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Review

Understanding the parts in terms of the whole

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Abstract

Metabolism is usually treated as a set of chemical reactions catalysed by separate enzymes. However, various complications, such as transport of molecules across membranes, physical association of different enzymes, giving the possibility of metabolite channelling, need to be taken into account. More generally, a proper understanding of the nature of life will require metabolism to be treated as a complete system, and not just as a collection of components. Certain properties of metabolic systems, such as feedback inhibition of the first committed step of a pathway, make sense only if one takes a broader view of a pathway than is usual in textbooks, so that one can appreciate ideas such as regulation of biosynthesis according to demand. More generally still, consideration of metabolism as a whole puts the emphasis on certain systemic aspects that are crucial but which can pass unnoticed if attention is always focussed on details. For example, a living organism, unlike any machine known or conceivable at present, makes and maintains itself and all of its components. Any serious investigation of how this can be possible implies an infinite regress in which each set of enzymes needed for the metabolic activity of the organism implies the existence of another set of enzymes to maintain them, which, in turn, implies another set, and so on indefinitely. Avoiding this implication of infinite regress represents a major challenge for future investigation.

Keywords: Systems biology; Metabolism-repair systems; *(M,R)*-systems; Metabolism

1. Introduction

More than half a century ago, Schrödinger (1944) raised the question of whether biology would require new laws of physics that could not be revealed by investigations in physics alone. He did not, of course, imply that biological systems were not constrained by the same laws of physics as physical and chemical systems; he did not doubt that adherence to physical laws was necessary, only whether it was sufficient to explain living systems. This profound question was ignored by most biologists, and received a mixed reaction from others. Jacob (1970), for example, quoted Schrödinger's opinion that "we must be prepared to find a new type of physical law prevailing in [life]" with apparent approval, but at the same time his collaborator Monod (1970) reacted with great hostility, finding such arguments "singularly deficient in rigour". Despite the essential distinction between a necessary and a sufficient condition, Monod apparently felt that admitting the possibility that biology might be more general than physics was tantamount to a return to the vitalism that had impeded the progress of life-chemistry in the 19th century.

Biochemistry has been at best indifferent to Schrödinger's ideas, when not frankly hostile, and the last half-century has been devoted to a strictly reductionist approach to research, accumulating large quantities of detailed information with little attempt to incorporate it into a broad view of the whole subject. Despite the current vogue for "systems biology", this term is often little more than a euphemism for gathering ever more details on an ever larger scale (see, for example, Ideker, 2004), and not, as it should be, the study of biological systems as systems rather than as collections of components.

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Here we shall try to show that understanding the organization of living organisms will only be possible with a genuinely systemic approach. Perhaps the most important justification for this view is the realization that, long after Schrödinger's lectures in Dublin in 1944, we are still far from a satisfactory answer to his question of what is life. All living organisms can do something that no machine that exists or that can currently be envisaged can do: they not only make themselves, but also they repair any damage to themselves that results from ordinary wear and tear. Efforts to understand how they do this have led to two major currents in biological thought, autopoiesis, as proposed by Varela et al. (1974), and *metabolism-repair systems*, usually called (*M*,*R*)-*systems*, developed during many years by Rosen (1958, 1991, 2000). The relationships between these two were examined in an earlier publication (Letelier et al., 2003); here we shall be concerned more with metabolism-repair systems.

2. Understanding metabolism is not simple

Metabolism has usually been regarded as the totality of all chemical reactions that operate in a living cell, but over the past two or three decades various developments have necessitated a more complicated definition. For example, transport of metabolites across biological membranes is an integral part of metabolism and is a transformation similar in function to a chemical transformation; it is often not helpful, therefore, to distinguish between chemical reactions, catalysed by enzymes, and transport processes, facilitated by transporters.

