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50th Anniversary Year Review

From *L'Homme Machine* to metabolic closure: Steps towards understanding life

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ABSTRACT

The nature of life has been a topic of interest from the earliest of times, and efforts to explain it in mechanistic terms date at least from the 18th century. However, the impressive development of molecular biology since the 1950s has tended to have the question put on one side while biologists explore mechanisms in greater and greater detail, with the result that studies of life as such have been confined to a rather small group of researchers who have ignored one another's work almost completely, often using quite different terminology to present very similar ideas. Central among these ideas is that of *closure*, which implies that all of the catalysts needed for an organism to stay alive must be produced by the organism itself, relying on nothing apart from food (and hence chemical energy) from outside. The theories that embody this idea to a greater or less degree are known by a variety of names, including (*M*,*R*) systems, autopoiesis, the chemoton, the hypercycle, symbiosis, autocatalytic sets, sysers and RAF sets. These are not all the same, but they are not completely different either, and in this review we examine their similarities and differences, with the aim of working towards the formulation of a unified theory of life.

Contents

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1. Introduction

Attempts to define the nature of the living state have been an important part of the scope of the Journal of Theoretical Biology since its first appearance 50 years ago. An important paper of Elsasser (1964) appeared in one of its first volumes; one of the leading theorists that we shall discuss, Robert Rosen, was a member of the Editorial Board for many years and published some of his work in the journal (for example, Rosen, 1975); another, Stuart Kauffman, was an Editor-in-Chief of the journal and first presented his theory of autocatalytic sets (Kauffman, 1986) in it. In recent years numerous papers related to the definition of life have appeared, not only written by ourselves (Letelier et al., 2003, 2006; Cornish-Bowden and Cárdenas, 2008; Cárdenas et al., 2010), but also by several others (Hordijk and Steel, 2004; Wolkenhauer and Hofmeyr, 2007; Meléndez-Hevia et al., 2008; Montero et al., 2008; Mossio et al., 2009; Manapat et al., 2010). However, the various threads in the development of understanding of this fundamental question have remained obstinately separate, with little or no interaction between the leading players.[1] In this review we try to bring them together under the common thread of metabolic closure, i.e. the fact that nearly all of the molecules that define the metabolic network of a cell, whether metabolites or enzymes, are produced by processes which are themselves mediated by other molecules produced by the very same metabolic network.

2. Mechanical and inorganic precursors

Mechanistic theories to explain the properties of biological organization in physico-chemical terms can be traced to the time of the *Encyclopédistes*, especially to the combative physician Julien Jean Offray de la Mettrie (1709–1751), whose book L'Homme Machine (de la Mettrie, 1748) was a mechanistic manifesto, with repercussions that forced him to escape France to obtain Frederick the Great's protection in Prussia, his views being seen as being in opposition to the religion-inspired vitalism of his time. His book is strongly argued, and illustrates the brief moment when clock automata were regarded as useful metaphors for living organization (see Langton, 1987). Such metaphors were, of course, crude by modern standards, but they reflected a very modern idea, that the properties of a living system arise from a *system* of interlocking components that act *locally* to produce a global behaviour without the intervention of a centralized controlling entity. Gears and shafts were the high technology of the 1760s, and about 130 years had to pass before the same idea resurfaced, by then based on molecules and chemistry.

By 1900, after the overthrow of vitalism a few years earlier (Buchner, 1897), it was becoming clear that living organization is based on cellular chemistry, and that this depends on metabolism, considered as a network of enzyme-catalysed chemical reactions, constrained by thermodynamics, that transform small molecules. Thus the idea of mechanical automata was changed by Leduc (1912) into his ideas of osmotic growth (*jardins osmotiques*, or osmotic forests). Instead of dynamic gears, he correctly saw living systems in terms of metabolism, and chose a simple system of coupled inorganic reactions as the metaphor for them. He introduced the term *Synthetic Biology*, which 100 years later has become an active area of research, though in its modern form it is mainly concerned with genetic engineering, or, more generally, biology engineering: see for example Endy (2005) or Brenner et al. (2008). Like the *Encyclopédistes* before him, he lacked a theory of biological organization, but rather he thought that if an artificial system was capable of producing the shapes (morphology) of living systems, then it was isomorphic with a living system. Leduc was widely misunderstood in his time because he was thought to be reviving vitalism, whereas in reality he was interested in the origin of life. We know now that the beautiful osmotic forests are not models of living systems *per se*, and that the reasoning behind his work was incomplete,[2] as it only focussed on a particular output set of coupled reactions (the overall morphology) and not on the properties of that network.[3] But Leduc was not alone in making the mistake of putting the emphasis on morphology, and many current computational models, such as the L-systems based on the work of Lindenmayer (1968a,b), suffer the same problem in focussing on form rather than on dynamics.

Production of osmotic forests requires simple chemicals and no special equipment, and Leduc's experiments are easily repeated today (Fig. 1).[4] They will always be associated with his name, but other scientists also studied them; in particular, Alfonso Herrera, a Mexican physiologist, systematically expanded the range of inorganic reactions that could produce lifelike structures. His science of "plasmogeny" has not survived, but he and Leduc can be seen as pioneers of current efforts in protocell research (see Negrón-Mendoza, 1995), and synthetic biology.

3. Nicolas Rashevsky and *relational biology*

Any account of the development of theoretical ideas in biology must refer to Rashevsky (1899–1972), the Ukrainian-born physicist who created an important group at the University of Chicago devoted to theoretical biology. He arrived in the USA in 1925, and worked initially at the Westinghouse Company. In 1935 he moved to the University of Chicago, where he remained until his first retirement in 1964. There he pioneered a quantitative and model-based approach to biological problems. His wide-ranging research included problems as diverse as cell division, neural conduction, population biology, muscle contraction, diffusion in cytoplasm, mathematical models of society and later *relational biology*. His huge activity went far beyond his personal research: he created the first journal devoted to theoretical biology, the Bulletin of

[1] Referring to Maturana and Varela, Gánti, and Kauffman, but Rosen can certainly be added, Luisi (2003) wrote "The three groups of authors … do not seem to be very well informed about each other's work".

[2] For a more enthusiastic assessment of Leduc's contribution to the theory of life, see Zeleny et al. (1987).

[3] The osmotic forest may still be useful, however, as an illustration of how complex shapes can appear spontaneously.

[4] Examples of the results that experienced chemists and professional photographers can obtain may be found in an article by Eastes et al. (2006), or at http://www.stephanequerbes.com/.

Fig. 1. Osmotic forest. This was created by seeding a solution of sodium silicate with crystals of copper sulphate and of ferrous sulphate, almost at first attempt, by Ricardo Rojas, a first-year undergraduate student at the University of Chile with no previous experience of the type of chemistry.

Mathematical Biophysics, founded in 1938, and he organized the first doctoral programme in mathematical biology, with around 14 doctorates awarded between 1949 and 1963 (Cull, 2007). His long period at Chicago was turbulent, with troubles both political[5] and scientific. Almost nothing survives of the large output of his group, and his work did not create a school of thought, being a curious mixture of mathematically detailed studies of simplified models that had almost no relation to experimental reality. His theory of nerve impulse propagation (Rashevsky, 1931), for example, has been totally replaced by the nerve axon model of Hodgkin and Huxley (1952), not only much simpler but also supported by abundant experimental evidence.

Rashevsky's contribution, together perhaps with most of his research, could be dismissed, but he made an important contribution that is slowly being recognized. By 1950 he was apparently having his own doubts about his earlier approach, and in 1954 he opened a new intellectual front devoted to the first principles of biological systems (Rashevsky, 1954), and in a series of papers spanning 10 years he introduced the notion of *topological analysis* of living system. By this he meant, as a metaphor, the use of analytical tools not dependent on measurements but on relations, and he named this approach *relational biology*. According to his own estimate, all of his work done before 1950 was a semi-quantitative approach focussing on the details of living systems, whereas what was needed was a new approach centred on the *organization* of living systems. Even today, theories vary considerably in the emphasis they place on the details and on the general requirements for organization, and we shall return to this in the Discussion. Rashevsky saw organization not as a property of matter, but as a systemic property of the system created by living matter. He did not make very much progress in this direction, but he laid the first stone, and one of his students (Robert Rosen, another atypical personality) was to advance much further along the path of relational biology, in developing, virtually alone, his theory of (*M*,*R*) systems (Section 8.2), but he had to face many of the same criticisms as Rashevsky.

