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Self-organization at the origin of life ☆

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Abstract

The concept of an (M, R) system with organizational invariance allows one to understand how a system may be able to maintain itself indefinitely if it is coupled to an external source of energy and materials. However, although this constitutes an important step towards understanding the difference between a living and a non-living system, it is not clear that an (M, R) system with organizational invariance is sufficient to define a living system. To take a further step towards defining what it means to be alive it is necessary to add to a simple (M, R) system some property that represents its identity, and which can be maintained and modified in subsequent generations.

Keywords: (M, R) systems; Metabolism-repair systems; Robert Rosen; Metabolic closure

1. Introduction

Reinhart Heinrich was one of the great biologists of recent years, and one of the founders of what is now called systems biology. His major contributions to our understanding of biological systems have yet to attain the full recognition that they deserve, but this will come: eventually it will be realized that the reductionist approach that has dominated biochemistry for a century is coming to the end of what it can achieve, even though it has been responsible for the tremendous advances in knowledge and understanding in biochemistry that have occurred since Buchner (1897, 1997) found that alcoholic fermentation could occur in cell-free extracts of yeast. In showing that at least some of the properties of living organisms could be explained in terms of their components Buchner made a major step away from the mystical idea of vitalism that had preceded him, but to go from this to the idea that *all* of the properties of organisms are just the sum of the properties of the components is to go too far. Heinrich's earliest papers (Heinrich and Rapoport, 1973, 1974a, b) were revolutionary, as he was one of the first to break away from the traditional way of thinking of metabolism as just a collection of reactions catalysed by a collection of more or less independent components. His ideas, together with those of Kacser and Burns (1973), forced biochemists to start looking at systems as integrated wholes.

Metabolic control analysis, which grew out of these landmark papers, is sometimes seen, for example by Atkinson (1990), as antithetical to the classical view of metabolic regulation in terms of cooperativity, allosteric interactions and feedback inhibition, and even some authors in metabolic control analysis write as if they regard the classical approach as irrelevant. In fact, however, although the classical approach puts the emphasis on individual enzymes, whereas metabolic control analysis puts it on systems of enzymes, they are both necessary for a full understanding of metabolic regulation (Hofmeyr and Cornish-Bowden, 1991, 2000; Letelier et al., 2005). Understanding metabolic regulation is itself only a step towards understanding the logic of a living organism, which involves much more than that. A vital point that is usually entirely absent from discussions of life is that nearly all of the catalysts used by organisms are themselves products of the organism. This not only makes the usual

☆This paper is dedicated to the memory of Reinhart Heinrich (1946–2006), who did much to foster the idea that living systems cannot be understood just as collections of components, because they need to be studied as complete systems, with properties that make sense only in terms of the complete systems.

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distinction between enzymes and metabolites more arbitrary than it seems to be; it also renders any attempt to understand organisms in terms of machine analogies far more difficult than it appears to be. Most authors have not even recognized the existence of a fundamental problem, let alone tried to resolve it. Preeminent among the few who have recognized the problem is Robert Rosen, exemplified by his (M,R) systems (Rosen, 1966, 1967, 1971, 1972, 1991), or *metabolism-replacement systems*.[1]

Central to Rosen's view of life is the concept of metabolic closure (Letelier et al., 2006), or, in his words, "organisms are closed to efficient causation". As we shall discuss, this idea is largely absent from current discussions of metabolism or life. It is, however, especially relevant to considerations of the origin of life, as the capacity of self-organization, and hence of metabolic closure, must have been a property of the first organisms able to survive. It was thus present at the beginning of metabolism, before its expansion towards the complex system of metabolic pathways that we know today, this expansion being a topic of research to which Heinrich made several important contributions (Ebenhöh et al., 2004; Handorf et al., 2005, 2006).

