

CATALYSIS AT THE ORIGIN OF LIFE VIEWED IN THE LIGHT OF THE (M,R) -SYSTEMS OF ROBERT ROSEN

ATHEL CORNISH-BOWDEN* AND MARÍA LUZ CÁRDENAS

Unité de Bioénergétique et Ingénierie des Protéines, Centre National de la Recherche Scientifique, 31 chemin Joseph-Aiguier, B.P. 71, 13402 Marseilles, France

E-Mail: *acornish@ibsm.cnrsrmsr.fr

Received: 1st September 2008 / Published: 16th March 2009

ABSTRACT

Living systems as we know them today are both complex, displaying emergent properties, and extremely complicated, with huge numbers of different components. At the origin of life they must also have had emergent properties, and hence must have been complex, but they cannot have been as complicated as modern organisms, because we cannot imagine that the first organisms started with anything as elaborate as a ribosome and all of the protein-synthesis machinery. Understanding how complexity could arise in even the simplest early organism requires, however, a theory of life, something that is largely lacking from modern biology. Various authors have contributed elements of such a theory, and the (M,R) -systems of Robert Rosen provide a convenient starting point.

INTRODUCTION

In an earlier contribution to this series [1] we commented that many phenomena are described as complex when in reality they are no more than complicated, because they can be fully accounted for in terms of the properties of their components: there is no “emergence”. It must be recognized, however, that it is not always easy to decide whether a property is truly emergent or not, in part because of disagreements about how emergence should be defined [2]. Living organisms in their totality, however, are complex, because it appears to

be impossible to deduce their properties solely by applying the reductionist programme of studying all of the properties of all of the components in sufficient detail. Some authors [3, 4] go further, and say that it not only appears to be impossible but it really is impossible even in principle, but this aspect remains controversial [5] and we shall not discuss it here.

We shall, however, try to resume the current state of understanding of the nature of life and the definition of a living organism. Although this might seem an essential component of biology, it is in practice ignored by nearly all biologists [6] and regarded as irrelevant to the practice of modern biology by many [7], as we discussed previously [8]. The famous question raised by Erwin Schrödinger [9] of what life is remains unanswered more than 60 years later. Parts of it, of course, have been answered: we can identify Schrödinger's "codescript" with the DNA in which protein sequences are recorded; we now rarely need to speak of organisms feeding on negative entropy because we understand that living organisms are thermodynamically open systems that can maintain themselves far from equilibrium without violating any thermodynamic principles. Nonetheless, the crucial question of what biological organization actually means and how it is maintained almost indefinitely remains inadequately studied. In 2005 the editors of *Science* [10] celebrated 125 years of existence of the magazine with a list of 125 questions, "the most compelling puzzles and questions facing scientists today". A high proportion of these were questions about biology, and included such vague items as "is an effective HIV vaccine feasible?", but Schrödinger's question was not among them.

The first modern attempts to understand biological organization were made by Stéphane Leduc [11]. His osmotic experiments produced impressively complicated and biological-looking structures (which can be reproduced in full colour illustrations today: see Querbes [12]), but few biologists today would accept that osmosis tells us much about the forms of real organisms. However, his more general belief that natural selection is not the only explanation of biological forms, and that chemical reactivity as well as physicochemical and mechanical forces also play major roles remains important, and was taken up by D'Arcy Thompson [13] in a closely argued book that has been very influential in modern thinking.

There are four principal current theories that try to explain biological organization, the (M,R) -systems of Rosen [3], the chemoton of Gánti [14], the autopoiesis of Maturana and Varela [15], and the autocatalytic sets of Kauffman [16]. Despite the fact that all of these theories contain some of the same ideas, they are by no means the same as one another, and none of the authors mentioned makes any reference to any of the others in their principal publications. Although all attach importance to "closure" and their definitions of this overlap, they underline different aspects. Rosen [3], for example, refers to closure to efficient causation, which means that all the catalysts required by an organism need to be products of its own metabolism; Maturana and Varela [15] stress structural closure, or the need for an organism to be enclosed within a membrane, cell wall, or skin; Kauffman [16] considers that catalytic closure is the consequence of very large sets of different polypeptides or polynu-

cleotides; Gánti [14] agrees on the necessity for structural closure, and also emphasizes the need for any theory of life to be rooted in an adequate knowledge of chemistry. Clearly, therefore, an important task for the future will be to integrate all of these threads into a single theory of life. Here we shall be less ambitious, concentrating on the ideas of Robert Rosen, which are the most abstract and difficult to understand of those we have mentioned, and will use them to analyse aspects of catalysis at the origin of life.

