynthetic processes.

Resource re-allocation is illustrated by the shifts from respiratory to fermentative metabolism by different organisms such as *Saccharomyces cerevisiae* and *Escherichia coli* [6]. Likewise, the onset of the Warburg effect in cancer cells serves as an example [6]. This so-called overflow metabolism is observed at high substrate concentrations and fast growth rates. The occurrence of overflow metabolism seems counterintuitive, as cells switch from efficient to inefficient use of substrate, thereby wasting extracellular resources. A metabolic shift to a fast and inefficient growth does however confer a fitness advantage when cells are selected for a high growth rate.

It is becoming increasingly evident that the maximization of growth rate can be achieved by optimal allocation of resources [6,8•,9,10,11,12]. However, it remains challenging to verify this experimentally, but ample circumstantial evidence does exist. A study with *E. coli* did recently quantify that at high substrate concentration the protein cost for energy generation through respiration exceeds that by fermentation [13••]. So overflow metabolism indeed appears to be the result of a tradeoff between energy yield and synthesis-rate of alternative pathways. Attempts to prevent fermentative acetate-production by fast growing *E. coli* is therefore likely failing, because respiratory metabolism is accompanied by a low growth rate [14–16].

Modeling resource allocation during metabolism adaptations

Mathematical models of metabolism have been developed to better understand how fitness parameters, such as growth rate and nutrient yield, are determined by underlying molecular circuits. They consider the allocation of intracellular resources over cellular processes and range in scale from highly simplified to genome-scale [5,6,7,8•].

Box 2 Relations between macroscopic parameters of bacterial growth: affinity for growth-substrate, biomass yield on substrate, and growth rate

The growth of unicellular organisms can be described by several mathematical models that take into account different levels of physiological and molecular detail. The goal of such models is to relate the growth properties of an organism, like rate and yield, to environmental, physiological and molecular properties. Perhaps the earliest and simplest model is the Monod model of growth that describes the growth rate as a function of the concentration of the limiting substrate in the medium [1]. It is an empirical relation, described by a hyperbola:

$$\mu(S) = \frac{\mu_{max} S}{K_S + S}$$

where K_S is the concentration at which half the maximal growth rate μ_{max} is reached. The empirical affinity constant (the inverse of K_S) is expected to bear some relation to the affinity constant of the first enzyme consuming the substrate (the transporter), although it will not be equal to it [1]. This equation, or modifications of it, is still used as part of models describing growth in chemostats, for example. Monod also described the empirical relation between growth yield and limiting substrate consumed, which was later adapted as

$$\frac{dX}{dS} = Y_S$$

where X is the concentration of biomass, S the concentration of limiting substrate consumed and Y_S a constant yield. Later, Pirt (1965) modified this relation for the case that S is the energy-yielding substrate, based on the hypothesis that organisms consume a constant amount of free energy per time unit, independent of whether they grow or not [2]. This was termed the maintenance rate, because it was thought to reflect the consumption of energy by processes that maintain cellular integrity. This energy substrate consumption, independent of growth, is proportional to the biomass concentration with a proportionality constant m, yielding for the total consumption of substrate:

$$\frac{dS}{dt} = \frac{dS}{dX} \cdot \frac{dX}{dt} + m \cdot X = \frac{1}{Y_S} \cdot \frac{dX}{dt} + m \cdot X$$

Dividing both sides by X yields the Pirt formula:

$$\frac{1}{X}\frac{dS}{dt} = \frac{\mu}{Y_S} + m$$

which states that the specific rate of consumption of the energy-substrate is a linear function of the growth rate with slope $1/Y_S$ and offset m.

Genome-scale stoichiometric models predict fluxes through metabolism from reaction stoichiometries, flux constraints and fitness objectives. Recent developments consider the allocation of limited resources to protein synthesis [7,17,18]. Such models are mostly restricted to yield calculations, as they usually do not consider enzyme kinetics.

Simplified models, which generally do consider kinetics, have mostly focused on explaining observed, linear phenomenological relations between cellular protein-fractions and growth rate [6,13••,19]. The most famous relation, known since the 1950s, is that the ribosomal protein-fraction increases linearly with specific growth rate. Similar relations appear to exist with other protein sectors, such as processes associated with cellular stress and catabolism [9,20,21].