2. Example of an (M,R) system

According to Rosen's theory the fundamental characteristic of a living organism is its ability to conserve its integrity of organization in spite of changes in its environment and in spite of the finite lifespan of all of its components. This capacity for autoconservation raises a major theoretical problem, because in present-day organisms the degradation and resynthesis of components involve the action of a series of interdependent macromolecules, which depend in their turn on another series, and so on, so generating a problem of infinite regress. We recently determined the limits within which this organizational invariance is possible and described a simple model of an (M,R) system (Letelier et al., 2006; Cornish-Bowden et al., 2007), because Rosen's publications unfortunately lack examples, and are also very difficult for non-specialists to read. Wolkenhauer and Hofmeyr (2007) also discuss how infinite regress can be avoided, but their approach is different: instead of trying to identify the elements of closure with material objects they identify them with sequences of events, or with processes.

Initially we considered that our simple model (Letelier et al., 2006; Cornish-Bowden et al., 2007) failed to be organizationally invariant, i.e. that it did not encode its organization within itself and hence could not maintain its organization. Subsequent analysis, however, suggested that this failure is only apparent, being a consequence of treating catalytic cycles as black boxes, rather than as they really are, cycles of chemical reactions (Cornish-Bowden and Cárdenas, 2007). Once these catalytic steps are shown explicitly the ambiguity disappears and the organization, now seen just as a manifestation of the chemical properties of the particular chemical entities present, is fully determined by these properties.

The arguments are summarized in Fig. 1, which represents a very simple example of an (M,R) system. As long as a metabolic system is drawn with unexplained catalysts (as in Fig. 1a), it is not closed to efficient causation, in Rosen's evocation of Aristotle's categories of causation. It can be closed, however, if the model is modified as shown in Fig. 1b: decay of the catalysts is now explicitly included and is counterbalanced by a set of replacement reactions, these being interpreted as properties of the molecules already included in the model or of additional molecules assumed to be present in the environment. This version of the model implies one new reactant J that was not shown in the simpler version, but all of the other functions are satisfied by molecules already present. Replacement of the catalyst B, for example, is assumed to be a secondary function of the molecule E to catalyse the production of B from A and I in addition to its primary function of reacting with C to produce G.

Fig. 1b thus represents an (M,R) system, but it does not appear to be an (M,R) system with organizational invariance, because it contains what appear to be arbitrary features. How does the system "know" that the replacement of B is catalysed by E and not, for example, by H? When all these arbitrary assignments are taken into account Fig. 1b represents just one out of $7^6 = 117,649$ ways in which catalysts could be assigned to reactions. However, this apparent arbitrariness is just a consequence of the usual way in which catalysed processes are shown in summary form, with a whole cycle of chemical reactions represented as a single "overall reaction" and the catalyst as an external effect rather than as an active participant. If the process is drawn more explicitly, as in Fig. 1c, with no catalysts as such but just a complicated series of chemical steps, the ambiguity disappears, because now there is no doubt about which molecule participates in which reaction, and no doubt about which catalysts catalyse which reactions. Instead the implication is that the particular steps shown represent nothing more than the chemical properties of the molecules concerned.

We now have an organizationally invariant (M,R) system, but can it be regarded as a model of a living system? With reluctance, perhaps, we must admit that it cannot. As long as it just represents the chemical properties of a set of molecules that happen to be available in the system it is no more "alive" than the inorganic mixtures studied by Leduc (1912) that could reliably generate complicated structures resembling living plants and fungi, as illustrated in Fig. 2. For a system to be "alive" it needs to have some sort of identity that is maintained in successive generations and distinguishes it from other systems that rely on the same chemistry in the same environment. In this paper we try to take this next step,

[1] As noted elsewhere (Letelier et al., 2006), we consider that *replacement* conveys the intended meaning better than Rosen's term *repair*.