LIMITS OF REDUCTIONISM

The reductionist approach has taken biochemistry a very long way since Buchner [17] first demonstrated that alcoholic fermentation could occur in a cell-free extract of yeast, and it is very unlikely that we should know much about biochemistry today, and still less about molecular biology, if the approach in the 20th century had not been overwhelmingly reductionist. However, it is one thing to recognize the progress that reductionism has brought, but it is another to suppose that this can continue indefinitely. One can certainly understand the behaviour of components of the cell, such as metabolic pathways, in terms of their components, enzymes in this case, and enzymes can be understood in terms of the properties of their side-chains. However, especially at the level of the cell or the whole organism, the reductionist approach cannot provide the whole truth, because these entities have complex and emergent properties. However, today, and in contrast to the early 20th century, we do try to understand the chemistry of whole organisms (systems chemistry) and, in the words of Henrik Kacser [18], “one thing is certain: to understand the whole you must study the whole”, an idea more picturesquely expressed by a Russian proverb, “a hundred rabbits do not make a horse”.

It may be illuminating to compare modern biology with modern physics. In the 19th century theory followed from experimental observations: thermodynamics, for example, developed from Sadi Carnot’s efforts to determine whether steam engines could be improved without limit. In this and other 19th century cases the experiments preceded the development of the theory, but in 20th century physics theory usually preceded experiment: Albert Einstein, for example, did not develop the theory of relativity after wondering how the satellite navigation system in his car worked; on the contrary, this and other applications of relativity came many years after the theory had been worked out. The comparison is not just with physics, and as Günter von Kiedrowski remarked earlier in this symposium, “a century ago chemistry was in the same situation as biology today”. We believe, in summary, that future advances in biology will require a more complete theoretical basis than is provided by the theory of natural selection, the only general biological theory that exists, which is valuable for interpreting observations, but is not the whole truth.

CLOSURE TO EFFICIENT CAUSATION

Metabolism is often represented as a large and complicated set of processes catalysed by enzymes or transporters, these processes including both chemical reactions and transport across membranes. This description, however, while true as far as it goes, is seriously incomplete. As biologists know, and as Rosen [3] emphasized, the enzymes and transporters (which for brevity we shall consider together just as enzymes) are subject to turnover, dilution by growth of the organism, and losses due to their finite stability, processes that we shall abbreviate to decay. So, even if all the necessary enzymes are present in one moment, they cannot continue to be present indefinitely unless mechanisms exist to replace them. From what, however, can they be made? Clearly the only possible answer is that they must be made from the products of metabolism, so they are themselves products of metabolism, and hence metabolites.

Not only must all “enzymes” be considered metabolites in this sense, but many “metabolites” are also enzymes, because they are biological molecules that act as catalysts: metabolic cycles require not only the protein catalysts usually regarded as enzymes, but also the molecules that are consumed and regenerated in the process: the urea cycle, for example, requires not only three proteins, citrullinase, arginine deaminase and arginase, but also ornithine, and so ornithine has just as much right to be called an enzyme as the three proteins. It has more right, even, as the cycle would still occur (slowly) if some or all of the proteins were missing, but it would not occur without ornithine or a molecule that could replace it, such as citrulline or arginine. It follows that the usual distinction between enzymes and metabolites is formally meaningless [19]. Similar considerations apply to other metabolic cycles, such as the tricarboxylate cycle.

To refer to this organized replacement Rosen [3] used the unfortunate term *repair*, inviting confusion with more standard notions of repair in modern biochemistry, such as DNA repair and action of chaperones, so we prefer to refer to *replacement* [20, 21]. Fortunately this begins with the same letter of the alphabet as Rosen’s word, so we can continue to use the term (M,R) -system as a short form of *metabolism-replacement system*, which summarizes Rosen’s view of an organism. The essential point is that catalysts need to be replaced internally, by the organism itself; they cannot be replaced by an external agency. For this reason an organism is fundamentally different from a machine, because regardless of how one defines a machine, whether a simple tool such as an axe or something as complicated as an airliner, or even as a complete factory, at no level of definition does the machine make itself or maintain itself. The machine analogy may be helpful for understanding certain properties of organism, for example how the heart work, but in general it fails, because an organism is not a machine.