Environmental effects on optimal resource allocation

Switching between metabolic strategies [22–25], such as from respiration to fermentation, occurs when conditions change from glucose limitation to excess. This indicates that environmental conditions greatly influence how resources are allocated.

Fitness optimization using mathematical models can help us finding explanations that can be tested experimentally. A recent mathematical model, linking gene expression and cell growth, considered tradeoffs in cellular energy, free ribosomes and proteins [26]. This provides explanations of growth-rate dependent regulation, and how (dynamic) nutrient availability determines evolutionary-stable strategies.

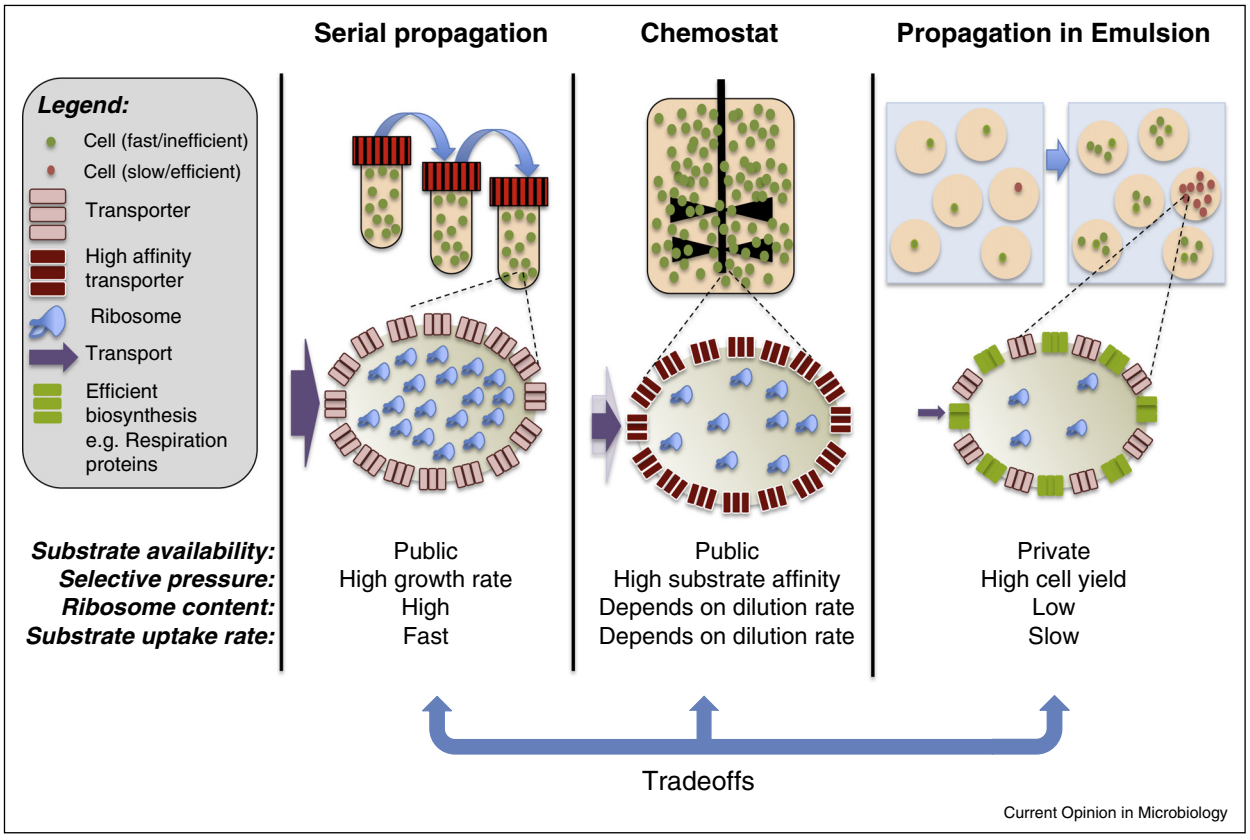
An experimental study [27••] investigated the response of different yeast strains to dynamic environments. It was found that some strains adapted quickly to the new conditions while others were much slower. Subsequent experimental evolution of those strains, in stable and dynamic environments, resulted in mutants with different catabolite repression properties. Evolution in dynamic regimes lead to the simultaneous expression of catabolic enzymes for multiple carbon sources, at the cost of a high growth rate on one carbon source and vice versa [27••]. Those 'generalist' strains turned out to be the strains that adapted quickly to new conditions, at the cost of growing slower in stable environments.

