Beyond reductionism: metabolic circularity as a guiding vision for a real biology of systems*

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The definition of life has excited little interest among molecular biologists during the past half-century, and the enormous development in biology during that time has been largely based on an analytical approach in which all biological entities are studied in terms of their components, the process being extended to greater and greater detail without limit. The benefits of this reductionism are so obvious that they need no discussion, but there have been costs as well, and future advances, for example for creating artificial life or for taking biotechnology beyond the level of tinkering, will need more serious attention to be given to the question of what makes a living organism living. According to Robert Rosen's theory of (M, R)-systems (metabolism-replacement systems), the central idea missing from molecular biology is that of metabolic circularity, most evident from the obvious but commonly ignored fact that proteins are not given from outside but are products of metabolism, and thus metabolites. Among other consequences this implies that the usual distinction between proteome and metabolome is conceptually artificial—however useful it may be in practice—as the proteome is part of the metabolome.

Keywords
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1 Introduction

The determination of numerous genome sequences, together with great advances in analytical techniques, including mass spectrometry, protein arrays, biochips, data-processing,

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etc., has opened the way to tremendous advances in our knowledge of the functions of proteins in living organisms. Nonetheless, making these advances a reality will require not only technology but also a broad vision of biological organization. In the words of Woese [1] in a recent article, "Without an adequate technological advance the pathway of progress is blocked, and without an adequate guiding vision there is no pathway, there is no way ahead."

Systems biology may seem to offer this vision. The term was scarcely known before the 21st century, and appeared fewer than 20 times in all of the biological literature before 2000; by contrast, in 2006 it appears about 20 times every ten days. As systems biology is currently practiced, however, there seems to be no guiding vision beyond a desire to accumulate ever more data, and it is easy to be reminded of a complaint made a long time ago that "the only goal of science appears to be analytical, i.e. the splitting up of reality into ever smaller units" [2]. In reality, however, a theory of biological organization will not appear spontaneously from beneath a mountain of experimental data. To obtain such a theory it is necessary to adopt a path of research focused on developing it—to go from data mining to constructing theories. Technology has now advanced so far that the limiting factor is no longer the difficulty of obtaining data but the difficulty of thinking productively about the data already available.

Although Schrödinger [3] raised his famous question about what is life more than half a century ago, it has excited virtually no interest among biologists during the era of molecular biology, most of whom would agree with Jacob [4] that "Today we no longer study Life in our laboratories", or with a more recent expression of a similar idea: "Today, a molecular biologist has no need, so far as his work is concerned, for the word 'life' " [5]. Unfortunately, however, although the reductionist approach has brought biology a very long way it cannot provide all the answers that will ever be needed; and the time has come to reopen the question of what life is. Certainly, at this time it would be difficult to argue that we understand life any better than Schrödinger did 60 years ago.

Nonetheless, if we want to modify the living world for medical or biotechnological purposes, we need a better understanding of the essence of a living being. In addition, the desire to study living organisms as global systems, i.e. to apply the central idea of systems biology, implies an interest in the nature of life. In recent years interest in it has been reawakened by work in several different fields, including the search for life on other planets, studies of the origin of life on earth, and ambitions to create artificial life. All of these presuppose a definition of the essence of life: to search for life on another planet we need to be able to recognize it if we find it; to talk about the origin of life on earth we need an unambiguous distinction between life and non-life, and so on.