As a different example, enzymes that catalyse separate reactions are sometimes physically associated with one another in the cell, sometimes even forming parts of the same molecule, and the effects of such association need to be taken into account in metabolic analysis. For example, complexes of enzymes that catalyse consecutive reactions allow (but do not require) direct transfer of the common intermediate from the active site of one enzyme to the active site of the other, without passing into the bulk solution, i.e. they allow *channelling* (Ovádi, 1991). This may have important metabolic effects, such as decreasing the concentrations of free intermediates, which would be of great importance for metabolic regulation. However, if the complex is leaky (as is often the case), so that less than 100% of the intermediate is channelled, channelling does not reduce its free concentration in conditions of steady state and constant flux (Cornish-Bowden and Cárdenas, 1993; Cascante et al., 1994).

In Fig. 1, we try to encapsulate the essential features of this view of metabolism schematically. In current research, it has become clear that knowing the complete set of genes that code for an organism, the genome, is not enough for understanding how it is organized. In addition, as a minimum, one needs to know the concentrations of the metabolites that exist in any metabolic state, the *metabolome*, as well as the concentrations of the proteins, the *proteome*. However, as we shall see, this minimum falls far short of being sufficient.

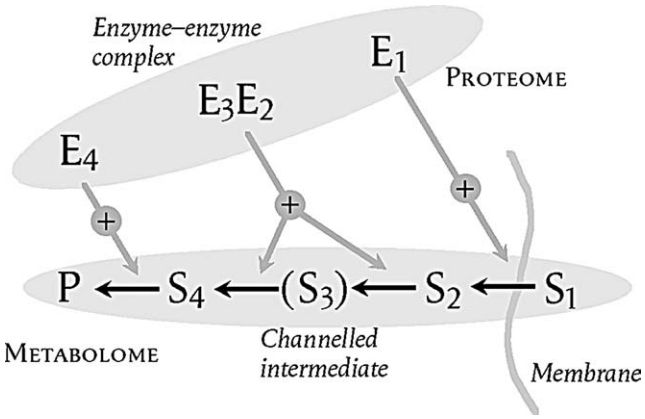

Fig. 1. Schematic representation of metabolism. A metabolism is commonly regarded as a set of chemical reactions that convert starting materials, here represented by the single reactant S_1, into products, here represented by P. However, several complications need to be considered (apart from the obvious one that real metabolisms involve more than a single starting material and lead to more than a single product). In all organisms, metabolism involves not only chemical transformations but also translocations of molecules across membranes, typically facilitated by transporter proteins analogous to enzymes. In addition, the enzymes catalysing successive steps can be physically associated into enzyme–enzyme complexes, and the metabolite that acts as product of one and substrate of the other may be channelled from one active site to the other without appearing free in solution. The totality of metabolites and their concentrations that exists in a particular metabolic state is called the *metabolome,* and the totality of proteins (mainly enzymes) and their concentrations is called the *proteome*.

3. Expressing metabolism in mathematical terms

Understanding the properties of the metabolic network is necessary for predicting the effects of genetic or other manipulations made with biotechnological or medical objectives. Ideally metabolism needs to be expressed mathematically, but this is made difficult by the fact that its elements are simultaneously both variables and functions. In addition to the complications already noted like enzyme–enzyme association, membrane transport, channelling, etc., there is another more serious one that has been evident for many years though ignored by most biochemists: the enzymes and transporters that catalyse biological processes are products of the organism itself, and are metabolites of the same standing as the substances that are usually regarded as metabolites. Moreover, as no protein is indefinitely stable, and many have rather short lifetimes, the resynthesis of enzymes is an essential part of metabolism. The resynthesis must itself be catalysed by enzymes that have limited lifetimes themselves, and are thus just as much in need of resynthesis as the enzymes they are responsible for (Fig. 2).

This analysis appears to lead inevitably to an infinite regress in which each level of enzymes implies the existence of another level of different enzymes necessary for their maintenance. Yet although a living organism is neither infi-