Besides dedicated experiments in dynamic conditions, the majority of studies using experimental evolution adapt microbial strains to growth in one particular environment. These studies usually find improved phenotypes on the medium in which strains were evolved and several of them showed that such specialization results in tradeoffs when cells are switched to other environments [27••,28,29].

A tradeoff related to growth on limited substrate concentrations appears associated with the observation that microorganisms encode multiple transporters for the same carbon source. These transporters typically have different substrate affinities and fitness optimization is associated with transporter expression that is substrate concentration dependent [30]. This suggests a tradeoff between transporter affinity and substrate levels, and is consistent with resource allocation models [31].

Summarizing, evidence is accumulating that highlights the importance of tradeoffs associated with how cells

Figure 1



Selective forces in different culturing systems. During serial propagation in suspension the fastest growing cell will outcompete slower ones (assuming propagation during exponential growth). Internal resources will be allocated for fast metabolism such as fermentation. Prolonged cultivation in a chemostat selects for cells with a high substrate affinity. Serial propagation in an emulsion like system favors cells with an increased number of offspring, which can be achieved by decreasing the cell size but also through using substrate efficiently.

allocate their resources and that they do this in an environment-dependent manner, presumably to maximize their fitness [26,32–35]. Understanding of metabolic strategies therefore requires a perspective on what the fitness objective likely is for the organism. This includes how it is cultivated and whether the environment is stable or not, what fitness value it can attain, given intracellular constraints associated with resource allocation, and which metabolic strategies attain those fitness values.

Public and private goods: selecting for rate or yield

Different culturing systems were developed over the years for the selection of macroscopic growth parameters such as growth rate, cell yield and substrate affinity. These protocols differ in how the external resources are made available such as carbon or nitrogen sources. In batch cultivation, for instance, the concentration of nutrients will vary, while in a chemostat their concentration is essentially constant.

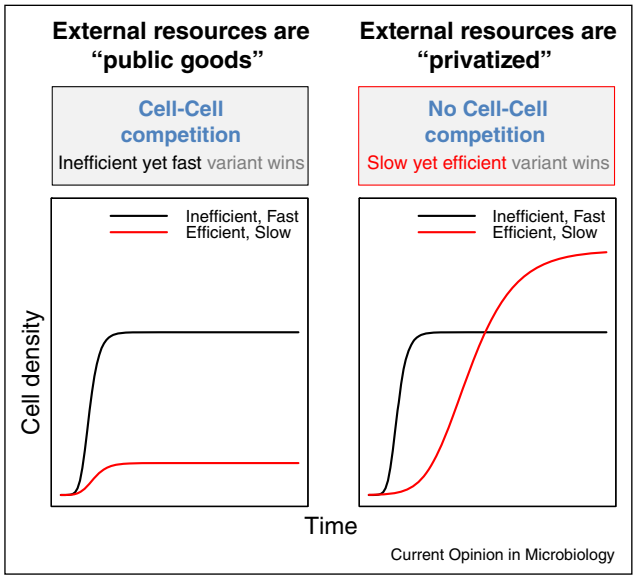
Batch and chemostat culturing methods have in common that the external resources are available to all cells in the culture — they are a public good — and individual cells compete for it [36]. Any mutant cell that is able to grow faster at saturating nutrient conditions (batch) or can attain a preset growth rate at a lower limiting nutrient concentration (chemostat) will outcompete the other cells, even if it comes at the cost of, for example, decreased metabolic efficiency [37••,38]. The Monod equation (Box 2) indicates that the growth rate is proportional to μ_{max}/K_S when the substrate concentration is low and hence either macroscopic growth parameter can be changed to achieve outgrowth of competitors. In practice, transport processes are often up-regulated in chemostat evolution experiments [39], suggesting that the K_S is lowered in many cases — although up-regulation of transport under nutrient limitation can equally affect μ_{max}.