4. Cybernetics and living organization

During the 1950s and 1960s *cybernetics*, originating with Wiener (1948), created excitement in many academic centres, as it seemed to promise a path to understanding brain function. An example of its impact was the creation of the Biological Computing Laboratory (BCL) at Urbana-Champaign (which may have absorbed much of the funding released when Rashevsky's Committee on Theoretical Biology was dissolved). It also had a powerful influence on ideas of self-organization, and the first scientific conference organized by the newly created Biological Computing Laboratory was the *Symposium on the Principles of Self-organization*, in June 1961. Not surprisingly, therefore, the first article describing *autopoiesis* appeared as a BCL internal report (Maturana, 1970), as we discuss in Section 8.3, and this history explains why the language of autopoiesis (*system*, *machine*, *organization*, *structure*, *process*) evokes its cybernetic origin.

Cybernetics later suffered a setback, virtually disappearing from US and European laboratories, but its rebirth as the Second Wave of Cybernetics retains the flavour of the original cybernetics literature. So, although the heralded revolution never happened, we find, rather surprisingly, that a side effect was the creation of a theory of biological organization: "cybernetics is the study of systems and processes that interact with themselves and produce themselves from themselves" (L. Kauffman, not formally published, but widely circulated).

The development of the theory of the chemoton (Section 8.4) has also been influenced by cybernetics. Gánti (1971) did not mention cybernetics in the first edition of his book, though he did emphasize the stability of the chemical cycle, but he referred explicitly to cybernetics in later editions from 1978 onwards,[6] including the English version (Gánti, 2003).

5. Molecular biology

Molecular biology can be considered to date from the isolation of DNA by Miescher (1871), and, more important, his contention that inheritance is a matter of chemistry (see Fruton, 1999). However, its early development was just as slow as that of theories of life, and the demonstration that DNA was the genetic material (Avery et al., 1944) was treated with scepticism until Hershey and Chase (1952) showed that when a bacterial virus infects a bacterium only the DNA enters the cell. Its explosive growth began, of course, with the recognition of the double-helix structure of DNA (Watson and Crick, 1953), and its subsequent history is so well known that it hardly requires a description here. Its importance for theories of life is that it has been so successful that it has relegated to the sidelines any idea that life may be more than a mechanical process in which DNA replication *is* life. So, although the number of molecular biologists increases every year, the number of biologists interested in closure and the idea that a living organism is more than a machine has been very small, in part because of a mistaken perception that denying that an organism is a machine is an appeal to vitalism. These few groups have worked in almost complete isolation, not only from molecular biology, but also from one another.

Fig. 2 shows an approximate time line for the two parallel histories. The book What is Life? (Section 6), which Schrödinger (1944) based on his public lectures in Dublin, did much to interest leading physicists in biological problems, and hence to stimulate the development of molecular biology, with its emphasis on individual molecules rather than on relational biology.

The two decades that began with a one-page article in Nature (Watson and Crick, 1953) on the three-dimensional structure of a nucleic acid were unique. The new field of molecular biology was

[5] Despite his history as an officer in the White Russian army, he was accused of communist sympathies when he refused to sign a loyalty oath.

[6] We are grateful to Dr. E. Szathmáry for informing us of this.

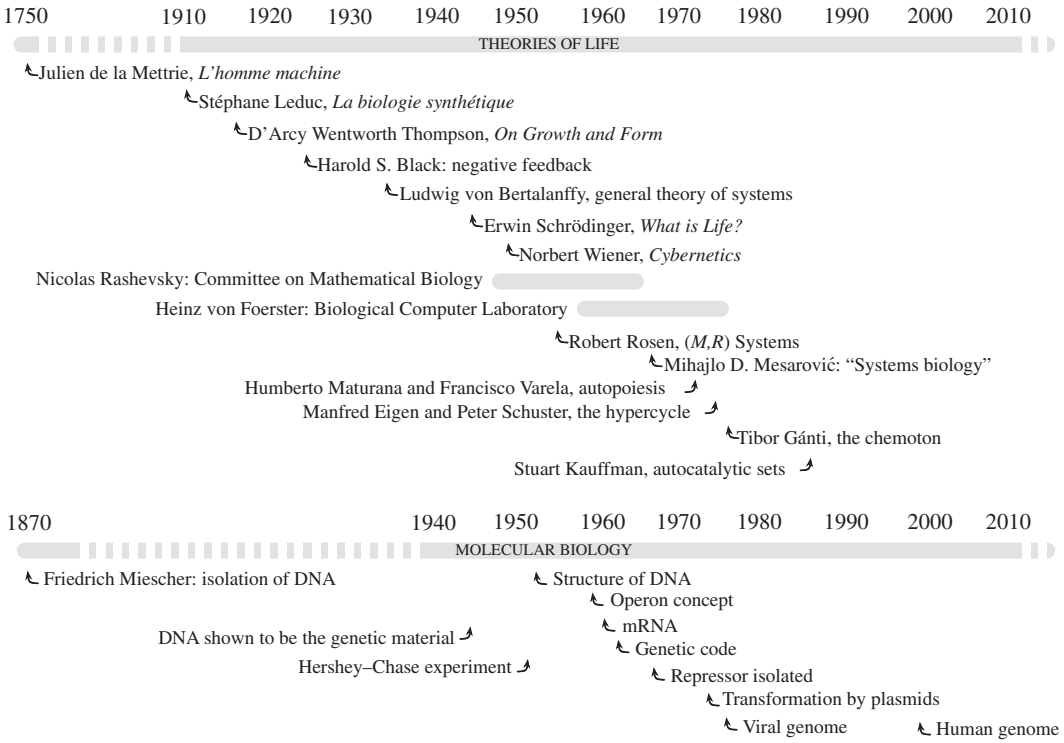

Fig. 2. Comparison between the development of theories of life with the growth of molecular biology.

created overnight (even if the phrase existed already) and became the dominant force in biological thinking as well as in biomedical applied research. The avalanche that had begun with a single page, and now enables us to sequence a bacterial genome in days, has been revolutionary. Progressively molecular biology (or more specifically biochemistry) has evolved, and today attention is being paid to organization, for example as "supercomplexes" (Prunetti et al., 2010).

An unwanted, and to us unwelcome, side effect of this revolution has been the almost universal adoption by most biologists of the seemingly powerful computer metaphor. In less than a decade, the operation of a metabolic network was interpreted in terms of *code*, *program*, *on/off switches* and *information*. The experimental facts behind these words were so compelling that no one cared to dissent: in its most simple-minded form the molecular-biology vision of metabolism resembles Mettrie's, with metal gears replaced by complex macromolecules, proteins and nucleic acids, working together by complex interlocking mechanisms conceptually similar to the rotation of shafts and movements of levers. During these years no one could challenge the computer metaphor, as one striking result after another appeared. This has tended to foster the idea that life is nothing more than the dynamics of nucleic acids.

In all this mechanistic view of life, one important insight of Mettrie was lost. He wrote that "the human body is a machine which winds its own springs. It is the living image of perpetual movement." Despite his obvious ignorance of thermodynamics, one can see here (perhaps with an element of wishful thinking) a suggestion of *closure*, an essential concept for self-organization (Section 8), and one that is entirely absent from the molecular-biology view of life, which neglects the networked nature of metabolism, relegates to a secondary position the role of proteins in maintaining the network, and exiles to the fringes of discourse theoretical approaches that are not based on the metaphor of computers and gears. It is not our intention, of course, to disparage the huge advances in knowledge and understanding that molecular biology has brought, but only to point out that it does not explain everything.

6. Erwin Schrödinger: What is Life?

We have mentioned What is Life? (Schrödinger, 1944) in the previous section, but its importance is such that we need to briefly describe the three principal ideas that it puts forward:

1. living organisms "feed on negative entropy",
2. a *codescript* is needed to encode information for transmission to progeny, and
3. biology is more general than physics, possibly needing physical laws that are not needed for physics itself.

From the perspective of 2011 the first of these seems an unnecessarily poetic way of asserting that organisms are subservient to the laws of thermodynamics. Nowadays everyone accepts that that is true, and even obvious, but, despite the contrary view of such distinguished commentators as Pauling (1987) and Perutz (1987), it was still worth saying to the audience for Schrödinger's lectures in the Dublin of 1944, for whom it may well not have been obvious. The idea of a codescript is now so thoroughly understood in terms of DNA that there seems little point in resurrecting Schrödinger's name for it.