In the years since Schrödinger's work, however, relatively few scientists have attempted to arrive at a theory of life [6–12]. None of their efforts has achieved a very wide following except among specialists in artificial life, and even there one finds a different group of researchers interested in each approach, with rather little communication between groups. As a result, the different approaches tend to overlap, and often cover rather similar ground with different language, but with emphases on different points that the authors see as crucial in accounting for the appearance of life. The hypercycle theory of Eigen and Schuster [7], for example, is mainly concerned with explaining the origin and evolution of RNA replication, and with escaping from what Maynard Smith and Szathmáry [13] have subsequently referred to as Eigen's paradox: "no enzymes without a large genome, and no large genome without enzymes". It proposes a two-tier model in which several different replicators (corresponding to RNA molecules) are organized as a cycle, such that each replicator

promotes replication not only of itself but also of the following replicator in the cycle. However, Ratner *et al.* [12] argued that the hypercycle does not provide the best model, which should instead be based on what they call *sysers* or *systems of self-reproduction*, in which translation and transcription are made explicit and clearly distinguished from replication. Both models address important questions about the nature of life, but they are not the only questions; in particular, they make no mention of metabolism, i.e. they ignore the fact that the macromolecules of living systems do more chemistry than just make one another, and they ignore the fact that macromolecules are not indefinitely stable, but need to be maintained. Moreover, as Volkenstein [11] has noted, the intricate problems relating to the origins of the complex functions needed in the syser model are far from being solved; it involves too many components to be considered as a plausible starting point for life. In any case, both approaches fail to account for the origins of metabolism.

There have been other efforts to understand life at a more fundamental level. Of these, the theory of autopoiesis of Maturana and Varela [8] has attracted a great deal of attention in some areas of biology, especially in neurobiology; as for the (M, R)-systems of Rosen [9], his admirers [14, 15] have so far had little success in making his name well known among biologists in general. It would be fair to say that the influence of all this work on modern molecular biology has been negligible. Nonetheless, we believe that (M, R)-systems may offer the "guiding vision" that Woese [1] was asking for in the words quoted earlier. There are some points in common between autopoiesis and Rosen's approach [16], but autopoiesis puts the primary emphasis on the structural organization of organisms and the necessity to enclose them with membranes, whereas Rosen is more concerned with the logical organization in terms of formal mathematics. Both approaches emphasize the importance of organizational closure, but in slightly different ways, as we have discussed elsewhere [17]: in brief, an autopoietic system is a network of processes in which any given process produces components that are eventually transformed by the rest of the network into the components transformed by that process, and this constitutes closure in the sense of autopoietic systems; (M, R)-systems focus instead on how enzymes are replaced, a slightly different idea of closure. Neither of these approaches, incidentally, has much to say about reproduction or evolution. Kauffman's autocatalytic sets [10] also have some similarities with (M, R)-systems; for example, the simple (M, R)-system that we describe later in this paper is also an example of an autocatalytic set. However, his principal aims are different from Rosen's: rather than trying to define the essential characteristics of life he has been concerned to explain how self-organization and order can arise spontaneously from random connections between large sets of elements.

We recently tried to clarify Rosen's vision of life, especially his view of metabolic circularity, to provide examples of how this might work, and to define the limits of applicability of his ideas [17, 18]. However, our articles were necessarily highly abstract and mathematical (albeit less abstract and mathematical than most of Rosen's own writing), and here we attempt to cover some of the same ground in as down-to-earth a way as possible.

2 What is metabolism?

Metabolism is usually regarded as a network of chemical reactions catalyzed by enzymes that occur in a specific compartment defined by a membrane. The enzymes may be associated in complexes with other enzymes, and when that happens there is the possibility (but not the necessity) of a direct transfer of an intermediate from one active site to another,

i.e. *channeling*. The set of enzymes and other proteins constitutes the *proteome*, and the set of metabolites constitutes the *metabolome*, the proteins being the principal agents that allow the flow of matter and energy through the metabolic network.

A problem with this view of metabolism is that enzymes (and all other proteins) are not given from outside; they are themselves products of metabolism. They are continuously degraded and synthesized, and to achieve this the cell requires complex machineries involving numerous macromolecules (nucleic acids, proteins, etc.) and regulatory mechanisms. Although the existence of this degradation and resynthesis of proteins is very well known there is a tendency to forget it and to regard the proteome as more static than it is. Thus the separation between proteome and metabolome, natural-sounding though it may be, is rather artificial and misleading.