If cells can benefit from each others activities, competition between them can lead to more counterintuitive

outcomes. Examples are the extracellular breakdown of macromolecules into growth precursors, by proteases or glucosidases [40,41], or the secretion of siderophores to capture poorly soluble iron [42,43]. In such cases, cheater cells can profit from the work of cooperating cells. Thereby cheaters safe resources and can grow faster. A number of laboratory evolution studies indicate that cheaters can take over the population at the cost of the overall fitness of the population. Both yield and rate can drop in such cases.

By contrast to well-mixed suspension cultures, where resource competition occurs, the culturing of cells in spatially structured environments such as emulsion droplets allows for 'privatization' of external resources. In such an emulsion based system [37••] a single cell is allowed to establish a population in a droplet. Generally, populations grown to the full carrying capacity of the medium do not exceed 100 cells per droplet. The small population sizes ensure a negligible likelihood of mutants arising and competing for external resources within a droplet. On the other hand, the millions of droplets in an emulsion ensure the supply of mutants that have an increased number of offspring. They will increase in overall frequency upon serial propagation. Serial propagation of cells, when external resources are privatized, therefore selects for mutants that can produce a higher number of viable offspring (Figure 2), which is likely associated with efficient metabolic strategies. This was shown by selecting lactococci that increased their biomass yield by shifting from homolactic to mixed acid fermentation [37••].

Figure 2



Effects of medium availability as public or private good. Assuming a yield/rate tradeoff inefficient, fast growing cells compete against efficient, slow growing cells. In a suspension where the external resources are a public good cell-cell competition occurs and the fast growing population will deplete external resources before the slow growing population reaches high cell densities (left panel). In the case of resource privatization (e.g. in emulsion droplets) no cell-cell competition occurs and the two types of cells grow undisturbed to the maximum carrying capacity of this medium for each cell. Slow but efficient cells increase in frequency during such a cultivation step. Figure adapted from [37••].

It is important to realize that during rate selection in suspension a cell with a faster growth rate increases in frequency with each generation. This is not the case in an emulsion-based propagation regime. The number of generations within a droplet does not matter to the increase in frequency of a variant cell with a higher yield. It is the number of serial transfers in emulsion that determines the rate at which a mutant with a higher cell yield increases in frequency. Only if the emulsion-culture would be propagated before the slowest individuals have reached their full carrying capacity, selection would favor cells that reach the highest cell yield within the given growth period. Such timing would put the selection pressure on rate and cell yield simultaneously.

There is an increasing appreciation that in nature the difference between private and public goods is not a strict one and that the metabolic competitions in such environments drive the evolution of microbial interactions [44].

Conclusions: fitness tradeoffs between yield, growth-rate and substrate affinity selection arise from a common mechanism

Microbial fitness and speciation is shaped by physicochemical, physiological and ecological constraints [44,45]. The consideration of the constraints associated with the allocation of limited resources and usage of novel selection protocols or macroscopic growth properties deepens our understanding of microbial evolution. A final, elegant example to illustrate this is the overexpression of a useless protein in *E. coli* which reduces the proteome fraction available for energy production and thereby leads to a shift toward acetate formation at lower growth rates than normally observed [13••]. This effect was shown to be dose dependent and it suggests, that a metabolic route that consists of fewer enzymes compensates for the space taken by the useless protein.

Yet, in recent work with *B. subtilis* [46] and *L. lactis* [47], it was shown that the expression of many proteins in core metabolism does not change appreciably with the flux. From a resource allocation perspective, it appears therefore that at low growth rate, there is an apparent enzyme overcapacity. This does not mean that resource allocation is not always a dominant strategy but indicates that other aspects of fitness, such as robustness [48] or readiness [27••] also required the investment of resources. It is furthermore conceivable that some suggested tradeoffs are rather observed correlations that can potentially be overcome given the right selective pressure. Yet, even if

such correlations could be altered by selection the question if the resulting organism would perform worse in a different environment remains.