Schrödinger's third suggestion, however, that biology is more general than physics, has been largely ignored, and only Elsasser (1964) and Rosen (1991) seem to have taken it seriously, Elsasser's article being severely criticized by Monod (1971). We shall not explore this question in this review, commenting only that until now no one has either provided examples of laws of biology that is not needed for physics, or shown that Schrödinger was wrong. If his suggestion had come from a biologist it would certainly have been ridiculed and forgotten, but coming as it did

from one of the foremost physicists of his time it could not be dismissed so readily. It has been greeted with embarrassment and scorn by those scientists who are even aware of it[7]: everyone agrees, of course, that obedience to the laws of physics is *necessary* for biology; the possibility for disagreement can only be over whether the laws known at present are *sufficient*. In view of the years that have passed one might expect to see some evidence by now if Schrödinger were right, so it does not seem likely that he was, but it will be premature to conclude that there are no fundamental laws still to be discovered until physics is complete: when the editors of Science (2005) compiled a list of the 125 "most compelling puzzles and questions facing scientists today"[8] the question "can the laws of physics be unified?" was the fifth on the list (and the second relating to physics).

7. Systems biology

Systems biology is commonly regarded as the child of the 21st century, and of the human genome project. However, the term "systems biology" is much older than is usually realized, being first used (unless a still earlier occurrence comes to light) by Mesarović (1968). It only occurred in a handful of publications before 2000, but in thousands since then.[9] The *ideas* of systems biology, however, are much older again, and can be traced at least to the *general system theory* of Bertalanffy, developed in the 1930s, but summarized in English in von Bertalanffy (1969), as well as to Rashevsky's relational biology and cybernetics, as already mentioned. In all of these the central idea is that complex systems can only be understood in terms of the interactions between their components, and for these one does not need new laws either of physics or of chemistry. In the kinetic understanding of metabolism, a related revolution was brought about by the realization by Kacser and Burns (1973), Heinrich and Rapoport (1974), and Savageau (1976) that analysis of multi-enzyme systems needed to go beyond the methods of analysing the kinetics of isolated enzymes. In particular, which enzyme, if any, controls the production of any metabolite is a property of the whole system, and must not be confused with the fact that some enzymes are essential for that production: an enzyme may be essential but that does not mean that in normal physiological conditions it controls the pathway. This revolution has been very important, but we shall not consider it further because it does not directly relate to life and metabolic closure, the principal focus of this review. Unfortunately much of the current enthusiasm for systems biology has led to the adoption of some of the terminology of systemic thinking while leaving its spirit largely ignored: systemic thinking means more than just accumulating huge amounts of data; the accent must be put on the organization more than on the details. In the Discussion we shall point out that the current theories of life reflect a spectrum from ones that concentrate mainly on details to ones that ignore details altogether.

8. Metabolic closure as the basis of living organization

The idea that life depends on the organization of the thousands of biochemical reactions that constitute metabolism was obvious, and accordingly little mentioned. The essential but often overlooked point is that enzymes, and all other proteins, are themselves products of metabolism, and thus metabolites.[10] The organization of metabolism is thus *circular*, a point that can be read into Mettrie's description of a "machine which winds its own springs", i.e. a machine that makes itself, and which Rosen expressed in the statement that "an organism is closed to efficient causation." Similar ideas are expressed in a variety of ways in the chemoton, autopoiesis, autocatalytic sets, hypersets and RAF sets. An 18th century metaphor has thus become the central concept for understanding biological organization.[11]

The principle that self-production is a fundamental component in theories of living systems has been a key element in most of the several models published since the Journal of Theoretical Biology first appeared in 1961. Two lineages can be distinguished within the models that have occupied the centre stage:

1. One lineage, represented by hypercycles, the chemoton, and sysers, emphasized the design of metabolic networks. The idea behind this approach was (and still is) that metabolic closure can be constructed by an appropriate choice of reactions and molecules, which will ensure that the system is self-maintained and robust enough to avoid being swamped by unwanted side reactions that clog the system with tar. This approach is akin to the period between 1800 and 1860 when steam engines were constructed empirically without application of the theoretical principles that were still in their infancy.
2. The other lineage, represented by (M,R) systems, autopoiesis, autocatalytic sets, autocatalysis in metabolic cycles, and RAF sets, was more concerned with understanding the fundamental principles of metabolism. Here the emphasis is not the production (even on paper) of an actual metabolic network, but a search for general principles. This approach resembles that of Carnot (1824) who proved, even before the first law of thermodynamics had been formulated, that the efficiency of a steam engine depends only on the temperature difference between the hot and cold heat reservoirs, a result that paved the way to fundamental discoveries like the definitions of temperature and entropy. Although this group of theories of life share the idea that closure is fundamental, they address this intellectual problem from various angles, all of them relevant.

We have introduced this dichotomy between design and principles because it seems that both approaches were natural responses to the question of how self-organized networks of creation, destruction and modification of molecules appeared. As we do not have a coherent and generally accepted theory of the stability (robustness) and origin of metabolic networks, we are experiencing a period similar to the first part of the 19th century, when incipient technologies were used and investigated independently of any general principles. We feel that as thermodynamics was the first systemic science it is helpful to consider its history when evaluating theories about living systems.

8.1. Infinite regress and closure

Before examining the various theories we first need to introduce the idea of *infinite regress*: any self-organized system needs specific catalysts, each of which requires other catalysts to

[7] Nonetheless, even today one can find it quoted by a prominent mathematician as a reasonable possibility, albeit with the qualification that "the 'other laws of physics' have not materialized" (Gromov, 2011).

[8] Schrödinger's question "What is Life?" is not among the 125, presumably because the editors of Science considered it either uninteresting or already solved.

[9] A search for "systems biology" (including quotation marks) at PubMed yields a total of 8741 publications up to December 2010.

[10] Not only are enzymes metabolites, but many metabolites can be regarded as enzymes: they are clearly catalysts, as they are regenerated by the metabolic cycles in which they participate, and if an "enzyme" is defined as being any biological catalyst, they are also enzymes (Cornish-Bowden and Cárdenas, 2007).

[11] The term *circular organization* is used in a weaker sense in the cybernetic literature, for example by Tsokolov (2010), referring only to the presence of feedback signalling loops, but with no implication of circular conversion of materials.

maintain it in the face of degradation, dilution and so on, each of which needs other catalysts, and so on, with no obvious way to prevent the system becoming infinitely complex. The various types of *closure* that we shall discuss can be regarded as attempts to solve this problem. As an illustration we can briefly refer to protein degradation, a topic that at first sight seems unrelated. For a long time this seemed to imply an infinite regress: as the different proteins have different rate constants for degradation, suggesting that each requires its specific degradation enzyme, and as these would also be proteins they would need their own specific enzymes, and so on. In the case of specific degradation of proteins the problem of infinite regress was resolved with the discovery of the ubiquitin system (Ciechanover et al., 1979). In the case of protein synthesis as it functions in present-day organisms the corresponding problem did not arise, as it was known that ribosomes synthesize all proteins, including their own. However, nothing as complicated as ribosomes and proteasomes can have been available to the first organisms, so they do not solve the fundamental problem of understanding life.

We shall now list the various threads in the development of ideas about closure, in the order in which the first publications appeared, and will try to weave them into a common fabric at the end.

8.2. (M,R) systems

The theory of (*M*,*R*) systems was developed almost entirely by Robert Rosen, in a long series of papers from 1958 onwards (Rosen, 1958a,b, 1959, 1966, 1971, 1973, 1975), and summarized in his book Life Itself (Rosen, 1991). To fully grasp the context of his effort we must consider the intellectual environment of the Committee for Theoretical Biology at Chicago between 1955 and 1960. During the previous 15 years Rashevsky's aim had been to explain biological phenomena one at a time and his approach was not well adapted to a search for general biological principles. The turning point was his introduction of relational biology (Rashevsky, 1954), as described in Section 3, and his subsequent papers suggest the influence that he and Rosen had on each other. However, and this is very important to emphasize, all the focus on relational biology was an activity confined to these two members of the Committee for Theoretical Biology: no one else seemed to have understood that it could lead to a theory of metabolic networks. These crucial years (when Rosen was writing his doctoral thesis) shaped the way in which the theory of (*M*,*R*) systems was presented to the community, and explain its almost non-existent reception.