Despite the current emphasis on the accumulation of facts, the facts being accumulated do not necessarily include the things we need to know in order to integrate them all into a system of interacting enzymes. In particular, a great deal of genetic information is being obtained in the absence of any studies of the kinetic or regulatory properties of the enzymes coded by the genes. For the moment, therefore, the absence of useful kinetic information is forcing us to pay attention to the purely structural (stoichiometric) properties of metabolic networks, leading to the development of methods such as the analysis of elementary flux modes [19, 20]: these methods, powerful and useful though they are, would be needed primarily as complementary techniques if adequate kinetic and regulatory information were available.

3 Inadequacy of the machine analogy

There has always been a desire to understand living organisms in terms of machines and other artifacts of technology. This is certainly useful for understanding certain functions of organisms, in particular mechanical ones such as muscle action and blood flow. The function of the human heart, for example, can be understood in considerable detail in terms of a sum-of-the-parts model [21]. There is, however, a fundamental difference between machines and organisms that renders this analogy much less general and useful than it may appear at first sight. All machines, at what ever level one defines the word "machine", whether a simple tool like an ax, a more complex machine such as an airplane or a computer, or even a complete factory, require external agencies to construct them and to maintain them—determining when defective components need to be replaced or repaired, and carrying out the replacement or repair when necessary. Note that here the words "replace" and "repair" describe the same process, but emphasize different levels of a hierarchy: repairing a computer, for example, usually involves identifying and then replacing the defective component. In his writing Rosen always referred to "repair", inviting confusion with better known and probably more appropriate uses of this term, such as DNA repair and chaperone function; we prefer the term "replacement".

In an organism, however, replacement is an internal function, involving no help (before the advent of modern medicine, at least) from an external agency, and death can be considered as a loss of this capacity. To a considerable degree even the construction of an organism is an internal function: a bacterium makes itself, but no machine does that, and at our present level of understanding we cannot even conceive of how a machine of the future might construct itself and maintain itself. Sophisticated modern instruments often incorporate some internal testing to detect faulty components and alert their operators to

Table 1: Causes in metabolism. Aristotle's term $\alpha\iota\tau\iota\alpha$ [22] is usually translated as *cause* (or is sometimes just transliterated as *aitia*), but the word *make* conveys the meaning better, and for that reason is italicized in the questions, each of which is a different way of putting the question "what makes glucose 6-phosphate a metabolite?", with a different answer according to which cause is being considered.

Cause	Question	Answer
Material	What is it *made* from?	Glucose and ATP
Formal	What *makes* it a metabolite?	Its status as the product of an enzyme-catalyzed reaction
Efficient	What agent *makes* it?	The enzyme hexokinase
Final	What is it *made* for?	Use as an intermediate in the harnessing of the energy available from glucose

them, and even to an extremely limited degree, to replace them. But what they can do is almost infinitely less than what living organisms can do, as they need to monitor the state of *all* of their components and maintain them *all* of the time.

Rosen summarized the essential property of organisms that makes them different from machines in the phrase "organisms are closed to efficient causation". The reference here is to Aristotle's four categories of causation [22]: the efficient cause (the only cause in the modern Humean sense) answers the question of what agent made a thing the way it is; the others (listed in Table 1 in relation to a specific example in metabolism) are the material cause (what is it made of?), the formal cause (what properties make it what it is?) and the final cause (what is it made for? what is its purpose?). Modern biology treats final causes with suspicion, because living organisms are the product of evolution, not of design. Nonetheless, as Atkinson [23], for example, has argued, a generalized rejection of teleological explanations is unhelpful, because so many properties of organisms can be understood *as if* they had been designed for a purpose that forbidding reference to function puts an unnecessary constraint on the discussion of metabolic organization: the absence of a designer does not imply the absence of design. Meléndez Hevia [24] has subsequently analyzed several metabolic pathways to show that perfection of design extends even beyond Atkinson's conception, as indeed it extends beyond the design of pathways [25].

