

# The harmony of the cell: the regulatory design of cellular processes

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## Abstract

The living cell is a complex system of interacting processes. The properties of the agents that facilitate these processes, such as enzymes, transporters and receptors, must be tuned to each other if the system is to behave harmoniously. The present chapter describes how the regulatory design of cellular subsystems that makes this harmonious behaviour possible can be visualized on a graph that combines the so-called log-log rate characteristics of these subsystems. The tools that are needed to create and analyse these graphs are metabolic control analysis, supply-demand analysis, enzyme kinetics and computer simulation.

## Introduction

An important aim of systems biology is to understand how all the components of the living cell interact harmoniously to give rise to emergent, systemic properties that characterize life, such as the homoeostatically maintained, far-from-equilibrium steady state. During the recent era of molecular biology the idea that all of this was directed by a genetic programme that resided in the DNA had widespread prevalence. However, it has become increasingly clear that DNA does not incorporate anything like an explicit

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program [1,2]. Compared with a recipe, which specifies both ingredients and the instructions for combining the ingredients into a dish, DNA provides only the basic data for making the ingredients (i.e. sequence data) and, of course, the range of available ingredients. The ability to make the ingredients and the rules for cooking the dish of life are embedded in the properties of the ingredients themselves (in the cell the active ingredients are, for example, the enzymes, ribozymes, transporters and supramolecular structures such as ribosomes, proteasomes, spliceosomes and chaperonins). In a loose sense it is like a cook combining the ingredients in a bowl and watching them transform themselves into a dish. But even this picture is misleading: there is no cook; there is only a cookbook, a very unusual cookbook that only specifies a range of ingredients, some of which are able to read from the book how to make themselves and the others and, ultimately, the dish [3]. In terms of the living cell this means that its 'programme' is not in the DNA but rather embedded in and distributed amongst the properties of all of the active agents in the cell, hence the more accurate idea of a 'cellular programme'. At face value it seems that such a programme must be so complicated that it is impossible to describe or visualize. In the present chapter I show that, even though it may prove too difficult, or even impossible, to give one encompassing view of the whole cellular programme, there is nevertheless a highly informative way of representing and understanding at least part of the programme. Just as a sphere can be described as the sum of an infinite set of planar approximations, so it should be possible to see the cellular programme as the sum of such partial views.

### **The molecular economy in the cell**

I have, in collaboration with Athel Cornish-Bowden, used a theoretical framework called metabolic control analysis [4–6] in conjunction with computer modelling to try and understand how cellular processes such as metabolism are functionally organized and regulated in the living cell. The problem with conventional metabolic studies is that they draw artificial boundaries around a metabolic pathway, forgetting that the function of such a pathway is to produce a product that is used by another subsystem in the cell. For example, amino acids are made for use in protein synthesis, and nucleotides are made for use in RNA and DNA synthesis. In fact, much of the cell is made up of such supply–demand systems. Conventionally, the rates at which these metabolic products are made are purported to be controlled by so-called 'rate-limiting' steps within the supply pathways. Biotechnology, for instance, often takes this textbook wisdom for granted and tries to manipulate these rates by engineering the levels of the 'rate-limiting' enzymes, usually with little success. Our analysis of supply–demand systems [7,8], in which the supply is subject to feedback inhibition by the product, shows that this view is wrong and that, as expected from a systemic and functional point of view, the

control of metabolic steady-state fluxes should lie in the demand processes. In contrast, the supply enzymes actually take on the role of maintaining homeostatically the intracellular steady-state concentrations of metabolites. Experimental verifications of our theory have recently been published [9–15]; see also the commentary in *Nature* by Oliver [16]. There may of course be supply–demand systems where, from a functional point of view, supply is expected to have control over the flux. A recent example is the muscle supply–demand system around glucose 6-phosphate, which is supplied from blood glucose and consumed by glycolysis and glycogen synthesis [17].