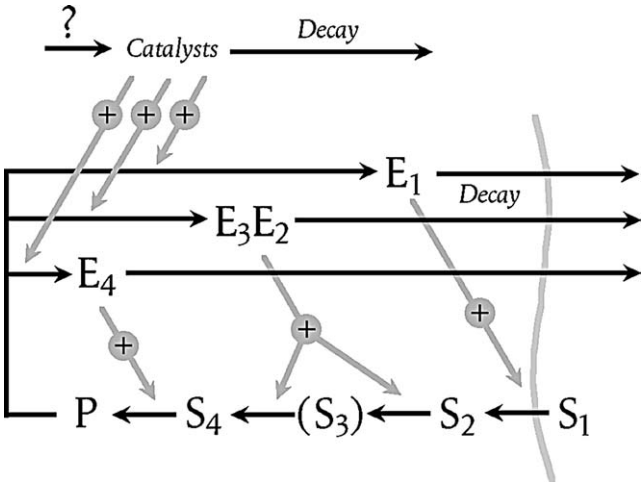

Fig. 2. A more realistic (but still highly simplified) view of metabolism. The enzymes that catalyse metabolic reactions are themselves products of the same metabolism, and ought to be regarded as metabolites. Moreover, they are never indefinitely stable, and need to be continually resynthesized. This resynthesis itself requires catalysts, themselves normally enzymes, and thus likewise not indefinitely stable, and equally in need of resynthesis. All of this implies an infinite regress, and understanding how to escape from it is an essential step for understanding life.

nitely large nor infinitely complex, it maintains its identity, and hence its internal organization, for periods that are very long compared with the time constants of the chemical reactions within it. Understanding how organisms escape from infinite regress is thus an essential part of understanding the nature of life, but it is a part that is far from complete at present, despite the important contributions made by Rosen (1991, 2000).

His work is almost totally unknown to biologists, but it is essential for placing in a broader context the idea of understanding the parts of a system in terms of the whole. He tried to analyse metabolism in terms of what he called *metabolism-repair systems*, or (*M*,*R*)-*systems*. "Repair" was an unfortunate choice of term, and what he meant by it was not repair but resynthesis. In other words, his (*M*,*R*)-systems were an attempt to give mathematical expression to the ideas outlined above in Fig. 2, where enzymes are explicitly considered as products of metabolism. An attempt to clarify the essential concept of metabolic closure may be found elsewhere (Letelier et al., 2004).

If metabolism is not treated as a whole, understanding it may appear simpler, but this is partly an illusion. Nonetheless, some predictions can still be made from incomplete information, and, in view of the lack of biochemical characterization of most of the enzymes in organisms with completely known genomes, there is current interest in methods such as analysis of "elementary flux modes" that are based only on the stoichiometric structure of the network, not needing kinetic information (Cornish-Bowden and Cárdenas, 2002; Stelling et al., 2002). Such methods may allow, for example, predictions of whether particular bacteria can grow in particular media, or which would be the best precursor of an end-product of commercial value. However, the fundamental question of expressing metabolism in mathematical terms remains unsolved.

4. Understanding the parts or understanding the whole?

In practice, most current research in biology is not directed towards solving fundamental problems, but towards accumulating more detailed facts, and unfortunately this may cloud understanding as much as it improves it. According to Bains (2001), for example, "the genome has turned out to be a relatively poor source of explanation for the differences between cells or between people". This statement may appear too negative, but there can be little doubt that the present capacity to generate mountains of new data has far outstripped our capacity to make sense of it all. Before many genomes had been elucidated, it was widely assumed that the function of an unidentified gene could be determined by examining the effects of deleting it. However, experience has now shown that many genes—perhaps as many as 80% in yeast, which does not appear to be an exceptional organism—are "silent": an organism remains viable when a silent gene is deleted, sometimes with normal growth and normal metabolic fluxes. Clearly, the solution here is not simply to gather more observations, but to search for a better understanding of the observations already available; an important step is to apply the lesson from metabolic control analysis that metabolite concentrations are far more sensitive to perturbations than are fluxes. Studying effects of gene deletion on concentrations, preferably combined effects on multiple concentrations, therefore provides a much more sensitive probe into gene function than studying effects on fluxes (Cornish-Bowden and Cárdenas, 2001a; Raamsdonk et al., 2001).