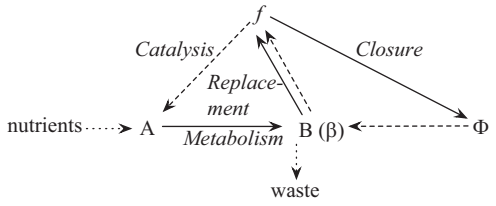

Fig. 3. Rosen's representation of closure. The continuous arrows represent *material causation* (transformation of matter by chemical reactions) and the broken arrows represent *efficient causation* (catalysis). Rosen referred to *repair* instead of *replacement* and *replication* instead of *closure*, but these terms are highly misleading for anyone familiar with the way these terms are used in modern biology. (For changes in terminology see Letelier et al., 2006). A represents the set of metabolites that are converted by metabolism into a second set B, catalysed by a set of enzymes *f* that are themselves products of metabolism. The catalysts needed for the replacement process is provided by a set of catalysts Φ that are produced from *f*. Closure is achieved by supposing that the efficient cause of Φ is a property of B (not B itself), represented as β. As the diagram is closed with respect to efficient causation (though not with respect to material causation) there is no external efficient cause, and no *final cause*. The dotted arrows from nutrients to A and from B to waste were not part of the diagram as drawn by Rosen, but are added here to emphasize that the system is an open system in the thermodynamic sense, and is not, therefore, closed to material causation.

The name (*M*,*R*) system stands for *metabolism-repair system*, in which *metabolism* has its usual meaning, but *repair* has no relationship with more familiar uses of the same term in modern biology, such as DNA repair or the action of chaperones; likewise Rosen's *replication* has no relationship with DNA replication. Quite apart from the obscure[12] terminology, his publications are very difficult for readers not versed in modern mathematics, in particular the theory of categories, and we have tried to make the theory more widely accessible (Letelier et al., 2006; Cornish-Bowden, 2006; Cornish-Bowden et al., 2007): as part of this aim we have introduced the term *replacement* instead of Rosen's *repair*, to recognize that the essential property of an (*M*,*R*) system is the capacity for continuous replacement of any catalysts that are lost by chemical degradation or the dilution that results from growth of the system; and we have called it *closure* rather than *replication*, to avoid any confusion with DNA replication. The central idea is that *organisms are closed to efficient causation*: this means that all of the catalysts required for organizing metabolism are products of the metabolism itself; no external catalytic activity is needed for maintaining the system. Catalysts in modern organisms are, of course, enzymes, but simpler catalysts must have existed at the origin of life; all of these constitute *efficient causes* in the terminology that Rosen adopted from Aristotle. Notice that there is no conflict with the thermodynamic necessity for organisms to be open systems, because this refers to *material causation*, or the flow of matter through an organism as the source of the chemical energy needed to maintain it in a state far from equilibrium (Cornish-Bowden and Cárdenas, 2007; Cárdenas et al., 2010). The production of efficient causes by the organism itself means that no appeal to a *final cause* is needed.

The concept of *hierarchy* has been very useful for analysing complex interactions in biology. For example, Westerhoff et al. (1990) showed that the previously intractable problem of applying metabolic control analysis to gene expression could be solved in terms of a hierarchy in which DNA causes mRNA, which causes proteins, which cause metabolites. However, a consequence of closure to efficient cause is that it eliminates the whole idea of hierarchy from theoretical biology. If all components in a living system, whether enzymes, nucleic acids or conventional metabolites, are products of the system, then there is no hierarchy. This does not of course deny the practical usefulness of applying hierarchical ideas to parts of systems, but it does imply that the hierarchy disappears when the whole system is considered. Nonetheless, hierarchical ideas are often introduced into biological discussions without a clear definition, and without a clear perception of the consequences of closure (Jagers op Akkerhuis, 2008).

8.3. Autopoiesis

During the 1960s, and still today in 2011, the principal metaphor for understanding the brain was the assumption that the nervous system is an information-processing device that decodes its sensory input, classifies it and then, according to the nature of the detected object, chooses a correct motor action. This positivist viewpoint still dominates conceptual thinking in the field of neuroscience, and it seemed a natural way of thinking, at least initially. One interpretation based on this computer metaphor was that every percept was coded (represented) by a specific

[12] We are almost tempted to call it obscurantist, but it does seem as if Rosen wanted his work to be understood.

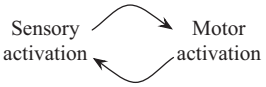

Fig. 4. Maturana's view of the nervous system. The decoding, classification and representation of "reality" is not a concept for Maturana's biology of cognition. Brain function is not about decoding an external reality but rather about producing coherent behaviors according to the ever-changing circumstances confronting the organism. Biology of cognition is a constructivist theory. The nervous system is always immersed in a never-ending senso-motor loop where sensory input defines motor output and *vice versa* (see Maturana and Varela, 1980).

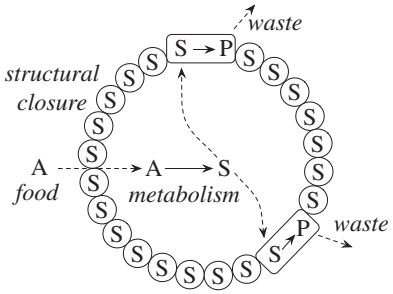

Fig. 5. Autopoiesis. Continuous arrows represent chemical reactions; broken arrows represent physical movement. The diagram is based on Fig. 8.3 of Luisi (2006). Although the idea of a network of processes is central to Maturana's vision of autopoiesis it is not clearly apparent in this representation.

neuron tuned to it (i.e. a grandmother cell that only fires when it sees its own grandmother).

However, Humberto Maturana, already well known in 1963 as an author of a seminal paper in neurophysiology concerned with visual perception (Lettvin et al., 1959), challenged this representationist viewpoint on many grounds, one of them the combinatorial explosion that it implies: not only grandmother neurons would be needed, but also neurons that detect not the percept *per se* (the grandmother) but the perception of the percept. We are thus at the beginning of an infinite chain of causes very similar to the infinite regress that Rosen set out to overcome (Section 8.2).

Many consequences followed from Maturana's fortuitous meeting in 1963 with Heinz von Foerster, the director of the Biological Computer Laboratory, including the attempt to "cybernetice" the Chilean economy (Medina, 2006). But from a scientific point of view, this interaction created a curious origin for a theory of metabolism. Maturana spent a sabbatical year in 1968–1969 at the Biological Computer Laboratory (Section 4), where, immersed in the daily discussions about systems, processes and the possibility of creating artificial intelligence, he wrote a Technical Report (Maturana, 1970) in which he stated that attempts at understanding the brain as a computer were fundamentally flawed, because the nervous system does not look out but in.

In effect he proposed a new metaphor: instead of assuming that the nervous system is a device that decodes reality, he assumed that it is a system whose main property is to produce movements coherent with the current situation of the organism. Instead of focussing on perception, and the perfect decoding and internal representation of this perception, he assumed that the nervous system is always in a particular state of senso-motor coordination. Thus the basic operation of the nervous system is an endless loop, as shown in Fig. 4. At every moment the total sensory input (vision, audition, touch, etc.), together with the internal state of the non-sensory parts of the nervous system, is used not to compute reality (a concept that has no meaning in his theory), but to define the transition to the next senso-motor state (or state of senso-motor coordination). The aim of neurophysiology is therefore not to understand how the brain decodes reality, but how it always manages to produce a senso-motor state that is compatible with the life-style of the organism. Under this viewpoint, which Maturana called the *biology of cognition*, the essential problem is to understand how the stream of senso-motor states is defined, taking account of both epigenetic and sensory aspects. As early as 1969 he posited that circular causation, which he called *closure*, was the core concept needed for understanding every aspect of living organization. A full account can be found in the first book in English dealing with autopoiesis, aptly titled Autopoiesis and Cognition (Maturana and Varela, 1980). In Maturana's approach the problem of infinite regress is not solved; rather it is dissolved, as it is no longer a valid question. In a footnote in the Technical Report he argued that the senso-motor loop was similar to the activity of metabolic networks in which every component participates, directly or indirectly, in its own production. With Varela he expanded this footnote into a small book in Spanish, De Máquinas y Seres Vivos[13] (Maturana and Varela, 1973) and with Varela and Uribe into a paper (Varela et al., 1974), where they introduced in a definitive way the notion of autopoietic systems as the central aspect of living organization.

An autopoietic machine is organized as a network of processes of production, transformation and destruction of components that

1. continuously regenerate and realize the network of processes (relations) that produced them through their interactions and transformations; and
2. constitute it (the machine) as a concrete unity in space in which they (the components) exist by specifying the topological domain of its realization as such a network.