Unfortunately, however, Rosen did not make it easy for his readers to study his work. He presented it in resolutely mathematical terms, making no concessions to readers without mathematical expertise, he provided no examples to illustrate his central points – not even mathematical examples, and certainly no biological examples, and he did not define the range of validity of his ideas. We have therefore tried to fill these voids [20, 21].

CATALYSIS AT THE ORIGIN OF LIFE

At the origin of life there was no natural selection as we understand it today, but there was certainly chemical selection resulting from differences in rates of reactions derived from kinetic or thermodynamic properties [22]. Thus (M,R) -systems (*metabolism-replacement-systems*) probably emerged in prebiotic conditions thanks to the presence of inorganic catalysts or simple organic molecules that could act as catalysts. Modern organisms are not only complex; they are also extremely complicated, with a wide array of regulatory mechanisms, both metabolic and genetic, that were surely absent at the origin of life. These mechanisms are not explicitly visible in (M,R) -systems (though they are not excluded, and can be considered to be implicit), and so the representation of an organism as an (M,R) -system may be closer to the reality at the origin of life than to the reality of today.

Catalysis is fundamental for the organization of living systems, and must have been necessary at the origin of life, to permit organized systems to appear, to maintain themselves, and to grow. Some degree of specificity was also necessary, to allow one system to be different from another. Thus although the first catalysts must have been much simpler molecules than the protein or RNA catalysts that we know today, they must have had properties closer to these than to highly unspecific catalysts like platinum black. Specificity could then have developed progressively, first through chemical evolution and then through natural selection, to arrive finally at present-day bio-catalysts. However, specificity cannot be complete, because it is not possible for a system to fabricate its own catalysts if each one needs its own unique catalyst.

TYPES OF CLOSURE

The idea of closure may be understood in three fundamentally different ways, as illustrated in Figure 1. The simplest is the structural closure (Fig. 1a) produced by the physical boundary (membranes, cell walls, skin) that encloses an individual: in a present-day organism this is always fabricated by the organism itself, but this may not have been true for the first organisms, which could perhaps have made use of already existing inorganic compartments. Organisms must be closed in this sense, because every individual must be distinguishable from every other, and it is in general clear where one individual ends and another begins (if we exclude consideration of *Dictyostelium discoideum* and other organisms that challenge any attempt at a simple definition of an individual). Note that there can be no competition between individuals, and no way of assigning an identity to an individual, without structural

closure. (This does not of course exclude the possibility that one individual may live inside another: the more than 10^{14} bacterial residents of a human body are all clearly distinct from one another and from their hosts.) Structural closure forms an essential characteristic of autopoiesis [15] and the chemoton [14], but it was given little or no emphasis by Rosen, who was more concerned with chemical and organizational considerations, which he called *material* and *efficient causation* (terms derived from Aristotle's analysis of causation). We return to the question of individual identity in the section on Individual Identity.

Organisms are *open to material causation*, which simply means that they use chemical molecules taken from their environment that are different from those they excrete into the environment (Fig. 1b). They use the conversion of food (and light, in the case of photosynthetic organisms) into higher-entropy excreta to maintain themselves in states far from equilibrium, and this is a thermodynamic necessity that has been well understood since Schrödinger [9] introduced the idea that organisms “feed on negative entropy”.

This thermodynamic necessity does not conflict in any way with Rosen's view that organisms are *closed to efficient causation*, which simply means that they make their own catalysts (Fig. 1c), because the two levels of causation are independent of one another: a system may be closed to one and open to the other without any contradiction.

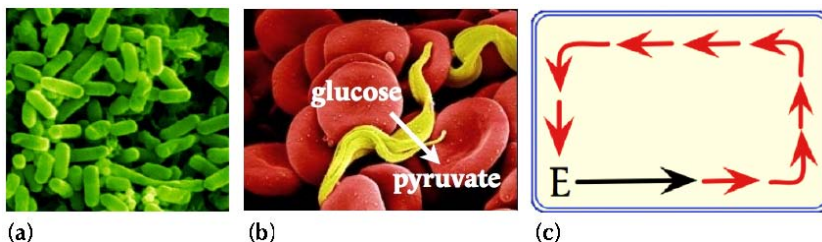


Figure 1. Three kinds of closure. (a) *Structural closure.* Any individual organism (here illustrated by a culture of *Escherichia coli*) is structurally closed, in the sense that it is separated from all other individuals by a physical barrier, such as a cell wall. (b) *No material closure.* No organism is a closed system in the thermodynamic sense. This is illustrated here by the parasite *Trypanosoma brucei* in the presence of erythrocytes, which can, to a first approximation, be regarded as a small chemical factory that transforms glucose into pyruvate. (c) *Organizational closure.* All organisms are closed in the sense that the catalysts that they need are products of their own metabolism.