This illustrates that despite the described progress, our current knowledge on intracellular constraints, phenotypic plasticity and evolvability is still rather limited. However, the concept that adaptation strategies are resource allocation strategies with only one objective — fitness — provides direction and a research agenda for many years to come. Their study will give insight into fundamental ecological and biological concepts but it will also be valuable for biotechnological applications such as metabolic and evolutionary engineering [49].

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Monod J: **The growth of bacterial cultures**. *Annu Rev Microbiol* 1949, **3**:371-394.
2. Pirt SJ: **The maintenance energy of bacteria in growing cultures**. *Proc R Soc Lond Ser B Biol Sci* 1965, **163**:224-231.
3. Stouthamer AH: **A theoretical study on the amount of ATP required for synthesis of microbial cell material**. *Antonie Van Leeuwenhoek* 1973, **39**:545-565.
4. Price ND, Reed JL, Palsson BØ: **Genome-scale models of microbial cells: evaluating the consequences of constraints**. *Nat Rev Microbiol* 2004, **2**:886-897.
5. Scott M, Klumpp S, Mateescu EM, Hwa T: **Emergence of robust growth laws from optimal regulation of ribosome synthesis**. *Mol Syst Biol* 2014, **10**:747.
6. Molenaar D, van Berlo R, de Ridder D, Teusink B: **Shifts in growth strategies reflect tradeoffs in cellular economics**. *Mol Syst Biol* 2009, **5**:323.
7. O'Brien EJ, Monk JM, Palsson BO: **Using genome-scale models to predict biological capabilities**. *Cell* 2015, **161**:971-987.
8. • Bosdriesz E, Molenaar D, Teusink B, Bruggeman FJ: **How fast-growing bacteria robustly tune their ribosome concentration to approximate growth-rate maximization**. *FEBS J* 2015, **282**:2029-2044.

This study presents a model which describes that ribosomes are expected to be saturated with substrate and subsequently suggests that expression optimization is sensed via inactive ribosomes.

9. Hui S, Silverman JM, Chen SS, Erickson DW, Basan M, Wang J, Hwa T, Williamson JR: **Quantitative proteomic analysis reveals a simple strategy of global resource allocation in bacteria**. *Mol Syst Biol* 2015, **11**:e784.
10. Wortel MT, Peters H, Hulshof J, Teusink B, Bruggeman FJ: **Metabolic states with maximal specific rate carry flux through an elementary flux mode**. *FEBS J* 2014, **281**:1547-1555.
11. Berkhout J, Teusink B, Bruggeman FJ: **Gene network requirements for regulation of metabolic gene expression to a desired state**. *Sci Rep* 2013, **3**:1417.
12. Flamholz A, Noor E, Bar-Even A, Liebermeister W, Milo R: **Glycolytic strategy as a tradeoff between energy yield and protein cost**. *Proc Natl Acad Sci USA* 2013, **110**:10039-10044.
13. •• Basan M, Hui S, Zhang Z, Shen Y, Williamson JR, Hwa T: **Overflow metabolism in bacteria results from efficient proteome allocation for energy biogenesis**. *Nature* 2015 http://dx.doi.org/10.1038/nature15765.

Shows that the protein cost for energy biosynthesis by respiration exceeds that by fermentation.