The space defined by an autopoietic system is self-contained and cannot be described by using dimensions that define another space. When we refer to our interactions with a concrete autopoietic system, however, we project this system onto the space of our manipulations and make a description of this projection. As this definition shows, autopoietic systems are encapsulated systems, as illustrated in Fig. 5, where a network of processes produces components that produce the same network of processes, a definition that is heavily dependent on the language of cybernetics.

Autopoiesis became very popular, especially after the book De Máquinas y Seres Vivos (Maturana and Varela, 1973) was combined with Maturana's original Technical Report and rewritten in English as Autopoiesis and Cognition (Maturana and Varela, 1980), but, surprisingly, mainly among non-biologists, as illustrated by an extensive literature discussing such questions as whether legal systems (Michailakis, 1995), music (Vieira de Carvalho, 1999) and waste management (Entwistle, 1999) are autopoietic. Interest from experimental biologists and chemists has been minimal (not only in autopoiesis but in all the theories of life that we discuss), with attempts to implement it in an experimental system essentially limited to the model of Fig. 5 studied by Zepik et al. (2001). Readers will have their own interpretations of this lack of interest, but one possibility is that the field has been too fragmented to be well understood by experimentalists. There are certainly contributions to the field that experimentalists can make, and a major part of our aim in this review has been to draw their attention to it.

[13] This title ("About Machines and Living Beings") has, of course, overtones from Mettrie's L'Homme Machine to Wiener's Cybernetics: or Control and Communication in the Animal and the Machine.

(*M*,*R*) systems (Section 8.2) and autopoiesis have very different origins and histories, and have been mainly studied by very different groups. For a long time they developed entirely independently of one another and their essential similarities remained unrecognized. However, it is now clear that they incorporate many of the same ideas – expressed quite differently – and that autopoiesis can be regarded as a subset of (*M*,*R*) systems (Letelier et al., 2003).

8.4. The chemoton

The *chemoton* is a model of an organism proposed by Gánti (1971, 1975) and thoroughly discussed in The Principles of Life (Gánti, 2003), a book in English based on books and papers published originally in Hungarian, and supplemented with many valuable notes by Szathmáry and Griesemer, together with additional chapters by the same authors (Griesemer, 2003; Szathmáry, 2003). Chapter 3 of Gánti (2003) is a translation of most of the 6th edition (1987) of Gánti (1971). The essential structure of the chemoton is illustrated in Fig. 6. It consists of a *metabolic* cycle A, an *information* cycle V and a *structural* cycle T. The driving force is provided by conversion of food molecules X_A, assumed to be available from the environment, into waste Y: the chemoton is thus a thermodynamically open system, as it must be. The metabolic cycle regenerates the intermediate A_1, as well as other molecules V′ and T, of which V′ enters the information cycle and produces a molecule R that reacts with T′ to produce T, which can polymerize and self-assemble to produce structural closure in the form of an enclosing membrane.

The chemoton is probably the most firmly based in chemistry of all the theories of life that we consider, and it also explicitly includes what Schrödinger (1944) called a "codescript" (Section 6), in the form of the information cycle V. The nature of the information coded by the cycle V is not very explicit in Fig. 6 but is somewhat clearer in the text of Gánti (2003), where the molecule pV_n is interpreted as an information-carrying polymer that acts as a template for production of T. In their account of the chemoton Maynard Smith and Szathmáry (1995) explain that the length of the pV_n molecule may vary in different chemotons, and it may contain two types of unit, V and Z, becoming thus a pV_nZ_m molecule. In these cases the values of *n* and *m* are inherited when the system divides. However, "although the sequence [of the pV_nZ_m molecule] is not utilized, either in coding or catalysis, it is inherited, ready for use at some later stage." This can perhaps be regarded as a small step towards coding useful information, but it remains far from being a complete solution. Our own attempt (Cornish-Bowden and Cárdenas, 2008) to introduce ideas of identity and heritability into (*M*,*R*) systems can also, no doubt, be regarded as rudimentary.

As the cycles regenerate their components they are catalytic, and they are also created by the system itself, so the system is closed to efficient causation. However, no catalysts are specified for the individual steps, and without these it is difficult to see how parasitic reactions that may cause the whole organization to collapse can be avoided, as discussed further in Section 9.2. On the other hand if individual catalysts are included the system will no longer be closed to efficient causation.

As we shall see in Section 8.6, the central role of catalytic cycles is a major feature of the theory developed by King, and was also proposed by Rössler (1971) in the same year as Gánti's book.

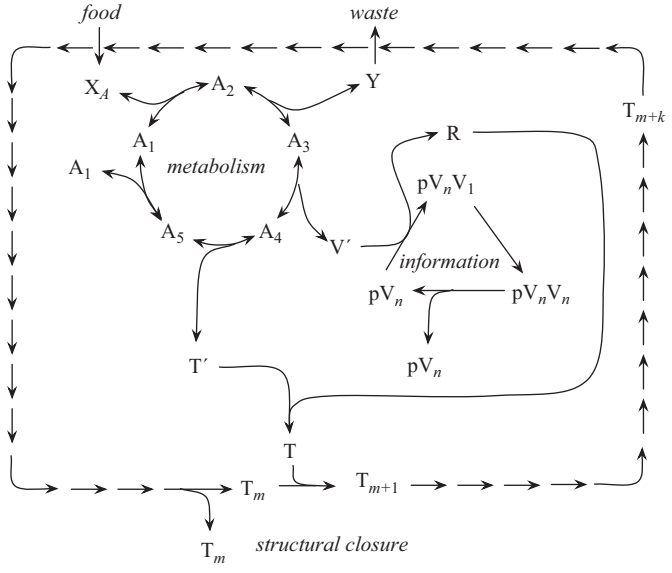

Fig. 6. The chemoton. All arrows represent chemical reactions (material causation), reversible in the case of double-headed arrows, irreversible otherwise. The diagram is based on Fig. 1.1 of Gánti (2003), redrawn to represent reactions involving multiple substrates in a more conventional way.

8.5. The hypercycle

Maynard Smith and Szathmáry (1995) used the name *Eigen's paradox* to refer to the puzzle that specifying the structures of enzymes requires a large genome, but producing and accurately replicating a large genome requires enzymes. All modern organisms have both enzymes and large genomes, so at some point in evolution the problem must have been solved, but organisms at the origin of life must have been far simpler, and it appears impossible for them to have satisfied both conditions simultaneously, so that all primitive organisms ought to have been subject to large errors, leading to collapse from an *error catastrophe*. Eigen and Schuster (1977) proposed the *hypercycle* as a way to escape this paradox. An example of what they called a "realistic model of a hypercycle of second degree" is illustrated in Fig. 7. It consists of a cycle of information-carrying RNA molecules I_i that specify the structures of enzymes E_i, each of which catalyses the replication of the information molecule of a different enzyme.

By means of detailed calculations of probabilities Eigen and Schuster (1977) showed that a system of this kind could escape from the error catastrophe, i.e. that it could be replicated with a sufficiently low error rate to avoid collapse. They also showed

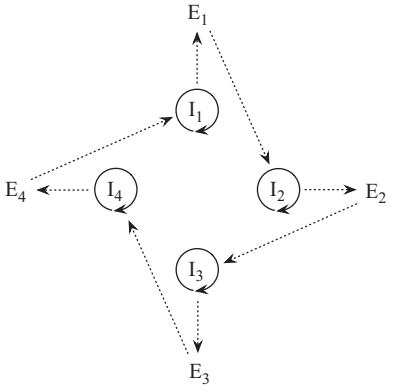

Fig. 7. A hypercycle of second degree. The system consists of four enzymes E_1–E_4 and four information-coding RNA molecules I_1–I_4. An information molecule I_i specifies the structure of the corresponding enzyme E_i, which, in turn, catalyzes the replication of the next information molecule in the cycle, I_{i+1}. Notice that there are no explicit chemical reactions in this scheme, and hence no metabolism.

that the *quasi-species* constituted by non-equivalent hypercycles occupying the same space and competing for the same resources would evolve by Darwinian natural selection.