The idea of viewing the living cell as an economy is not particularly new. There is, however, a vast difference between using such a description in a metaphorical sense and, as we have done, using it as the basis for a quantitative explanatory theory. What is the justification for this view? How does the network of connected chemical reactions in a cell become a molecular economy? The answer lies in the realization that living cells are subject to environmental pressures that force them to adapt through the process of evolution. Through genetic variation and subsequent natural selection, the properties of the enzymes that catalyse the individual metabolic reactions become optimized to fulfill specific systemic functions, the most important of which is to control the rates of metabolic reactions and the concentrations of the metabolic compounds that link these reactions. This adaptive process has resulted in the metabolic networks inside cells becoming functionally differentiated into, on the one hand, supply processes (think of them as molecular factories) that produce key metabolic commodities such as chemical energy, reducing equivalents and small building-block molecules, and, on the other hand, demand processes that consume these commodities to make large molecules such as proteins, nucleic acids, complex carbohydrates and lipids for growth. In essence, this is exactly what happens in human economies—as the environment changes, market pressures cause adaptation in the behaviour of the economic agents so that they become functionally organized into producers and consumers. Human and molecular economies of course differ in the details of their agents and the rules by which they interact; in the molecular economies that we study, the agents are enzymes that are governed by the laws of enzyme kinetics and thermodynamics. The greatest difference, which, ironically, makes classical supply–demand economics more applicable to biomolecular than to human economies, lies in the premise of rational behaviour of agents being perfectly valid for enzymes, but for humans at best an optimistic approximation, at worst a pipe-dream. Under any specific set of conditions all molecules of a particular enzyme behave the same; try that for humans!

How does our theory differ from existing metabolic theories? Imagine yourself an economist, producing a report on some manufacturing industry without considering consumer demand in your analysis. You will be laughed out of the boardroom. However, this is exactly what the classical theory of metabolic regulation does. Open any modern biochemistry textbook and you

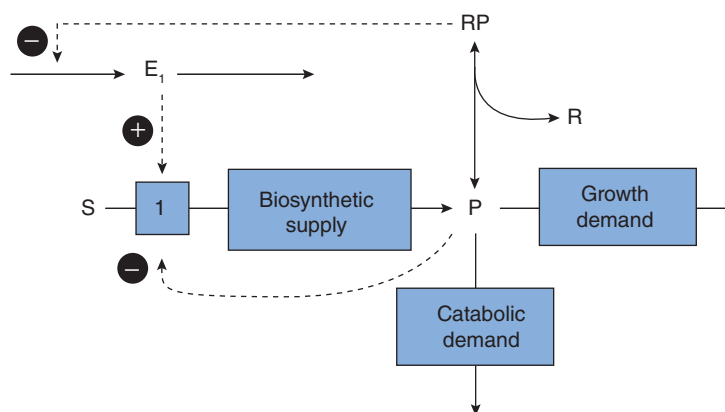
will find that metabolic regulation is discussed only in terms of the supply parts, those parts that manufacture the end-products of metabolism. In fact, the discussion usually focuses only on a few so-called 'key' steps in the supply. As discussed in the beginning of this section, we have shown that when one takes into account the demand parts the whole picture changes and the classical theory of metabolic regulation is turned on its head. Our theory explicitly recognizes that the evolved properties of any part of a system can only be understood in relation to the whole: it is, therefore, a systems theory of the cellular processes. Although it is perfectly possible to describe any part of a system fully in terms of its constituents and their interactions, it is impossible to understand why that part of the system has the properties it has without considering it in the context of the intact, whole system. More importantly, we also have shown how embedding a part in the whole changes the control and regulatory properties that one observes when studying the part in isolation.

### The design of the cellular program: an example

What we have learnt from supply–demand analysis is how to consider, simultaneously, the quantitative properties of the subsystems from which a more complex system is constructed. Using log–log rate characteristics, we have developed a graphical visualization that highlights in a novel way the integrated functional organization of that system. This is, in my opinion, the closest we can get at present to a view of at least part of the cellular programme. In this section, rate characteristic analysis is applied to a system that should be familiar to most biochemists and molecular biologists.