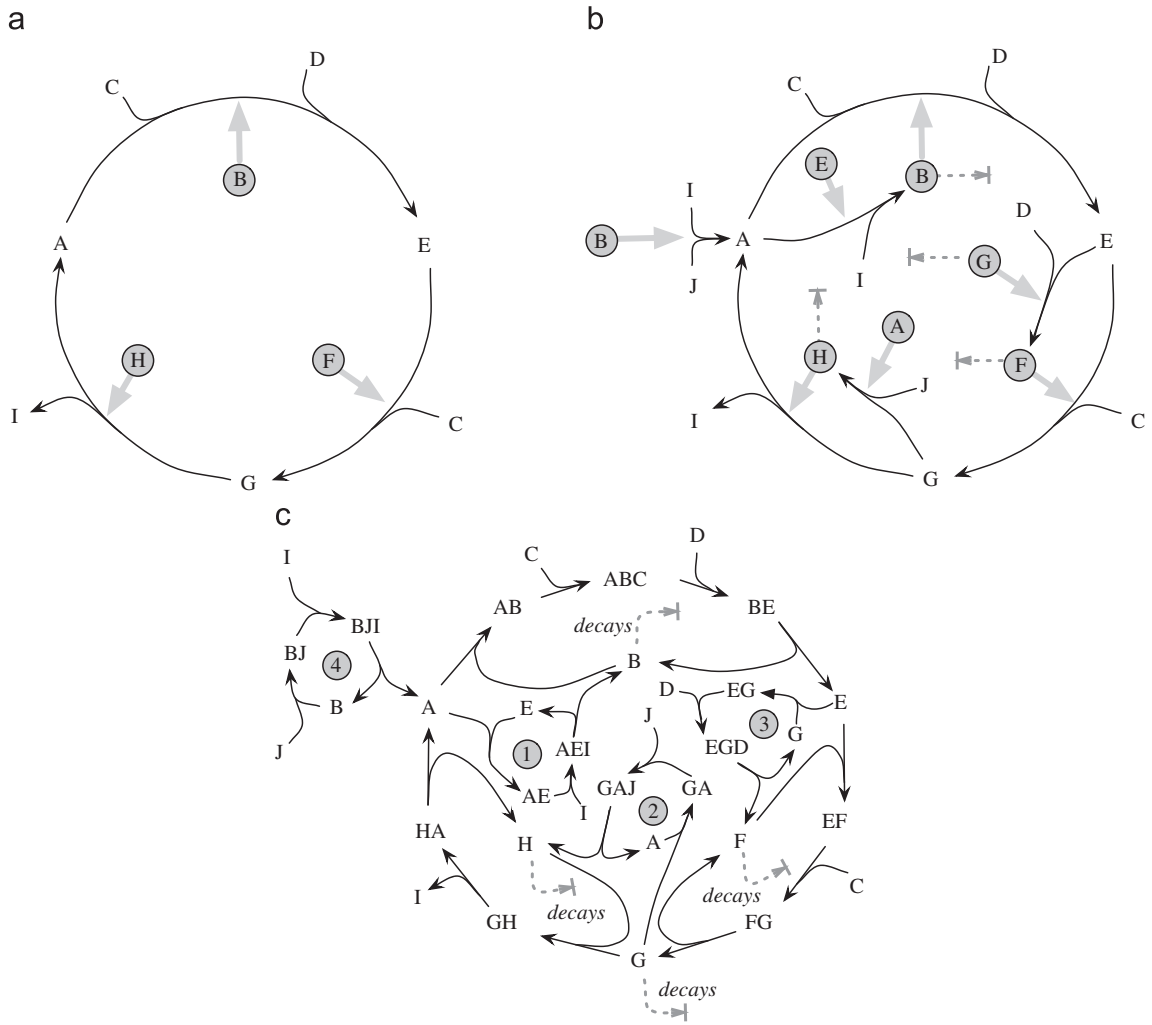

Fig. 1. An (M, R) system. This Figure summarizes the argument that the apparent lack of organizational invariance in a simple model of an (M, R) system is an artefact of the standard way of representing catalytic processes as black boxes rather than as explicit sequences of chemical steps (Cornish-Bowden and Cárdenas, 2007). (a) The simplest version of this model of metabolism provides no explanation of the existence of the catalysts B, F and H, or of the composite catalyst composed of A, E and G. (b) Replacement of the catalysts is indicated explicitly, but as summarized catalytic processes rather than as sequences of chemical steps. In this representation the assignment of particular catalysts to particular steps is arbitrary, just one out of 117,649 possibilities. As A, E and G are different states of a single catalyst the decay of A and E is implicit in the decay of G. (c) All steps are shown explicitly and the ambiguity disappears.

and to show how a model similar to Fig. 1 might have such an identity.