The formal cause, though important, played little part in Rosen's thinking, and we shall not refer further to it here. The interplay between material and efficient causes, however, is central to the understanding of (M, R)-systems. Notice that there is no suggestion that organisms are closed to *material* causation: organisms certainly need molecules available in the external milieu for their internal functions; what they do not need are external catalysts to oversee them.

4 Algebraic formulation of metabolism

In metabolism each reaction is catalyzed by an enzyme. For example, valyl-tRNA synthetase catalyzes the following transformation:

$$\text{ATP} + \text{L-valine} + \text{tRNA}^{\text{Val}} \rightarrow \text{AMP} + \text{pyrophosphate} + \text{L-valyl-tRNA}^{\text{Val}} \qquad (1)$$

To arrive at an algebraic formulation of metabolism each enzyme can be viewed in a general way as an operator, M, that transforms a set of molecules (input materials) into another one (output materials):

$$a_1 + a_2 + a_3 \xrightarrow{M} b_1 + b_2 + b_3 \tag{2}$$

The catalyst M acts formally as a *mapping*, because it transforms some variables (from the admissible set of input materials) into other variables belonging to the set of admissible output materials:

$$M((a_1, a_2, a_3)) = (b_1, b_2, b_3) \tag{3}$$

Rosen generalized this mathematical model of a single metabolic reaction into one that takes account of the entire network that constitutes metabolism. In other words, he interpreted the complete metabolism as a kind of generalized enzyme or operator (mapping), M_{met}, that transforms all of the input materials (all of the left-hand sides of all of the chemical equations) into all of the output materials. Moreover, enzymes are not totally specific [26], for example valyl-tRNA synthetase will accept L-threonine as an alternative to L-valine as substrate. So a_1, a_2 etc. must be understood as sets of admissible metabolites rather than as individual substances. Ultimately, therefore, we arrive at a formulation like

$$A \xrightarrow{M_{met}} B \tag{4}$$

as a summary of the whole of metabolism. As the enzymes represented here by M_{met} are being degraded (or damaged), they need to be continuously replaced in order for the living system to have more than a transient existence. There are, in fact two problems to be solved. The first is to achieve some permanence, but the second is to maintain an identity, that is to say to maintain an organization, even though many details may need to be continuously revised to take account of variations in the environment in which the organism has to live. How do living systems solve these problems? Rosen's view, illustrated in Figure 1, is that a circular organization is needed, and this will be analyzed in the next section.

5 Metabolic circularity

The considerations discussed in the previous section lead to the idea that metabolism is part of a *metabolism-replacement system*, or *metabolism-repair system* in Rosen's terminology, both con-•veniently represented by the same shorthand as (M, R)-*system*. Such a system is closed ("organisms are closed to efficient causation"), and the way in which closure is achieved is explained in terms of a diagram of the type shown in Figure 1. The essential point is that the enzymes that catalyze metabolism are themselves products of metabolism, and closure can be represented by the following equation

$$\beta(f) = \Phi \tag{5}$$

in which β is a property of the metabolites B that acts on the enzymes f. Understanding the nature of this function β is perhaps the most difficult idea in Rosen's analysis, one that he failed to make fully clear, and one that some critics have claimed to be impossible, as we discuss below. This equation may be written in the following form [17, 18]:

$$f(f) = f \tag{6}$$

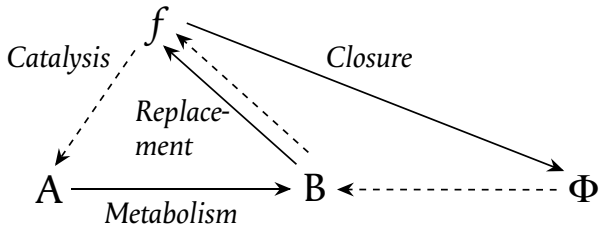

Figure 1: Metabolic circularity. The diagram illustrates the closed nature of metabolic systems, in which there are no external influences, and no final causes in Aristotle's sense. Material causes are represented by full arrows, efficient causes by broken arrows. The diagram is best understood in four stages: (i) the arrow A $\longrightarrow$ B represents a generalized *metabolism*: A stands for the left-hand sides of all of the reactions, and B for the right-hand sides. As B is made from A, A is the material cause of B. (ii) The arrow f - - $\rightarrow$ A represents *catalysis*, the effect of enzymes f on A to produce B; f is thus the efficient cause of B. (iii) As the enzymes are themselves the products of metabolism, the arrows Φ - - $\rightarrow$ B $\longrightarrow$ f represents *replacement* ("repair" in Rosen's terminology), the effect of the replacement system Φ on B to yield f. (iv) Finally, as the replacement system is itself subject to decay, and needs its own replacement system, *closure* is achieved by the arrows B - - $\rightarrow$ f $\longrightarrow$ Φ.

to convey the idea that in a sense metabolism is a mathematical function (or mapping) that acts on an instance of itself to produce another instance of itself. Nonetheless, the fact that metabolism is producing not itself but a new instance of itself may appear puzzling, and some may prefer the first formulation in which catalysis and closure are kept separate. In any case, a biologist will naturally ask why it is necessary or useful to have an abstract mathematical formalism at all. The answer is that until the problem is fully formalized it is not possible to subject any proposed solution to it to profound logical analysis, to move the discussion beyond mere hand-waving. Moreover, one requires a formal expression of Rosen's ideas before one can decide whether objections to them, such as those offered by Landauer and Bellman [27] and by Chu and Ho [28], have any merit.

Equation 6 is an application to metabolism of an equation derived by one of us [29] that is related to the concept of fixed-point combinators in the theory of computer languages [30], and it defines a remarkable property. Ordinary mathematical functions do not behave like this, and, in particular, operations on sets do not behave like this. Trying to understand metabolic circularity can thus be formalized as the search for functions that can be regarded as solutions of equation 6.

The major conceptual difficulty in circular organization is implicit in the last step: how does B, the product of metabolism, induce the system to maintain the primary replacement system Φ? There are, in fact two difficulties here: is it possible, even in a formal mathematical sense, for a generalized operation on sets to be inverted? Even if it is, how does the system "know" how it is organized? How does knowledge of B imply knowledge of Φ? The first of these difficulties was raised by Landauer and Bellman [27], who went as far as to claim that "unfortunately, the mathematics [of Rosen's analysis] is incorrect, and the assertions remain unproven (and some of them are simply false)". Fortunately this conclusion is incorrect in formal mathematics [18]. Although it is unusual for set operations to be invertible, it is not impossible. For example, for any set i that is a sub-set

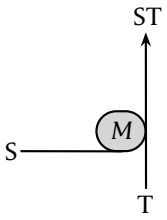

Figure 2: A naive view of metabolism. In this simple example the only metabolic function of the organism is to produce a single metabolite ST from precursors S and T available in its environment. This requires a catalyst, the molecule M, but the illustration makes no allowance for the fact that M will have an inevitable tendency to become degraded, and that the system can therefore have only a transient existence. It is in that sense that we call it naive, and a more realistic view is shown in Figure 3.

of the set $\{0, 1, 2, 3 \ldots 11\}$ the operation $j = i \times 7 \bmod 12$ is invertible, i.e. it is possible to deduce i from j; for example, the equation $\{3, 7, 9\} = i \times 7 \bmod 12$ has the unique solution $i = \{1, 3, 9\}$, and there is a unique solution regardless of the set on the left-hand side of the equation [18]. This argument is of course too abstract to carry much weight in a biological discussion; the essential point is that it disposes of the main claim of Landauer and Bellman [27]. Similar criticisms have been made more recently by Chu and Ho [28], but these are also groundless.