Figure 1 shows the functional organization of cellular processes around a metabolic end-product that serves as a building block for macromolecular synthesis (think of an amino acid for protein synthesis, or a nucleotide for DNA or RNA synthesis). This scheme should be seen as generic, in that it incorporates our generalized knowledge about the biochemical properties of such systems, in particular the biosynthetic part: the first biosynthetic enzyme  $E_1$  is usually not only allosterically inhibited by the end-product  $P$ , but its synthesis is also controlled by the intracellular concentration of  $P$ , which acts as a co-repressor (often some or all of the biosynthetic enzymes form part of an operon under control of a repressor/co-repressor complex, but, for simplicity, only the first enzyme is here considered to be controlled at the genetic level). In this particular model,  $E_1$  is considered to be insensitive to its immediate product and is therefore rate-limiting in the biosynthetic block (but, as will become clear, not necessarily in the full system). The growth demand is considered to bind  $P$  strongly, so that it is readily saturated at relatively low physiological levels of  $P$  (look ahead to Figure 2 where near-saturation of growth demand by  $P$  already occurs at 0.1 concentration units).

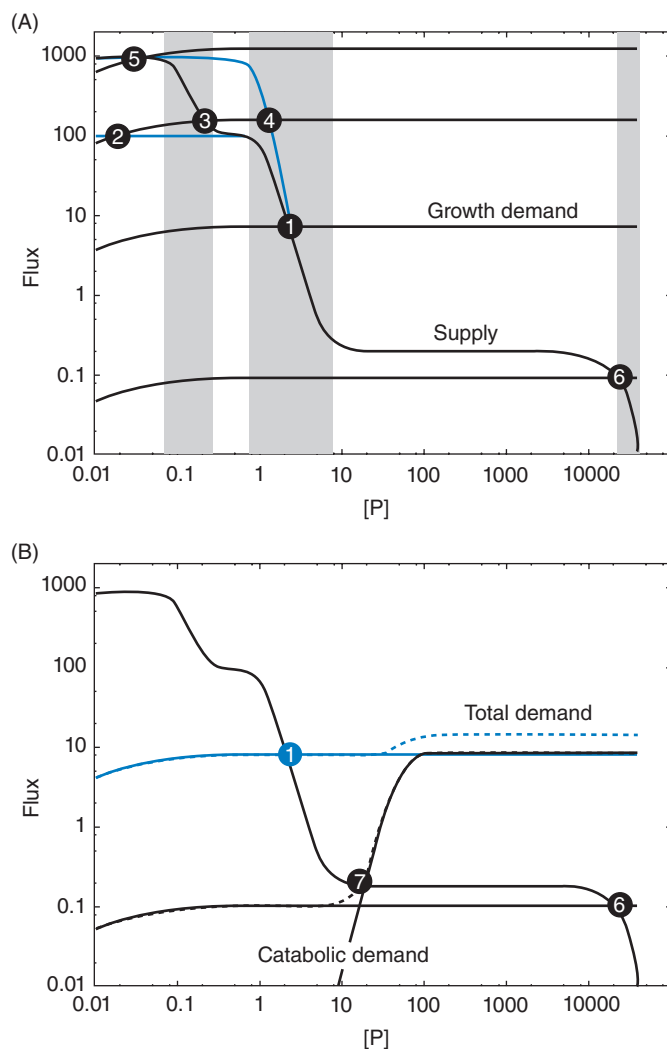
We have shown previously that the regulatory design of such a metabolic junction can be understood in terms of a graph of combined log–log



**Figure 1. A typical metabolic junction**

A biosynthetic end-product P (e.g. an amino acid or nucleotide) is both catabolized (catabolic demand) and used for macromolecular synthesis (growth demand). P is synthesized from a substrate S by a biosynthetic supply pathway of which the first enzyme  $E_1$  is subject to allosteric inhibition by P ( $E_1$  is shown as a separate entity; the plus sign next to the dotted arrow that connects  $E_1$  to the box representing the first biosynthetic step indicates catalysis). In addition, P acts as a co-repressor, binding to the repressor R to form the RP complex that prevents transcription of the structural gene for  $E_1$  and therefore synthesis of  $E_1$ .  $E_1$  is also degraded, so that it can reach a steady-state level which is determined by the properties of the system.