It is difficult to know to what extent Leduc (1912) considered his inorganic structures to be alive, but he certainly considered them to have much more than a superficial similarity to living organisms. For him the essential characteristic of the living state was its "form" that resulted from purely physical forces, i.e. osmotic pressure, rather than from any particular combination of substances and chemical processes, and he minimized the importance of using any particular material during the search for the synthetic forms. This emphasis on physical forces to the exclusion of other considerations is extreme, of course, but there is also the opposite danger of ignoring the importance of physical forces altogether in determining the forms of organisms.

In discussing the fungi in Fig. 2b, Leduc commented that "the feet of the osmotic mushrooms are fibrous, the surfaces of the caps are smooth like those on the right, or covered with little scabs like those on the left, and the lower surface is layered or perforated. This similarity, compared with natural mushrooms, of general form, of details and of structure, is extremely worthy of attention." In effect he provided early examples of a principle now thoroughly understood from studies of fractals (Mandelbrot, 1977), that repetitive application of very simple rules can generate complex structures. Observation of a complex structure is thus no guarantee of a complex foundation; more important for the understanding of life, the existence of a complex foundation does not guarantee the emergence of a complex property such as life either.

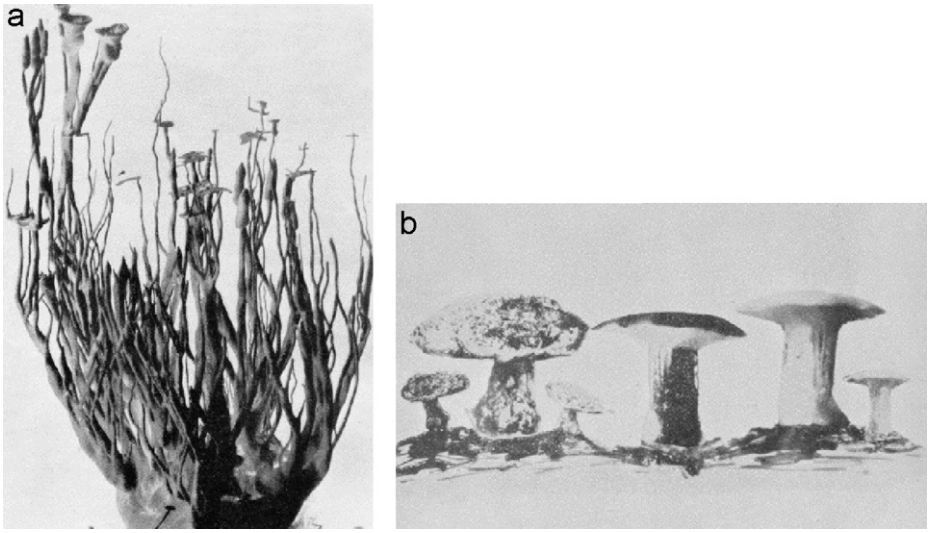

Fig. 2. Examples of inorganic "vegetation". If crystals of inorganic salts are left undisturbed in suitable environments they grow systematically into structures that superficially resemble living organisms, though they are not living entities at all. The illustrations are reproduced from (a) Fig. 48 and (b) Fig. 41 of Leduc (1912).

3. Conditions for an organizationally invariant (M, R) system

A number of important assumptions that are implicit in Fig. 1 need to be made explicit:

1. A particular set of molecules C, D and J are available from the environment in essentially unlimited quantities.
2. A suitable partition exists to separate the system from its environment.[2]
3. The necessary external molecules are capable of diffusing across the partition into or out of the system.
4. The molecules C, D, I and J have chemical properties that allow them to participate in the reactions shown in Fig. 1c, which imply the capacity to support the existence of a cycle.
5. The molecules C, D, I and J do not have chemical properties that would allow them to participate in any reactions not shown in Fig. 1c at rates high enough to interfere with the processes shown.
6. The thermodynamic properties of the overall reaction $2C + D \rightarrow I$ are such that it is essentially irreversible in the direction shown.

The last point is equivalent to assuming that C and D are compounds of sufficiently high energy to satisfy the energetic needs of the system. In more complex models energy management would need to be considered in more depth, as for example in a recent model of a "self-maintaining system" (Olasagasti et al., 2007), which is discussed in relation to the stoichiometric analysis of metabolic networks elsewhere in this issue (Montero et al., 2008).