ROSEN'S RELATIONAL DIAGRAM

Rosen [3] summarized his view of an organism with a diagram topologically equivalent to that shown in Figure 2. Although at first sight this diagram is unintelligible, it can be understood by following the various steps in a clockwise direction (as we explained earlier [8] in the context of Rosen's own unsymmetrical version of Figure 2), starting with $A \rightarrow B$,

which represents the whole of metabolism, as full arrows represent material causation: the substrates A of the whole set of metabolic reactions are the material cause of the products B of the same set of reactions. The broken arrows represent efficient causation, and so $f \dashrightarrow A \rightarrow B$ means that metabolism $A \rightarrow B$ is catalysed by the whole set of catalysts f . These catalysts are replaced from the only pool available, the set of metabolic products B under the influence of a replacement system Φ , so $\Phi \dashrightarrow B \rightarrow f$.

This system must itself be replaced, because it is subject to the same problems of decay as the metabolic catalysts. At this point Rosen avoided the incipient infinite regress by supposing that the efficient cause of this replacement of Φ from f could be caused by an entity β that was considered as a property of B , so $B \dashrightarrow f \rightarrow \Phi$ allows the whole diagram to be closed to efficient causation, though, as noted above, it is open to material causation, with a net non-cyclic (or only partially cyclic) process $A \rightarrow B$. Rosen always made it clear in his writing, and especially in his papers of 1966 and 1971 [24, 25], that although β is related to B it is not the *same* as B , and in mathematical terms it is best understood as the inverse of B [20], but he was less careful to be clear about this in the diagram on which Figure 2 is based, an oversight that has caused a great deal of confusion and misunderstanding in the literature.

The whole diagram is thus closed to efficient causation, or in other words it shows metabolic circularity or organizational invariance. It contains no *final causes* – no explanations of *purpose*, or of *why* anything happens as it does. The fourth of Aristotle’s categories of causation, the *formal cause* (what makes a metabolite a metabolite? What makes an enzyme an enzyme?), is also absent from the diagram, as it played little role in Rosen’s thinking.

We have already noted that Rosen’s term “repair” has nothing to do with conventional uses of this term in modern biochemistry. Perhaps even more misleading, his term “replication” for what we call organizational invariance has *nothing* to do with DNA replication, etc. We include both of these terms in Figure 2 to facilitate comparison with Rosen’s publications, but they are otherwise best avoided.

Recent years have seen an enormous growth in “systems biology”, with greatly increased interest in “small-world” models of metabolism [26, 27] and the “bow-tie model” of metabolic regulation [28]. However, the idea of metabolic closure is completely absent from these. The small-models are completely concerned with material causation and do not address the question of where the catalysts come from, and although the bow-tie model does imply the existence of catalysts it also does not address the question of where they come from. So although Figure 1 of Csete & Doyle [28], for example, contains numerous feedback loops it has a clear left-to-right reading direction, with no suggestion of closure.