14. Wolfe AJ: **The acetate switch**. *Microbiol Mol Biol Rev* 2005, **69**:12-50.
15. De Mey M, De Maeseneire S, Soetaert W, Vandamme E: **Minimizing acetate formation in *E. coli* fermentations**. *J Ind Microbiol Biotechnol* 2007, **34**:689-700.
16. Eiteman MA, Altman E: **Overcoming acetate in *Escherichia coli* recombinant protein fermentations**. *Trends Biotechnol* 2006, **24**:530-536.
17. Lerman JA, Hyduke DR, Latif H, Portnoy VA, Lewis NE, Orth JD, Schrimpe-Rutledge AC, Smith RD, Adkins JN, Zengler K *et al.*: **In silico method for modelling metabolism and gene product expression at genome scale**. *Nat Commun* 2012, **3**:929.
18. O'Brien EJ, Palsson BO: **Computing the functional proteome: recent progress and future prospects for genome-scale models**. *Curr Opin Biotechnol* 2015, **34**:125-134.
19. Scott M, Gunderson CW, Mateescu EM, Zhang Z, Hwa T: **Interdependence of cell growth and gene expression: origins and consequences**. *Science* 2010, **330**:1099-1102.
20. You C, Okano H, Hui S, Zhang Z, Kim M, Gunderson CW, Wang Y-P, Lenz P, Yan D, Hwa T: **Coordination of bacterial proteome with metabolism by cyclic AMP signalling**. *Nature* 2013, **500**:301-306.
21. Li G-W, Burkhardt D, Gross C, Weissman JS: **Quantifying absolute protein synthesis rates reveals principles underlying allocation of cellular resources**. *Cell* 2014, **157**:624-635.
22. Postma E, Verduyn C, Scheffers WA, Van Dijken JP: **Enzymic analysis of the crabtree effect in glucose-limited chemostat cultures of *Saccharomyces cerevisiae***. *Appl Environ Microbiol* 1989, **55**:468-477.
23. Huberts DHEW, Niebel B, Heinemann M: **A flux-sensing mechanism could regulate the switch between respiration and fermentation**. *FEMS Yeast Res* 2012, **12**:118-128.
24. Garrigues C, Loubiere P, Lindley ND, Cocaign-Bousquet M: **Control of the shift from homolactic acid to mixed-acid fermentation in *Lactococcus lactis*: predominant role of the NADH/NAD+ ratio**. *J Bacteriol* 1997, **179**:5282-5287.
25. LaCroix RA, Sandberg TE, O'Brien EJ, Utrilla J, Ebrahim A, Guzman GI, Szubin R, Palsson BO, Feist AM: **Use of adaptive laboratory evolution to discover key mutations enabling rapid growth of *Escherichia coli* K-12 MG1655 on glucose minimal medium**. *Appl Environ Microbiol* 2014, **81**:17-30.
26. Weiße AY, Oyarzún DA, Danos V, Swain PS: **Mechanistic links between cellular trade-offs, gene expression, and growth**. *Proc Natl Acad Sci USA* 2015, **112**:E1038-E1047.
27. •• New AM, Cerulus B, Govers SK, Perez-Samper G, Zhu B, Boogmans S, Xavier JB, Verstrepen KJ: **Different levels of catabolite repression optimize growth in stable and variable environments**. *PLOS Biol* 2014, **12**:17-20.

Describes how catabolite repression optimizes resource allocation in stable and dynamic environments.

28. Schick A, Bailey SF, Kassen R: **Evolution of fitness trade-offs in locally adapted populations of *Pseudomonas fluorescens***. *Am Nat* 2015, **186**:S48-S59.
29. Solopova A, van Gestel J, Weissing FJ, Bachmann H, Teusink B, Kok J, Kuipers OP, Gestel JVan, Weissing FJ, Bachmann H *et al.*: **Bet-hedging during bacterial diauxic shift**. *Proc Natl Acad Sci USA* 2014, **111**:7427-7432.
30. Gresham D, Hong J: **The functional basis of adaptive evolution in chemostats**. *FEMS Microbiol Rev* 2014, **39**:2-16.
31. Bosdriesz E, Magnúsdóttir S, Bruggeman FJ, Teusink B, Molenaar D: **Binding proteins enhance specific uptake rate by increasing the substrate-transporter encounter rate**. *FEBS J* 2015, **282**:2394-2407.

32. Ferenci T: **Trade-off mechanisms shaping the diversity of bacteria**. *Trends Microbiol* 2015 http://dx.doi.org/10.1016/j.tim.2015.11.009.

33. Caspeta L, Nielsen J: **Thermotolerant yeast strains adapted by laboratory evolution show trade-off at ancestral temperatures and preadaptation to other stresses**. *mBio* 2015, **6**:e00431-e515.

34. Maharjan R, Nilsson S, Sung J, Haynes K, Beardmore RE, Hurst LD, Ferenci T, Gudelj I: **The form of a trade-off determines the response to competition**. *Ecol Lett* 2013, **16**:1267-1276.