8.6. Autocatalysis at the origin of life

The role of autocatalysis at the origin of life has been analysed in particular by King (1977a,b, 1982), who pointed out that autocatalysis is an inevitable property of any system in which molecules consumed in one step in a network are regenerated in another. He maintained that in the early stages of life symbiosis[14] (rather than mutation) was the main evolutionary process. By this he meant interaction between autocatalytic cycles of reactions such that each cycle depended for its continued operation on output from another. In this way he thought it possible to overcome the obvious problem that a single autocatalytic process must collapse after the catastrophic depletion of its substrates that occurs, for example, in the explosion front in a combustible mixture of gases. In this way a system of autocatalytic cycles could achieve a long-term stability that would not be possible for a single reaction. The essential idea, later put in more precise terms by Fernando (2005) is that a stable society of symbiotic autocatalytic reactions can be reached if molecules are recycled. This interdependence is similar to the idea of closure that we advocate, though King (1977a) did not himself use this term.

8.7. Autocatalytic sets

Dyson (1982) and Kauffman (1986, 1993) set out from a different starting point from most of the other authors considered here. Rather than asking what properties were necessary for a system to be regarded as living, they asked what sort of conditions might allow self-organization to arise from purely chance properties of sets of molecules. The most important part of Kauffman's definition of an *autocatalytic set* is the following:

> Catalytic "closure" must be achieved and maintained. Thus it must be the case that every member of the autocatalytic set has at least one of the possible last steps in its formation catalyzed by some member of the set, and that connected sequences of catalyzed reactions lead from the maintained food set to all members of the autocatalytic set.

This definition is illustrated in Fig. 8. Consider, for example, the molecule ABCC, produced by the following reactions:

$$A+B \overset{ABC}{\rightarrow} AB; \quad AB+C \overset{AABABCB}{\rightarrow} ABC; \quad ABC+C \overset{ABCBABCC}{\rightarrow} ABCC$$

where molecules acting as reactants are shown in roman type, whereas molecules acting as catalysts (which may be the same molecules) are shown in italic type. However, this is not the only way in which ABCC can be produced, as it can also result from the following reactions:

$$A+B \overset{ABC}{\rightarrow} AB; \quad C+C \overset{ABCC}{\rightarrow} CC; \quad AB+CC \rightarrow ABCC$$

in which the last step is spontaneous and has no catalyst. However, note that the definition does not require all steps to be catalysed, only that there must exist at least one route to every member of the set in which all steps are catalysed, and this requirement is satisfied for ABCC. On the other hand the molecule AABABCAAAAB is *not* a member of the autocatalytic set, because there is no series of catalysed reactions that reaches it. There is no requirement for every member of the set to be a catalyst, as misinterpreted by Chemero and Turvey (2006), only for every member to be reachable by a series of catalysed reactions. There are several molecules in Fig. 8 (for example ABCB) that catalyse no reactions, but they are members of the autocatalytic set.

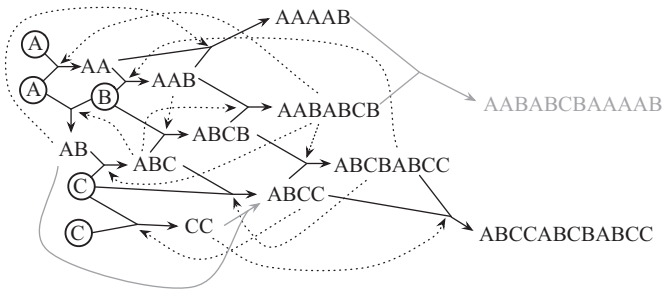

Fig. 8. An autocatalytic set. The precursors A, B and C (circled) are available from the environment, and every polymer except one (shown in grey and discussed in the text) can be made from a series of reactions (solid lines: material causation) catalysed (broken lines: efficient causation) by members of the set. Uncatalysed reactions are shown in grey. The untidy appearance of this illustration compared with the "designed" look of some of the others is intentional, to emphasize the expectation that order can arise spontaneously from chance properties of molecules.

An autocatalytic set as originally conceived by Kauffman (1986) must inevitably be large: orders of magnitude larger than what is shown in Fig. 8, because the probability that a randomly chosen member of the set can be capable of catalysing a randomly chosen reaction must be very small. For example, if this probability is 10^{-9} there must be at least 3×10^8 members of the set before there is a high probability that the entire set can arise spontaneously (Kauffman, 1993). In drawing Fig. 8 we have been faithful to the definition in assuming that any molecule can just happen to be a catalyst for any reaction, with no tendency to be more effective for some sorts of reaction than for others. So, for example, we have assumed that $A+B\rightarrow AB$ is catalysed by ABC, but the similar reaction $AA+B\rightarrow AAB$ has a quite different catalyst, AABABCB, which also catalyses a quite different reaction, $ABCB+ABCC\rightarrow ABCBABCC$. Knowledge of chemistry and enzyme catalysis, however, suggests that some molecules should be completely ineffective for catalysing any reactions, with others capable of catalysing many similar reactions: for example, the proteolytic enzyme trypsin catalyses hydrolysis of most peptide bonds on the carboxyl side of arginine or lysine residues, and although it also catalyses hydrolysis at other bonds it does so with much lower activity. If this is allowed for, it would allow a spontaneously arising autocatalytic set to have many fewer than 3×10^8 members even if the average probability is 10^{-9}.

An important refinement of autocatalytic sets is the concept of GARD (*Gradual Autocatalysis Replication Domain*), which formalizes the cooperative non-covalent integration of single molecules into heterogeneous molecular assemblies (Segrè et al., 1998). The initial GARD model is particularly suited to simulation of the incorporation of lipids into micelles, where the rate of incorporation of a single molecule is synergistically modulated by the molecules already present in the assembly. This scheme produces a collective autocatalysis, but one that is restricted to the process of building assemblies. In the GARD model the elementary molecules are given de facto, along with their catalytic properties, and thus are not produced by any metabolic network; only joining (or leaving) a given supramolecular assembly is catalysed. Thus although a GARD assembly shows a type of population catalytical closure it does not allow for metabolic closure.

[14] King shared with Rosen a tendency to assign new meanings to well understood biological terms. His term "symbiosis" is especially unfortunate, as it has nothing to do with symbiotic relationships between different organisms, a very important feature of evolution. The idea of symbiosis can perhaps, however, be related to the merging of separate autocatalytic cycles into a larger system.

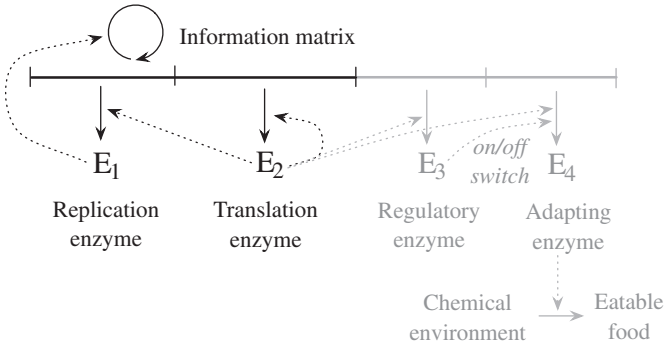

Fig. 9. A model of a syser. A matrix molecule contains the information necessary for synthesizing two enzymes, a replication enzyme E_1, which catalyses replication of the matrix, and a translation enzyme E_2, which catalyses synthesis of both enzymes. The minimal syser includes only these elements, but it can be expanded into an *adaptive syser* that also includes the elements and processes shown in grey: the regulatory enzyme E_3 then acts to switch on or off the synthesis of the adapting enzyme E_4, which catalyses the production of usable substrates from the chemical environment. The diagram is based on Fig. 2b of an unpublished manuscript kindly provided by Dr. V.G. Red'ko as an English version of two papers in Russian (Red'ko, 1986, 1990).

8.8. Sysers

In contrast to the other theories compared in this paper, *sysers*, or *sy*stems of *se*lf-*r*eproduction, were explicitly introduced as a development of another theory, namely that of hypercycles (Section 8.5). Sysers were proposed independently by White (1980), Ratner and Shamin (1980) and Feistel (1983), the name being given by the Russian group, and were intended as more realistic and complete than hypercycles. A syser is illustrated in Fig. 9, based on an analysis by Red'ko (1986, 1990).[15]

Even in its minimal form the scheme in Fig. 9 is closed to efficient causation, because all catalysts are products of the system itself. However, it is also closed to material causation, so it cannot grow or maintain itself, but this objection is overcome in the *adaptive syser*, which includes the elements shown in gray in the figure, consisting of an adapting enzyme E_4 that catalyzes the production of usable molecules from the chemical environment. Notice also that E_2 is a "moonlighting" protein, as it catalyzes at least two different processes: this is an essential requirement for closure (Cornish-Bowden et al., 2007), and in the context of Fig. 9 it is clear that if E_2 could only catalyze translation of the matrix into E_1, with another enzyme needed for catalyzing translation into E_2, we should need to explain how this other enzyme is produced, and unless at some point there was at least one enzyme with more than one function there would be infinite regress.