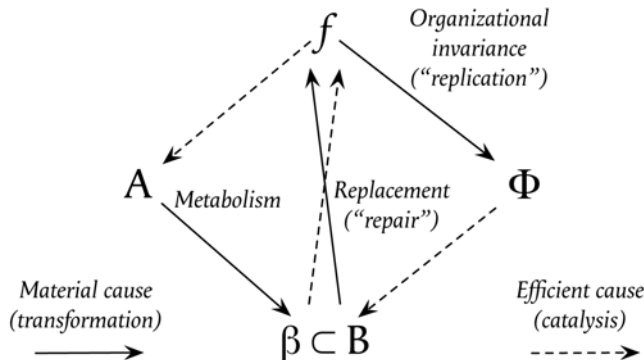


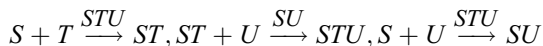
Figure 2. Closure to efficient cause (metabolic circularity). The diagram is based on Figure 10C.6 of Rosen [3], redrawn in the more symmetrical way suggested by Cottam *et al.* [23]. Broken arrows show efficient causes, or catalysis, whereas full arrows represent material causes, or chemical transformations. The diagram is highly abstract: the transformation $f \rightarrow A \rightarrow B$ represents the whole of metabolism; the transformation $\Phi \rightarrow B \rightarrow f$ represents replacement of enzymes from available products of metabolism, and $\beta \subset B \rightarrow f \rightarrow \Phi$ represents the processes need to maintain organizational invariance. Rosen's terms "repair" and "replication" shown in parentheses for these last two processes are misleading, as they have *nothing* to do with the ordinary uses of these words in modern biochemistry, for example for DNA repair and DNA replication. Note that although β is related to B it is not the same as B.

REPRESENTING ROSEN'S MODEL IN A MORE BIOLOGICAL WAY

As noted, Rosen's representation as illustrated in Figure 2 is highly abstract, and not easy to relate to ordinary ideas of biological models. In an attempt to make it more concrete, therefore, we extended a model outlined by Morán *et al.* [29] to arrive at the minimal biological model of an (M,R) -system illustrated in Figure 3 [8, 19–21]. In this representation the three catalytic processes are shown as three cycles of chemical reactions:

1. $S + STU \rightarrow STUS; STUS + T \rightarrow STUST \rightarrow STU + ST$ (metabolism)
2. $ST + SU \rightarrow SUST; SUST + U \rightarrow SUSTU \rightarrow SU + STU$ (replacement)
3. $S + STU \rightarrow STUS; STUS + U \rightarrow STUSU \rightarrow STU + SU$ (organizational invariance)

These can alternatively be written as three catalysed reactions:



but the greater simplicity is only apparent, because writing the cycles as catalysed reactions just hides the chemical reality of what is happening. An important point to note here is that closure was achieved by requiring one molecule STU to catalyse two different processes.

We believe that this will be generally true, that for models of arbitrary complexity it will always be necessary to include multifunctional catalysts if closure is to be possible. This in turn implies a general principle: that “moonlighting”, or multifunctionality of proteins, as well exemplified by the many different functions of glyceraldehyde 3-phosphate dehydrogenase, is not simply an interesting property of biochemical systems that is being increasingly observed [30, 31], but is an absolute necessity for life. In present-day organisms the ribosome, which participates in the synthesis of a wide variety of different types of protein, certainly contributes to the closure though it is far from being the only example. For consideration of the organisms shortly after the origin of life we need to envisage vastly simpler solutions than that represented by the ribosome and modern catalytic proteins.

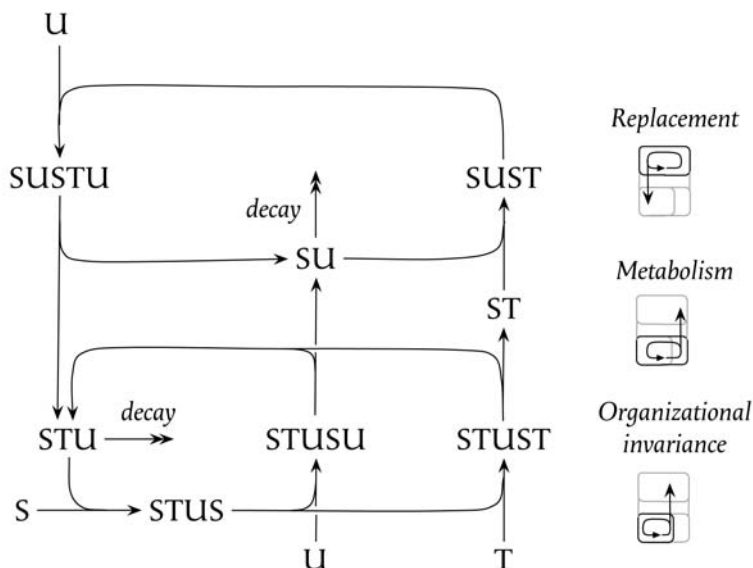


Figure 3. A model of an (M,R) -system. This is the biological model suggested previously [8, 19–21]. The two decay reactions shown with double arrowheads are considered to be irreversible. Unfortunately, Figure 3b of [21], which should have been very similar to this one, was printed incorrectly, as three intermediates were 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). It was printed correctly in the other papers that have similar diagrams [8, 19].