This sensitivity of metabolite concentrations to perturbations has major implications for the regulatory design of metabolism in living organisms. As fluxes need to be adjusted to satisfy changes in demand for metabolic products, there would be a danger in an unregulated system of accompanying every change in flux with enormous and damaging changes in metabolite concentrations, and avoiding this is the principal function of regulatory mechanisms such as cooperativity and feedback inhibition by end-products (Cornish-Bowden et al., 1995). To understand this, it is necessary to represent biosynthetic pathways in a way that allows analysis in terms of supply and demand (Hofmeyr and Cornish-Bowden, 2000), that is to say in a more complete way than is usual in textbooks of biochemistry. These typically show, for example, the biosynthesis of lysine as a series of reactions that begin with aspartate and end with lysine (Fig. 3a). However, lysine is not in any meaningful sense the end-product of this process: it is made not as an end in itself but as a starting material for other processes, principally, in this

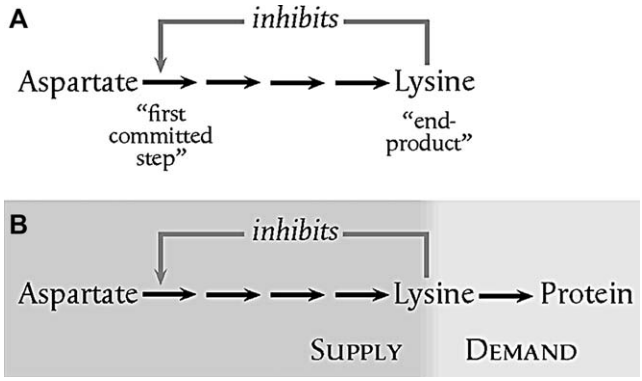

Fig. 3. Biosynthesis of lysine (a) as illustrated in typical biochemistry textbooks, (b) as it needs to be illustrated if the feedback inhibition of aspartokinase by lysine is to make sense. In the conventional representation, with the end-product shown as the end of the process, feedback inhibition of the first committed step is essentially arbitrary. However, if it is recognized that the end-product is in reality the link metabolite between a *supply block* responsible for its synthesis and a *demand block* responsible for its utilization, it can be understood that the effect of the feedback inhibition is to allow the rate of synthesis of lysine to vary according to the rate at which it is used in protein synthesis without accompanying large changes in metabolite concentrations.

case, protein synthesis. As the consumption of lysine for protein synthesis is the main component in the metabolic demand for lysine, it determines the rate at which it needs to be synthesized from aspartate. Omitting the conversion of lysine into protein from the pathway means omitting the one step that explains the feedback inhibition of aspartokinase by lysine. This inhibition cannot be explained solely in terms of the components concerned, aspartokinase and lysine, but requires consideration of the whole system, including protein synthesis (Fig. 3b).

The case of phosphofructokinase is striking: despite having an activity that is modulated by several effectors, it has very little control over the glycolytic flux: all the attempts made by different research groups to increase glycolytic flux in diverse organisms by genetically increasing its activity have been unsuccessful (see, for example, Schaaff et al., 1989). This appears surprising if the phosphofructokinase reaction is considered in isolation, but it makes sense as a property of the whole system: as for the biosynthesis of lysine just discussed, the glycolytic flux is not determined by phosphofructokinase, but by the metabolic use that is made of the products of glycolysis. The regulatory properties of phosphofructokinase allow changes in flux to occur with minimal changes in metabolite concentration.

A final example of the need to analyse systems as systems, and not as mere collections of parts, is provided by studies of the effects of combining deletions of each of nine galactose-utilization genes with two dietary states, with and without galactose (Ideker et al., 2001). Comparison of the effects of 20 perturbations on cellular gene expression showed that the levels of 997 mRNAs varied significantly with one or more of these perturbations. The results were thus far from the naive expectation, still common, that deletion (or overexpression) of a gene should affect expression of just that gene alone. Integrating mRNA and protein expression responses with the global set of protein–protein and protein–DNA interactions allowed a network involving hundreds of genes to be deduced. This generalized pleiotropy, predicted many years ago by Kacser (1963) but long ignored, underlines that genes act in concert with one another and with the environment. The more complex the level at which one seeks to explain a living system, the greater the need to examine the network of interactions that lie behind the genome (Cornish-Bowden and Cárdenas, 2001b). The fact that a complex network of interactions connect genes to phenotypes emphasizes the idea that only through the understanding of the whole can we understand the function of the parts.

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