8.9. RAF sets

Hordijk and Steel (2004) introduced RAF sets ("*R*eflexive *a*utocatalytic systems generated by a *f*ood set") in an effort to construct a formalism for studying the autocatalytic sets (Section 8.7) of Kauffman (1993) so that they could be described and analysed in the computer. In a RAF set every reactant is either produced by the system or harvested from the environment, a definition that does not exclude the possibility that some catalysts are not produced internally. Thus although they can be closed to efficient causation that is not a necessary part of their definition, and so they provide a weaker definition of life than (*M*,*R*) systems: any (*M*,*R*) system is a RAF set, but not all RAF sets are (*M*,*R*) systems. Nonetheless, as we have discussed elsewhere (Jaramillo et al., 2010), they have much in common with (*M*,*R*) systems (Section 8.2), and as a theory of life they add little to the theories discussed above.

On the other hand Hordijk and Steel (2004) presented powerful algorithms that will be very useful for analysing (*M*,*R*) systems and other models of life, for example, they make it feasible to generate and analyse all possible metabolisms of a specified size.

[15] These papers are in Russian, but our comments are based on an unpublished English version kindly supplied by the author.

Table 1
The game of the two lists.

Living	Non-living
Fly	Radio
Tree	Automobile
Mule	Virus
Baby	Crystal
Mushroom	The Moon
Amoeba	Computer

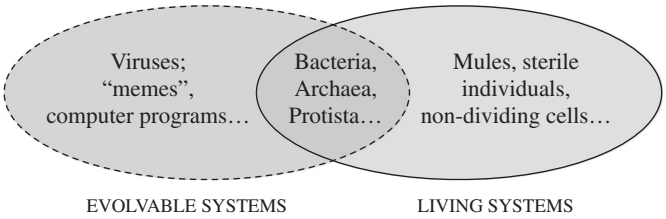

Fig. 10. Evolution and life. Evolvable systems are not coextensive with living systems, because non-living entities such as computer programs can evolve, and not all living organisms can evolve. The figure is based on Fig. 4.1 of Szathmáry (2003).

9. Discussion

9.1. The definition of life

Recognizing a living organism is easy to do, but very hard to define, as Luisi (2003) has cogently discussed in his review of autopoiesis in the context of the "game of the two lists" illustrated in Table 1. Everyone will agree that the items in the left-hand column are living, and, apart from some residual argument about the status of viruses,[16] that those in the right-hand column are not. Any acceptable theory of the living state must be capable of leading to the right classification, but it is arguable that none of the current theories does so. Luisi (2003) himself considers that autopoiesis does, but the omission of catalysis seems important. At the end of his book Barbieri (2003) lists more than 60 attempts to define life, from Lamarck until the 21st century. About 40 of these were written after Schrödinger (1944), and cover a range from the utterly obscure ("Life on earth today is a highly degenerate process in that there are millions of different gene strings (species) that spell the one word 'life'") to the absurdly precise ("Life consists of proteinaceous bodies…"). Several consider reproduction and capacity for natural selection (rather than just staying alive) as essential, but although these are certainly characteristic of life as we know it today we do not see them as part of the *definition* of life (Fig. 10).

[16] See for example the comments of a reviewer appended to a recent article (Tsokolov, 2010). Interestingly, viruses do not appear in either of the two columns of a more recent version of the table prepared by Luisi (2006). Unfortunately he gives no reason for the omission, but later in the book (p. 159) he makes it clear that he does not consider viruses to be autopoietic systems.

We think that metabolic closure is the core aspect that must be understood for a working theory of living systems and that Rosen's formulation of it is a path for achieving this. Furthermore, in the preceding pages we have shown how most theories about living systems, since 1960, are centred on the idea of closure. Although we claim that of the many versions of metabolic closure Rosen's viewpoint is the most promising, we recognize that his ideas are difficult to interpret and to use, but they permit the creation of theoretical tools that help us to advance. It is important to emphasize that when we talk about theory we are not equating theory with mathematization, but instead bringing forward new concepts (like a circular chain of causation implemented by networks of chemical reactions) that could be, but does not need to be, put in the language of symbols and equations. In this sense his intuitions seem to us very important, as they put the problem in a new light and separate it from questions of the specificity of molecules and processes. Metabolic closure in this sense is quite different from the systems biology models that depend on many feedback loops or from the approach of systems chemistry based on the specific properties of molecules. We believe that the fundamental property will prove to be a network property and not a property of a single component. Finally, our insistence on putting metabolic closure at the centre of the stage is our conviction (shared by the many theoretical frameworks surveyed in the review) that it is the central (and oldest) aspect of biological organization, and represents the core phenomenon that allows a living system to be alive. No one would set out to learn the basic principles of aircraft design by studying a modern airliner such as an Airbus A380: the original biplane of the Wright brothers would be far more suitable, as it lacked millions of components – light bulbs, video screens, reclining seats, ovens, call buttons, escape hatches, and so on – that do not contribute to its airworthiness. Yet even the simplest organism that we know today is complex in a way that a modern airliner is not, but we have no choice but to try to deduce the basic principles of life from examples that are not basic at all. It is for this reason that theory is needed, to define the minimum conditions that a living system must satisfy.

There is considerable overlap between the various theories of life that we have considered in this review, despite the almost total absence of communication and cross-referencing between their authors. All of them incorporate some idea of closure, but they do not all mean the same by this term. Catalytic closure is regarded as crucial by Rosen (1991), but absent from the work of Maturana and Varela (1980); structural closure is crucial for Maturana and Varela (1980), but absent from (or at best only implicit in) the work of Rosen (1991). Although some of the authors seem to be saying the same thing in different words, they are sometimes saying different things with similar words, and always emphasizing different aspects even when saying the same things.

In Table 2, therefore, we compare the main points in the different theories with one another, and with the points that we think ought to be explicit in an "ideal" theory. None of the current theories incorporate all of them, so in that sense all of them lack essential features. Several other points are missing from the table, though some will consider them essential. We do not regard coding, reproduction, metabolic regulation or capacity for evolution by natural selection as necessary features of a definition of life: even though all of them are characteristic of life as we know it today they will not have been necessary for the original emergence of life. The essential problem to be solved before any other arose was the problem of staying alive. Only when the first organisms became capable of staying alive long enough to reproduce did evolution become possible. We disagree, therefore, with the definition of life adopted by NASA (Joyce, 1994): "*Life* is a self-sustained chemical system capable of undergoing Darwinian evolution".[17] Although Gánti (2003) specifically mentions that "a living system must have the capacity for hereditary change, and furthermore, for evolution", Szathmáry (2003) points out in a note to the same statement that this is a mistake.[18] In general his view of the living state is close to ours, and he is particularly critical of a definition that Luisi (2003) considered to represent the views of adherents of the "RNA world": "life appears as a population of RNA molecules (a quasi-species), which is able to self-replicate and to evolve in the process." In a recent paper (Vasas et al., 2010) he describes the capacity for Darwinian evolution as a "basic property of life", without, however, implying that it is a defining characteristic of life. We see this as a crucial distinction: all living populations that we know today are capable of evolution, but that follows from the impossibility of error-free replication, not from any inherent merit in evolution.

Table 2
Principal characteristics of theories of life. The bottom line lists the points that we believe a satisfactory theory ought to contain.

Theory	Section	Thermo-dynamically open	Catalyzed	Catalytic closure	Structural closure
(*M*,*R*) systems	8.2	Yes	Yes	Yes	No
Autopoiesis	8.3	Yes	No	No	Yes
Chemoton	8.4	Yes	No	No	Yes
Hypercycle	8.5	Implied	Yes	Yes	No
Symbiosis	8.6	Unclear	Yes	Yes	No
Autocatalytic sets	8.7	Implied	Yes	Yes	No
Syser	8.8	Implied	Yes	Yes	No
RAF sets	8.9	Yes	Yes	No	No
"Ideal theory"		Yes	Yes	Yes	Yes

None of the theories in Table 2 include all of these additional features, though one, the syser, includes regulation as a supplementary feature, albeit not part of the basic model, and several allow for coding. However, it seems hardly possible for the first organisms to have had coding of proteins, whether by nucleic acids or anything else, so they must either have used RNA to fulfil their catalytic functions, or managed without coding of their catalysts. Likewise, metabolic regulation is an essential function of modern organisms, but may not have been crucial at the origin of life, when vast amounts of time were available for metabolism (but also for parasitic reactions) and there were no competitors to eliminate highly inefficient organisms. In this context we have shown (Piedrafita et al., 2010) that a computer model of a simple (*M*,*R*) system can reach a steady state in the absence of any regulation, and, more significantly, can regenerate itself after catastrophic loss of a catalyst.