If all the assumptions are true then the system will exist and will maintain its organization. It may be objected that these assumptions, taken together, are not very plausible. That is not the point, however, because nobody supposes that life itself is so likely to occur that it will arise spontaneously as soon as the right molecules are available. The point is that none of the listed assumptions is contrary to any law of chemistry.

4. What is life?

The title of this section reproduces the question put by Schrödinger (1944) in his epoch-making book of the same name, which Peretó et al. (2005) have recently put in the context of studies over the subsequent 60 years. Schrödinger identified some major characteristics of living systems and pointed the way to understanding how life was compatible with the laws of physics, but he did not satisfactorily define how a living system is different from a non-living one. As Maturana and Varela (1980) pointed out, no one has any difficulty in making the distinction in practice, despite the great difficulty of supplying an unambiguous definition. If we exclude occasional aberrations such as the belief of some physiologists in the early part of the last century that inorganic structures similar to that illustrated in Fig. 2 could be considered "alive", no one, given a list such as {human, tree, worm, mule, fly, coral, radio, car, computer, robot, moon, tide}, will hesitate to class the first six elements as living and the last six as non-living; how is this classification to be justified, however? Many biologists will assert that the capacity to reproduce and evolve by natural selection is the defining characteristic of a living organism, but such a definition would exclude the mule, though no one doubts that a mule

[2]In modern organisms this partition is of course made by the organism itself, but we assume that in the primitive world suitable inorganic partitions occurred spontaneously (see Section 6).

can be alive. Other simple-minded definitions likewise fail to include all the living elements in the set, or fail to exclude all the non-living elements.

The theory of autopoiesis (Maturana and Varela, 1980) takes the argument to a more profound level, as does that of the organizationally invariant (M, R)-system (Rosen, 1991). These two approaches have more in common (Letelier et al., 2003) than is often realized, and both give importance to the need of a living system to maintain its organization in the face of changes in its environment and degradation of its components. This is crucial, certainly, but, as Fig. 1 illustrates, it is insufficient because a living system has an identity that distinguishes it from other systems that have similar properties. One earthworm is not identical to another earthworm. Even a wild-type bacterial cell is not identical to another one of the same species (except by chance), because any mutations that may have occurred at its formation allow it to be identified, and variations between the individual cells in a bacterial culture are typically large (Kaprelyants and Kell, 1992).

A system of the kind illustrated in Fig. 1, however, has no identity. If the conditions necessary for it to come into existence occurred in two different places there would be nothing apart from location to distinguish one of the two systems from the other. Even if chance variations in the environment caused some differences to appear there would be no reason for these differences to be maintained. We need to ask, therefore, how to impart identity to an organizationally invariant (M, R) system.

5. Identity in an organizationally invariant (M, R) system

There is no reason to doubt that the identity of a modern organism resides in its nucleic acids, which are, nonetheless, molecules that obey the ordinary laws of chemistry with nothing added: if, and only if, a cell contains a particular DNA molecule in its genome then this molecule will also appear in daughter cells produced by cell division, but no such characteristic applies to any other components of the cell. The presence of chlorophyll, for example, in a cell provides no guarantee that all daughter cells will also contain chlorophyll, or that the parent cells did. Even a molecule such as glucose that is probably present in the overwhelming majority of metabolizing cells is there not simply because it was in the parent cells but because the whole of metabolism is there.

Fig. 3 shows a way in which the (M, R) system of Fig. 1c might be modified to address the question of identity. Despite its simpler appearance Fig. 3a represents exactly the same system as Fig. 1c except that it is now separated from its environment by a barrier or membrane through which the molecules C, D and J can pass, together with I if it is considered, as drawn in the figure, to be external, but the other molecules in the system cannot. The decay reactions that were shown explicitly in Fig. 1b must be assumed to occur in Fig. 3a even though they are not drawn. Likewise, although catalytic cycles are not drawn explicitly in the manner of Fig. 1c they should be assumed. We leave unanswered the question of how the molecules B and G[3] came to be inside the membrane in the first place. The point is that once they are there the system as specified will maintain them without external influence: the system is closed to efficient causation. As noted previously (Cornish-Bowden et al., 2007; Cornish-Bowden and Cárdenas, 2007), it is important not to confuse efficient causation with material causation, or to suppose that closure to efficient causation, a description of the logical organization of an organism, implies closure to material causation. The latter is not merely obviously false from the observation that all organisms consume some chemical materials and excrete others; it would also be impossible, as the energetic constraints on organisms require them to be thermodynamically open systems.[4]