35. Litchman E, Edwards KF, Klausmeier CA: **Microbial resource utilization traits and trade-offs: implications for community structure, functioning, and biogeochemical impacts at present and in the future**. *Front Microbiol* 2015, **06**:254.

36. Barrick JE, Lenski RE: **Genome dynamics during experimental evolution**. *Nat Rev Genet* 2013, **14**:827-839.

37. •• Bachmann H, Fischlechner M, Rabbers I, Barfa N, Branco dos Santos F, Molenaar D, Teusink B: **Availability of public goods shapes the evolution of competing metabolic strategies**. *Proc Natl Acad Sci* 2013, **110**:14302-14307.
Describes that the privatization of substrate for single cells in emulsion droplets allows to select for lactococci with increased cell yield.

38. Jasmin J-NJ-N, Dillon MM, Zeyl C: **The yield of experimental yeast populations declines during selection**. *Proc Biol Sci* 2012, **279**:4382-4388.

39. Brown CJ, Todd KM, Rosenzweig RF: **Multiple duplications of yeast hexose transport genes in response to selection in a glucose-limited environment**. *Mol Biol Evol* 1998, **15**:931-942.

40. Gore J, Youk H, van Oudenaarden A: **Snowdrift game dynamics and facultative cheating in yeast**. *Nature* 2009, **459**:253-256.

41. Bachmann H, Molenaar D, Kleerebezem M, Vlieg JET, van H, van Hylckama Vlieg JET: **High local substrate availability stabilizes a cooperative trait**. *ISME J* 2011, **5**:929-932.

42. Kümmerli R, Santorelli LA, Granato ET, Dumas Z, Dobay A, Griffin AS, West SA: **Co-evolutionary dynamics between public good producers and cheats in the bacterium *Pseudomonas aeruginosa***. *J Evol Biol* 2015, **28**:2264-2274.

43. Cordero OX, Ventouras L-A, DeLong EF, Polz MF: **Public good dynamics drive evolution of iron acquisition strategies in natural bacterioplankton populations**. *Proc Natl Acad Sci* 2012, **109**:20059-20064.

44. Estrela S, Morris JJ, Kerr B: **Private benefits and metabolic conflicts shape the emergence of microbial interdependencies**. *Environ Microbiol* 2015 http://dx.doi.org/10.1111/1462-2920.13028.

45. Johnson DR, Goldschmidt F, Lilja EE, Ackermann M: **Metabolic specialization and the assembly of microbial communities**. *ISME J* 2012, **6**:1985-1991.

46. Chubukov V, Uhr M, Le Chat L, Kleijn RJ, Jules M, Link H, Aymerich S, Stelling J, Sauer U: **Transcriptional regulation is insufficient to explain substrate-induced flux changes in *Bacillus subtilis***. *Mol Syst Biol* 2014, **9** Article No 709.

47. Goel A, Eckhardt TH, Puri P, Jong A, Branco dos Santos F, Giera M, Fusetti F, Vos WM, Kok J, Poolman B *et al.*: **Protein costs do not explain evolution of metabolic strategies and regulation of ribosomal content**. *Mol Microbiol* 2015, **97**:77-92.

48. van Heerden JH, Wortel MT, Bruggeman FJ, Heijnen JJ, Bollen YJM, Planqué R, Hulshof J, O'Toole TG, Wahl SA, Teusink B: **Lost in transition: start-up of glycolysis yields subpopulations of nongrowing cells**. *Science* 2014, **343**:1245114.

49. Bachmann H, Pronk JT, Kleerebezem M, Teusink B: **Evolutionary engineering to enhance starter culture performance in food fermentations**. *Curr Opin Biotechnol* 2015, **32**:1-7.

50. Campbell A: **Synchronization of cell division**. *Bacteriol Rev* 1957, **21**:263-272.

51. Novick A, Szilard L: **Description of the chemostat**. *Science* 1950, **112**:715-716.

52. Gresham D, Dunham MJ: **The enduring utility of continuous culturing in experimental evolution**. *Genomics* 2014, **104**:399-405.