The second question is more difficult to answer in satisfactory biological terms, and for the moment needs to be left open. In more precise mathematical terms, it means that the system must be able to invert the evaluation map at B, with a unique Φ such that $\Phi(\mathrm{B}) = f$. However, we were able to define the limits within which Rosen's conclusions could be valid, i.e. that a unique Φ could exist [18]. The biological example of an (M, R)-system that we shall give in the next section goes only part of the way towards explaining how knowledge of the organization of a system can be coded within the system itself.

6 An example of an (M, R)-system

Figure 2 shows a naive attempt to illustrate the metabolism of an organism whose entire metabolic activity consists of producing a single molecule ST by the action of a catalyst M on two molecules S and T available from its environment. It fails to be a satisfactory model, however, because it treats the catalyst as given, but even if it happened to be available at the beginning it would have an inevitable tendency to become degraded, and therefore the process illustrated in the Figure could exist only transiently.

An improvement on this example is provided by the complete (M, R)-system shown in Figure 3a. The entire metabolic activity again consists of producing a single metabolite ST from molecules S and T, but now the model allows for the catalyst M to be replaced by the action of a second catalyst R on the product ST together with a third molecule U available from the environment. As R is likewise subject to decay it also needs to be replaced, and this is achieved by supposing that M can accept U as an alternative substrate that leads to production of R. Stoichiometric considerations suggest that M and R have the structures STU and SU respectively, and in Figure 3b these identifications are made explicitly; in addition, each catalytic process is represented as a cycle of three chemical

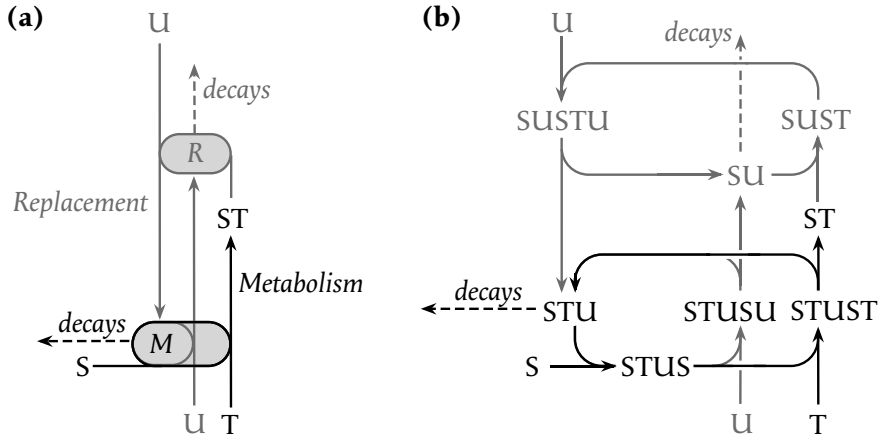

Figure 3: A metabolic example of an *(M, R)*-system. **(a)** The model of Figure 2 is extended to allow for decay of the catalyst M and the need for it to be replaced by action of a second catalyst R acting on a third external molecule U, R being itself replaced in a secondary activity of M acting on U as an alternative substrate to S. The replacement module is shown in grey. **(b)** The catalysts are represented by the structures STU and SU, and each catalytic process is represented as a cycle of three reactions, for example STU → STUS → STUST, so as to make the chemical nature of the catalysis explicit.

reactions. Thus although Figures 3a and 3b[1] represent the same model, Figure 3b does so in a way that makes its chemical nature explicit. The example is based on a slightly simpler one proposed by Morán *et al.* [31].