Another important point to note is that the “solution” to the problem of closure shown in Figure 3 is not unique, because the assignment of STU , SU and again STU as catalysts of the three processes is just one of the $3^3 = 27$ ways in which the three possible catalysts STU , ST and SU could be distributed among the three reactions. Thus the structure of the network does not by itself define Rosen’s function β needed for organizational invariance.

A possible way out of the difficulty comes from reexamining the definition of an enzyme or of a catalyst. Any catalysed reaction can be written as a cycle of uncatalysed reactions, as was done in the preceding section, and, as noted in the section Closure to Efficient Causation, it is only convention, not logical necessity, that leads us to called arginase but not ornithine a catalyst of the urea cycle, for example. Catalysis is nothing more fundamental than a human interpretation of chemical cycles, and if this is recognized then Figure 3 represents not a set of three catalysed reactions, but a set of eight uncatalysed chemical reactions, and on this interpretation the question of which catalyst catalyses which reaction does not arise [19]. This means that the catalytic properties follow simply from chemical reactivities, and makes it easier to understand how an organism can “know” which molecule, whether catalyst or other metabolite, is needed for maintaining its metabolism.

INDIVIDUAL IDENTITY

Natural selection implies competition between individuals, and needs identifiable and distinguishable individuals to do the competing. However, Figure 3 does not provide any indication of how the system is contained, and thus “individual”, and does not suggest how one individual might be distinguished from another. An obvious step to overcome the first objection will be to incorporate the obligatory formation of a membrane, already explicitly included in autopoiesis [15] and the chemoton [14], into the representation of (M,R) -systems. We have discussed elsewhere [32] how the second objection may be overcome. Although this was in terms of a model somewhat more complicated than the one in Figure 3, the main idea can still be presented in terms of Figure 3. First we need to suppose that the whole system is contained within a barrier (structural closure), as without this there can be no competition. Suppose now that a chance accident causes one of the catalysts, for example SU, to appear in a variant form SU' that has similar properties to the normal form but catalyses the production of a slightly different form of another catalyst (which must be STU in this case as that is the only possibility). Although initially there may be only one molecule of SU' the number will increase if it happens that the variant system reacts faster than the normal one and will eventually replace it.

CONCLUSION

Future development of systems chemistry and systems biology will need what Woese [33] has called a guiding vision: the main theories about the nature of life [3, 14–16], in particular the (M,R) -systems of Robert Rosen could constitute this guiding vision. For example, current research efforts to define the minimal genome that allows a system to be autonomous should benefit greatly from the incorporation of such concepts as metabolic closure and organizational invariance.

Efforts must be made to integrate the theories about the nature of life in a single coherent one, which could serve as a basis for understanding how life originated and how it is maintained. Catalysis at the origin of life probably made some use of external agents, so prebiotic systems probably did not fully satisfy the criterion of metabolic closure, but only when they acquired it did they become truly alive, even if the structural closure still depended on external inorganic support. It is tempting therefore to speculate that metabolic closure was acquired before structural closure.

REFERENCES

- [1] Cornish-Bowden, A., Cárdenas, M.L. (2003) Metabolic analysis in drug design: complex, or just complicated? In: *Molecular Informatics: Confronting Complexity* (ed. M. Hicks & C. Kettner), pp. 95 – 107, Beilstein-Institut, Frankfurt.
 - [2] Bedau, M.A. (2003) Downward causation and autonomy in weak emergence. *Principia* **6**:5 – 50.
 - [3] Rosen, R. (1991) *Life itself: A comprehensive inquiry into the nature, origin and fabrication of life*. New York, Columbia University Press.
 - [4] Louie, A.H. (2007) A living system must have noncomputable models. *Artif. Life* **13**:293 – 297.
 - [5] Stewart, J., Mossio, M. (2007) Is “life” computable? Proc. ENACTIVE/07 (Proceedings of the 4th International Conference on Enactive Interfaces 2007, Grenoble, France), <http://www.enactive2007.org>.
 - [6] Harold, F. M. (2001) *The Way of the Cell*, Oxford University Press.
 - [7] Atlan, H., Bousquet, C. (1994) *Questions de Vie*, Le Seuil, Paris.
 - [8] Cornish-Bowden, A., Cárdenas, M.L. (2007) Bring chemistry to life: what does it mean to be alive? In: *Molecular Interactions: Bringing Chemistry to Life* (ed. M. Hicks & C. Kettner), pp. 27 – 39, Beilstein-Institut, Frankfurt.
 - [9] Schrödinger, E. (1944) *What is Life?* Cambridge University Press, Cambridge.
 - [10] *Science* **309**: 1 – 204 (2005).
 - [11] Leduc, S. (1912) *La Biologie Synthétique*, Poinat, Paris.
 - [12] Querbes, S. (2007) <http://www.stephanequerbes.com/books/recherche/recherche.html>.
 - [13] Thompson, D’A.W. (1961) *On Growth and Form* (ed. J.T. Bonner, abridged edition of a book originally published in 1917, with second edition in 1942), Cambridge University Press, Cambridge.
-