rate characteristics [8,18–20], which has proved to be a powerful explanatory tool. Rate characteristics not only allow one to understand how the steady state responds to perturbations, but they also provide a broad picture of how the system behaves over a large range of variation in the concentration of P and in the activities of supply and demand. The mathematical basis for this type of analysis is developed in [7,8]. However, in the present chapter we do not consider the mathematics because the rate characteristics in themselves provide a clear enough picture. Figure 2 provides the example that will serve as the basis for discussion. Figure 2(A) shows how the fluxes local to the biosynthetic supply and growth demand respond to changes in the concentration of P. Consider first the rate characteristic of the biosynthetic supply (with S, the supply substrate, buffered at a constant value). It is a complicated curve that can be divided into a number of regions that will be identified with the numbered points on the graph. The region around point 1 is determined by allosteric inhibition of  $E_1$ . The steepness of the curve depends on the degree of co-operativity with which P binds to  $E_1$ ; the higher the degree of co-operativity, the steeper the slope of the curve. The concentration range in which P inhibits the enzyme kinetically is determined by the half-saturation constant of the enzyme for P (which in this case is 1). At concentrations of P above 10 the inhibitory effect is abolished (the reasons for this are discussed in [21,22]). As P nears its equilibrium value (which depends on the set concentration of S and the overall equilibrium constant for the supply block), the rate of biosynthesis rapidly falls to near zero (the region around point 6).



**Figure 2. Visualizing with combined log-log rate characteristics the part of the cellular program that pertains to the biosynthetic end-product P in Figure 1**

Each curve shows how the steady-state flux local to one of the three reaction blocks in Figure 1 responds to a change in the concentration of P. In (A) the behaviour of the system at various growth demand activities is depicted with and without induction of the first biosynthetic enzyme. The grey bands depict ranges where the concentration of P is homeostatically maintained in the face of changes in growth demand. In (B) the effect of adding catabolic demand is shown for two growth demand activities (the total demand for P, i.e. the sum of growth and catabolic demands, is depicted by the dotted lines). The numbered disks indicate points where the rates of supply of and demand for P are equal and therefore where the steady state is obtained. The different situations are discussed in the text. The numerical simulation, details of which can be obtained from the author, was performed using the open-source metabolic modelling program PySCeS [26]. All reactions were modelled with realistic, reversible enzyme kinetic rate equations. The first biosynthetic enzyme, which determines the response of the biosynthetic reaction block to P, was modelled using the reversible Hill equation with one allosteric modifier [27].

Whereas, around point 1, the inhibitory effect of P is purely kinetic, around point 6 it is thermodynamic and independent of the kinetic properties of the supply enzymes. At very low concentrations of P there is of course no inhibition and the activity of  $E_1$  is determined by its limiting velocity,  $V_{\max}$ , and the concentration of its substrate S. In the absence of genetic regulation, this concentration of P would be at point 2 (the grey curve through point 2 showing the supply rate characteristic under these conditions). However, when P acts as a co-repressor of  $E_1$  synthesis, low concentrations of P (where the repressor-co-repressor complex dissociates) relieve repression and increase the concentration of  $E_1$ , leading to the supply rate characteristic that passes through points 3 and 5. The concentration of P at which this increased synthesis of  $E_1$  kicks in is determined by the affinity of the repressor protein R for P. At a lower affinity, the supply rate characteristic would change to, for example, the grey curve that passes through points 4 and 5.

Figure 2(A) also shows the rate characteristic of the demand for P at four different demand activities. Note that, in the log-log representation used, the demand curve retains its shape when demand activity changes. Wherever the supply and demand rate characteristics intersect the rates are equal and the full system reaches a steady state; the steady-state flux and concentration of P can be read directly from such an intersection point. The beauty of log-log rate characteristics is that the degree of control that supply and demand have over the steady-state flux and over the concentration of P can be deduced directly from the graph: the ratio of slopes (more accurately, the absolute value of this ratio) determines the distribution of flux-control between supply and demand (if the demand slope is shallower than the supply slope, the demand has a higher degree of control over the flux, and vice versa). The mathematical control theory behind this statement is described in [8]. In Figure 2(A) demand clearly controls the flux at points 1, 3, 4 and 6, whereas supply controls the flux at points 2 and 5.