Suppose, however, that these are not the only chemical species that can participate in appropriate reactions. Suppose, more precisely, that G′ is a molecule that resembles G and is also capable of reacting with C to produce I + A, but when it catalyses combination of D with E the product is F′ rather than F, which resembles F but causes G′ instead of G to be produced when C reacts with E. This different system is illustrated in Fig. 3b. Again, we do not ask how G′ rather than G came to be inside the membrane, but if it is there the system will maintain itself. As an example of what we have in mind here, consider the existence in nature of two different forms of the enzyme lactate dehydrogenase: both use the same reactants, (achiral) pyruvate and NADH, but the product may be either L-lactate or D-lactate, depending on which form of the enzyme exists in the organism concerned; likewise hexokinase and fructokinase can both act on fructose and ATP as substrates, but with hexokinase the products are fructose 6-phosphate and ADP, whereas with fructokinase they are fructose 1-phosphate and ADP. These enzymes are, of course, highly complicated molecules, and whether a similar variation in products from the same reactants could occur with the very simple catalysts likely to have been available at the origin of life is a point that will need further study.

It follows that Fig. 3 shows two different (M, R) systems, both capable of similar chemistry, but both maintaining their distinct identities. As they use the same raw materials and release the same product they can in principle compete with one another if they occur in the same environment. If, for example, F′ and G′ are better catalysts than F and G then the alternative version of the system will react faster

[3]It is not necessary to have the complete set {A, B, E, F, G, H} present from the outset, because if B and G are present (as well as the external molecules C, D, I and J) the others can be produced internally by network expansion (Handorf et al., 2005).

[4]The image of the Ouroboros, the dragon that nourishes itself by consuming its own tail, is visually appealing when it is used as a symbol of life (Gnaiger et al., 1994), but it is seriously misleading if taken too literally, because it uses a false idea of material causation to represent a true (but subtle and difficult) idea of efficient causation.

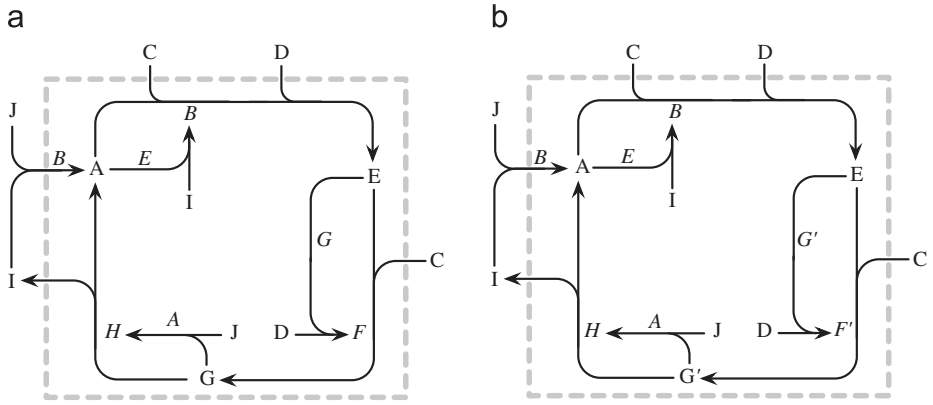

Fig. 3. Two distinguishable (M, R) systems. (a) At left is shown a representation of the model of Fig. 1c with the addition of an enclosing barrier or membrane (assumed to occur naturally, and independently of the system itself: see Section 6). The decay reactions that were shown explicitly in Fig. 1b should be assumed to be present even though, for the sake of simplicity, they are not drawn. Action of A, B, E, F, G and H as catalysts is shown simply by writing them in italics next to the reactions they catalyse, but all the catalytic processes should be understood as cycles of chemical reactions, exactly as in Fig. 1c. (b) At right is shown an alternative system that uses the same external molecules C, D and J, and releases the same molecule I into the environment. Inside the barrier, however, it differs in using molecules F′ and G′ instead of F and G, respectively. In both cases I is drawn as an external species, but nothing in this paper depends on that, and it could equally be considered as confined to the interior.

than the original one and will generate a higher fraction of the total of I produced. Note that the barriers separating the two systems are essential for competition to occur between them: if all the molecules mentioned were present in a single compartment they could illustrate alternative pathways within one organism, but they could not illustrate competition between two different ones.