- [14] Gánti, T. (2003) *The Principles of Life*, Oxford University Press, Oxford.
- [15] Maturana, H.R. and Varela, F.J. (1980) *Autopoiesis and Cognition: the Realisation of the Living*, D. Reidel Publishing Company, Dordrecht, The Netherlands.
- [16] Kauffman, S.A. (1986) Autocatalytic sets of proteins. *J. Theor. Biol.* **119**:1–24.
- [17] Buchner, E. (1897) Alkoholische Gahrung ohne Hefezellen. *Ber. Dt. Chem. Ges.* **30**:117–124.
- [18] Kacser, H. (1987) On parts and wholes in metabolism, in *The organization of cell metabolism* (ed. G.R. Welch & J.S. Clegg), pp. 327–337, Plenum, New York.
- [19] Cornish-Bowden, A., Cárdenas, M.L. (2007) Organizational invariance in (M,R)-systems. *Chem. Biodiversity* **4**:2396–2406.
- [20] Letelier, J.-C., Soto-Andrade, J., F. Gunuez Abarzua, F., Cornish-Bowden, A., Cárdenas, M.L. (2006) Organizational invariance and metabolic closure: analysis in terms of (M,R)-systems. *J. Theor. Biol.* **238**:949–961.
- [21] Cornish-Bowden, A., Cárdenas, M.L., Letelier, J.-C., Soto-Andrade, J. (2007) Beyond reductionism: metabolic circularity as a guiding vision for a real biology of systems. *Proteomics* **7**:839–845.
- [22] Melendez-Hevia, E., Montero-Gomez, N., Montero, F. (2008) From prebiotic chemistry to cellular metabolism – the chemical evolution of metabolism before Darwinian natural selection. *J. Theor. Biol.* **252**:505–519.
- [23] Cottam, R., Ranson, W., Vounckx, R. (2007) Re-mapping Robert Rosen’s (M,R)-systems. *Chem. Biodivers.* **4**:2352–2368.
- [24] Rosen, R. (1966) A note on replication in (M,R)-systems. *Bull. Math. Biophys.* **28**:149–151.
- [25] Rosen, R. (1971) Some realizations of (M,R)-systems and their interpretation. *Bull. Math. Biophys.* **33**:303–319.
- [26] Fell, D.A., Wagner, A. (2000) The small word of metabolism. *Nat. Biotechnol.* **18**:1121–1122.
- [27] Jeong, H., Tombor, B., Albert, R., Oltvai, Z.N., Barabasi, A.L. (2000) The large-scale organization of metabolic networks. *Nature* **407**:651–654.
- [28] Csete, M. & Doyle, J. (2004) Bow ties, metabolism and disease. *Trends Biotechnol.* **22**:446–450.
-

- [29] Morán, F., Moreno, A., Minch, E., Montero, F. (1996) Further steps towards a realistic description of the essence of life. In Langton, C. G., Shimohara, K., editors, *Artificial Life V*, pp. 255 – 263. MIT Press, Cambridge, Massachusetts.
- [30] Tipton, K.F., O’Sullivan, M.I., Davey, G.P., O’Sullivan, J. (2003) It can be a complicated life being an enzyme. *Biochem. Soc. Trans.* **31**:711 – 715.
- [31] Sriram, G., Martinez, J.A., McCabe, E.R.B., Liao, J.C., Dipple, K.M. (2005) Single-gene disorders: what role could moonlighting enzymes play? *Am. J. Hum. Genet.* **76**:911 – 924.
- [32] Cornish-Bowden, A., Cárdenas, M.L. (2008) Self-organization at the origin of life. *J. Theor. Biol.* **252**:411 – 418.
- [33] Woese, C.R. (2004) A new biology for a new century. *Microb. Molec. Biol. Rev.* **68**:173 – 186.
-