The combined rate characteristics also show that when one block controls the flux, the other block takes over the function of homeostatic regulation of the concentration of P. Consider for example, the grey banded region around point 1. Imagine moving the demand rate characteristic curve up or down until the intersection point reaches the limit of the band (this of course represents what would happen if the demand activity increases or decreases). Clearly the flux increases directly in proportion to demand, because, with a slope of near zero, demand has complete control over the flux. It is also clear that the narrowness of the band (and therefore the effectiveness of homeostatic maintenance of P) is solely determined by the supply rate characteristic, more specifically by its slope. Increasing the demand beyond the capacity of the supply, whether the supply is (point 5) or is not (point 2) genetically regulated, causes a switch in the flux-control profile: the supply now controls the flux, whereas the demand determines how the steady-state concentration of P responds. From the point of view of regulatory design, it would be desirable for the allosteric inhibition

of the activity of  $E_1$  and the genetic regulation of the concentration of  $E_1$  to be tuned to one another to give a supply rate characteristic such as the one through point 4. The supply characteristic through point 3 shows how a mismatch leads to two regions in which P is homeostatically maintained (these two regions would of course operate on different timescales, the one around point 1 responding much faster than the one around point 3).

Whereas derepression of the synthesis of  $E_1$  allows the system to respond adaptively to high demand activity, it is clear that the system as depicted in Figure 2(A) cannot cope with very low demand activity: when demand falls too low,  $E_1$  cannot keep the concentration of P in the far-from-equilibrium, homeostatic range around point 1, so that it jumps to near-equilibrium values (point 6). For most biosynthetic systems (which have large equilibrium constants) this would be disastrous, because the cell, having a limited solvent capacity, cannot cope with the resultant high concentrations of end product (and of other biosynthetic intermediates).

If this were not so, then the response profile around point 6 would seem highly favourable: observe that under these conditions demand still controls the flux and the homeostasis of P is excellent. However, thermodynamic properties cannot be controlled by enzymes. In addition, not only can the cell not cope with high near-equilibrium concentrations, but we have also shown [19] that, near equilibrium, the system responds sluggishly compared with one under kinetic control at P concentrations far from equilibrium. This points to one disadvantage of rate characteristics: there is no time dimension.

Figure 2(B) shows how the addition of a catabolic demand circumvents the jump to the near-equilibrium domain at low growth demand by adding a bypass that redirects P into catabolism, thereby creating a metabolic overflow valve. In order for catabolic demand to start functioning only when P increases beyond a certain point again requires tuning of the binding properties of the first catabolic enzyme. Figure 2(B) depicts such a properly tuned system operating at normal demand and at low demand. Note that the steady state is now obtained where the supply flux matches that of the combined demands (this amounts to adding the rate characteristics of growth and catabolic demand to give the total demand, which is depicted by the dotted lines on the graph). Consider the situation at low demand: instead of the concentration of P jumping to point 6, the presence of the catabolic branch now keeps it from increasing beyond point 7 even if the demand decreases further. At demand activities in the range for which the system is designed to operate (the grey rate characteristic for growth demand through point 1) the catabolic demand is effectively switched off. Note again how important it is for the correct functioning of the system that the properties of the different subsystems are attuned to each other. If, for example, the first catabolic enzyme were to bind P with higher affinity (displacing the catabolic rate characteristic to the left), it could cause a wasteful diversion of P into catabolism, or, alternatively, provide a regulatory mechanism for partitioning flux between growth and catabolism.



## Conclusion

Although systems biology means many things to many people, these, often seemingly divergent, views are all compatible and can be consolidated into something along the lines of “explaining or understanding the emergence of systemic functional properties of the living cell as a result of the interactions of its components” [3]. Whether this is attempted via an ‘omics’ approach [23] or a ‘silicon cell’ approach [24], we still need conceptual tools with which to analyse the experimental or numerical results. The present chapter describes a set of such tools that, together, provide a quantitative framework that can be used to design experiments, guide computer simulations and explain results.

A seeming shortcoming of the analysis as presented above is that the coupled processes should communicate through the coupling metabolite only. Recently, we have devised a method called generalized supply–demand analysis [25] which extends the analysis to any metabolite in a system of arbitrary complexity, allowing for communication between supply and demand blocks other than through the coupling metabolite.

## Summary

- *Nothing in an organism makes sense except in the light of functional context.*
- *The harmonious behaviour of coupled cellular processes depends on the properties of the molecular agents that facilitate these processes being finely tuned to each other.*
- *The regulatory design of cellular processes can be visualized with combined log–log rate characteristics.*
- *Supply–demand analysis (and, by implication, metabolic control analysis) allows the quantification of the degree to which the functions of flux-control and the homeostatic maintenance of metabolite concentrations are distributed among coupled cellular processes.*

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