The theories differ on how much they focus on organization of an entire system, and how much on its details. At one extreme, (*M*,*R*) systems focus on organization to such an extent that the details appear completely lost. At the other, the theory of autocatalytic sets considers only the organization that arises by chance, and focusses on the details that could allow this.

[17] According to Luisi (2003) this definition originated much earlier with Horowitz and Miller (1962). However, although their definition is related, it is not the same: "An organism, to be called living, must be capable of both replication and mutation; such an organism will evolve into higher forms." For them, therefore, Darwinian evolution was a consequence of being alive, not a prerequisite for it.

[18] He stated that "a living system cannot have the capacity of evolution; only a population of living systems has this capacity", and in general his main concern is to avoid confusion between living individuals and populations.

9.2. Vulnerability of self-organized systems to parasitic processes

As Hofmeyr (2007) has pointed out, the chemoton model (Section 8.4) contains no evident mechanism to avoid collapse due to parasitic reactions, because there is no explanation of the specificity that could prevent this. However, this problem is not unique to the chemoton, as all current theories of life, including (*M*,*R*) systems, autopoiesis, hypercycles and autocatalytic sets, require highly specific catalysts. Szathmáry (2003) has recognized this problem, calling it the "paradox of specificity", but adds that "nobody has yet provided a satisfactory solution." So far as modern organisms are concerned we can attribute enzyme specificity to 4 billion years of natural selection, but this will not do for a proto-organism that arose from chance properties of the chemical compounds that compose it. If a model contains very large numbers of different molecules, as in autocatalytic sets, we find it implausible that all of those that act as catalysts can be specific purely by chance. The chemoton and hypercycles assume much smaller numbers of components than autocatalytic sets, but they still require many more distinct components than are assumed to be necessary in autopoietic models, or in our minimal version of (*M*,*R*) systems. So, although we certainly do not claim that the problem of specificity is solved, we do argue that its solution will be found in very small models that are capable of becoming more complex. Only then can one attribute the initial appearance of metabolic closure to chance.

Although it is commonplace to emphasize the power of enzymes to accelerate reactions, a property they share with metals such as platinum and with heating the reactants to high temperatures, this is far less important than the fact that they are totally ineffective as catalysts for the overwhelming majority of other reactions that could potentially occur in a cell: in other words, they have *specificity* (Cornish-Bowden and Cárdenas, 2010), a property that heating lacks completely, and which platinum has to no great extent. This is important for considering autocatalytic sets, which not only require that 3×10^8 molecules just happen to be capable of catalysing 3×10^8 different reactions, but also that they catalyse *no other reactions* that the 3×10^8 molecules could potentially undergo. If an autocatalytic set of 3×10^8 members can spontaneously spring into existence, what would stop it from adding further members indefinitely until it degenerated into tar? In other words, could such a set possess the organizational stability that is characteristic of an organism?

The importance of specificity is so great that the early organisms can hardly have become more complex without it. Its appearance must have been a powerful driving force for early evolution.

9.3. The way ahead

Our summary of 50 years of theories of living organization has been compressed, but it has highlighted the unanimity that closure (or self-construction) is central to developing a useful theory of biological organization. All of the theories we have discussed touch on this, but Rosen's concept of (*M*,*R*) systems is special, because he introduced a level of analysis of closure not found in any of the others. He succeeded, albeit in a complex and puzzling manner, in deconstructing closure by segmenting it into three processes, metabolism, replacement and metabolic invariance. We emphasize that Rosen's segmentation, once understood and liberated from over-mathematization, is useful for generating new viewpoints, as illustrated in our interpretation of a simple three-reaction network in terms of these three processes (Letelier et al., 2005, 2006; Cárdenas et al., 2010). This is not the place to repeat a long but interesting argument, and we simply draw attention to four points:

1. Rosen's construction underlines the usually ignored fact that enzymes are products of the very same metabolic network in which they act as catalysts.
2. Rosen's three-element segmentation is useful as a natural division of the set of processes into the subnetworks f, Φ and β shown in Fig. 3.
3. Escaping the otherwise inevitable regress to infinity requires closure, and we have found that this requires some catalysts to have multiple functions (Cornish-Bowden et al., 2007). This is a powerful result that indicates that a systemic function, closure, imposes multifunctionality on at least some of its components, so this is a genuine systemic property in which a whole system defines properties of its components (Cornish-Bowden et al., 2004; Cornish-Bowden and Cárdenas, 2005).[19]
4. Closure is incompatible with a hierarchical organization, so even if it may be convenient to consider hierarchies within parts of organisms there can be no overall hierarchy.

Rosen's segmentation has been widely misunderstood, as illustrated by the prolonged controversy over his contention that an organism cannot have computable models (McMullin, 2004; Wells, 2006; Chu and Ho, 2007a,b; Louie, 2007; Wolkenhauer, 2007; Wolkenhauer and Hofmeyr, 2007; Mossio et al., 2009). We have reviewed this elsewhere (Cárdenas et al., 2010), and will not repeat the discussion here.

We are convinced that progress with theories of biological organization will depend on understanding metabolic closure, and the concept of β will constitute an essential step in arriving at such understanding: clarification of β is thus a fundamental task in which people interested in this problem will need to converge. Rosen's three-element analysis may prove to be incomplete, but understanding metabolic invariance (β) appears to be the only key that exists at present. All other models, apart from RAF sets, suffer from having closure too remote from their intellectual centers (as with hypercycles), or from treating it in too narrow a context (as with autopoiesis) or, in the special case of the closure-operator theory of Jagers op Akkerhuis (2010), they are formulated at such a level of generality that they have at best a metaphorical value. (*M*,*R*) systems and RAF sets, when reanalysed from the point of view summarized in this review, have the advantage of generating new questions and suggesting possible answers.

In this review we have surveyed efforts to understand the essence of metabolic organization. These span at least the last seven decades, and it is remarkable how most of the scientists involved have worked during this long period in a rather strict isolation from each other and, in consequence of this, depressingly little real progress has been made. Theoretical biologists should perhaps learn from the history of quantum mechanics, which, in a much shorter period between 1900 and 1940 was constructed three times, the original quantum mechanics of Planck and Bohr being radically reshaped as Heisenberg's matrix mechanics and again as Schrödinger's wave mechanics, and this cross-fertilization produced unarguable advances in basic understanding, with commercial applications as early as the first electron microscope in 1939. One relevant fact, very well illustrated by physics, is how results from one theory can be transferred into another without complex explanations: the final judge of the appropriateness is how much prediction and understanding are advanced. For example, Einstein explained the photoelectric effect by taking the idea of energy quantization and applying it almost unchanged to photons.

[19] This corresponds to a *top-down* approach in cognitive neuroscience.

Nothing similar exists in theoretical biology: it seems that every researcher wants to solve the basic problem alone, without becoming liable for intellectual debts. Even today it is a mystery how the fundamental results from metabolic control analysis are understood by only a small fraction of the people (especially biotechnologists) who need to know them. This autarkic approach hinders progress, especially in a field where the fundamental idea of metabolic closure has surfaced and resurfaced numerous times in different guises, and very few seem willing to recognize it. Our aim in this review, and our previous work, is to underline the extent to which most of the ideas about metabolic organization are centred around the notion of metabolic closure. Thus in the special moment of current biology, where the need for a theory of metabolism is implicitly recognized by many engaged in systems biology, researchers should understand the paths already explored and concentrate on understanding the fundamental concept of closure, an understanding that will also extend our notions about linear causality. The question of whether closure to efficient causation obviates the concept of hierarchy in biological systems, mentioned briefly in Section 8.2, will need to be analysed further in the future.

As for whether biology really needs a theory of the living state, we conclude by quoting Woese (2004), who wrote that "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." Of course we need the technological advances that we have seen in the past 60 years, but we also need a guiding vision.

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