Despite the trivially minimal nature of the metabolism achieved by this system, with just one metabolite produced from two precursors, the system as a whole appears remarkably complicated, especially as shown in Figure 3b. The version in Figure 3a may appear a little simpler, but this just disguises the complication; it does not eliminate it. The reason for making the example so complicated is that the catalyst is not directly available from the environment and it has an unavoidable tendency to decay with time. So even if STU happened to exist initially it would not exist indefinitely unless replaced. However, as SU is just as liable to decay as STU, its existence implies the existence of another replacement process. In principle, each enzyme requires a replacement system, but as each replacement system is itself an enzyme it requires its own replacement system, and an infinite regress, or combinatorial explosion, seems inevitable. To escape from this, the example suggests that SU is produced from S and U in a secondary activity of the first catalyst STU. The result is a complete (M, R)-system, closed to efficient causation. Note that the need for one catalyst to have more than one function emerges automatically from the need to escape from infinite regress. This implies that the multifunctionality of proteins that is being increasingly observed [32, 33] is more than just an interesting fact about living systems; it is an absolute necessity for life.

Complicated though it is, Figure 3 only partly satisfies the need for an example of an (M, R)-system, because although it is indeed an (M, R)-system it does not have organiza-

[1]**IMPORTANT NOTE.** The published version of Figure 3b contained a serious error, with three intermediates named incorrectly, STUST as SUST, STUSU as SUSU, and SUSTU also as SUSU (with the result that the names SUST and SUSU occurred twice each).

tional invariance: it does not contain the information needed for the arrows B $- - \rightarrow f$ $\longrightarrow \Phi$ of Figure 1, and is thus not guaranteed to maintain its identity indefinitely. In effect, the missing information is the knowledge of which of the three metabolites STU, SU and ST are the enzymes, i.e. which intermediate in the network acts to catalyze each reaction. Although the illustration assumes that STU catalyzes two reactions and SU catalyzes the third, this is not inevitable: in principle, with three metabolites and three reactions one could conceive of 3^3 different assignments, even in this minimally small system, so there is not the unique solution that would be needed for organizational invariance. In a model of more reasonable size (though still very small compared with a real organism), such as the stoichiometric model of *E. coli* metabolism studied by Stelling *et al.* [19], which contained 89 metabolites and 110 reactions, the corresponding numbers become huge, 89^{110}, or more than 10^{214}, in this case. In the simple example of Figure 3 we [18] offered some arguments about how the total of 3^3 possibilities might be decreased, but it is clear that further study will be needed to understand how organizational invariance can be achieved in a living organism.

7 Conclusions

A criticism that may arise in relation to equation 6 as an expression of metabolic circularity is that it makes no allowance for reproduction and evolution: why $f(f) = f$ rather than, say, $f(f) = 2f$? The point here is that staying alive was the problem that needed to be solved first: the early living entities could not begin to reproduce or evolve until they had learned how to stay alive, maintaining organizational invariance in the face of changing conditions. Rosen's theory of (M, R)-systems does not in its present form solve all of the problems of how that is possible, or of what constitutes a living system, but it represents a major step. In particular, it addresses the right questions, questions that have been overlooked in nearly all studies of modern biology, when the essential nature of life has been set aside as having little interest or importance [4, 5]. The idea that the proteome is not a separate entity from the metabolome, but a part of it, is obvious once pointed out, but is easily ignored. Its importance is in recognizing that enzymes and indeed all proteins are not given from outside but are themselves products of metabolism, and hence metabolites, like any other. The ideas that we have tried to analyze are abstract, and it may be many years before they can be translated into practical applications, but such applications will be necessary before there can be significant progress towards creating artificial life; they will also be very useful if modifying existing organisms for biotechnological purposes is to move beyond tinkering, and useful also for defining criteria for recognizing whether candidates for living systems that may eventually be found elsewhere in the universe are truly living or not. A complete theory of life is unlikely to be based solely on (M, R)-systems. It will also need to incorporate ideas from autopoiesis [8] and autocatalytic sets [10], for example, as the important role of the membrane for defining the limits of an organism needs to be taken into account. Once the problems related to staying alive are fully understood, one can begin to deal with evolution, extending the understanding to incorporate the ideas of information storage and error correction that form the focus of other theories of life, such as hypercycles [7] and sysers [12].

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