6. Self-organization at the origin of life

The system illustrated in Fig. 1 is, of course, vastly simpler than the metabolism of any modern organism, and it should be compared not with present metabolisms but with systems like the cycles proposed by Wächtershäuser to have existed at the origin of life, at the beginning of the transition from abiotic geochemistry to biochemistry, when no enzymes or nucleic acids existed (Maden, 1995; Wächtershäuser, 1990). Indeed, it must be much simpler than Wächtershäuser's cycles, which already include such complexities as an archaic version of the tricarboxylate cycle. Likewise the primitive metabolism discussed by Meléndez-Hevia et al. (2008) elsewhere in this issue is much more complex than Fig. 1.

As present-day networks of biochemical reactions are the result of natural selection they can be expected to show characteristic features that distinguish them from chemical reaction networks of inanimate matter or random networks (Ebenhöh et al., 2004). The only reactions that can take place are those that can use the available substrates and these will produce new compounds that may be used by further reactions, and the interdependencies of the reactions in a network can be evaluated (Handorf et al., 2005). It is generally accepted that the primaeval conditions allowed the formation of some aminoacids and nitrogen bases (Orgel, 1998), carbohydrates being much less common than aminoacids (Larralde et al., 1995). These compounds could have been part of the initial blocks, with metal sulphides such as FeS and NiS, and some organic compounds, as catalysts. However, the major problem of the low concentrations of all available reagents has led to the view that the early organisms were chemoautotrophic in origin rather than heterotrophic, i.e. they could subsist on purely inorganic sources of carbon and other elements and required no organic molecules from their environment.

Appearance of an organizationally invariant (M, R) system needs more than just a set of reactions; it also requires a semipermeable boundary to separate it from the aqueous environment. Martin and Russell (2003), for example, have argued that pyrite (FeS) precipitates could have satisfied this crucial requirement for compartmentation, but other hypotheses, such as the spontaneous self-organization of amphiphilic molecules into vesicles, are preferred by others (Deamer, 1997; Hanczyc and Szostak, 2004; Luisi, 2007). In any case, inorganic compartmentation could have played a crucial role at the very beginning, favouring the subsequent appearance of other types of compartment; the different hypotheses are not mutually exclusive. To favour the possibility of reaction, compounds must be bound to the surface where they are contained but be capable of lateral migration to permit encounters with other reactants, conditions well satisfied by ionic binding. To bind to pyrite a hydrophilic compound requires at least two negative charges on the same or different groups as in PO_3^{2-} or S^{2-}. The metabolic pathways known to be ancient contain many such polyanionic metabolites and cofactors, which therefore have potential for binding to surfaces. Studies by Heinrich's group have revealed that metabolites crucial for allowing the complexity of the network to increase include adenosine 5-phosphosulphate and 3-phosphoadenosine 5-phosphosulphate, both of which play an important role in the sulphur metabolism of many microorganisms (Handorf et al., 2005), and this

metabolism is considered ancient. Even more interestingly, with their method of simulating network expansion in which each "generation" allows incorporation of compounds that can be synthesized from those already in the network, they found that exactly the same set of compounds could be generated from a set of very simple seed compounds, CO_2, NH_3, H_3PO_4 and H_2SO_4 as could be obtained from adenosine 5-phosphosulphate, the only difference being that 61 "generations" would be needed instead of 55. It follows that simple chemicals that are likely to have been present under prebiotic conditions are possible seeds for very complex biochemical reaction networks.

A qualification is necessary, because the criterion of which reactions were possible used by Handorf et al. (2005) was the existence of an enzyme catalysing it in the KEGG (Kyoto Encyclopedia of Genes and Genomes) database (Kanehisa and Goto, 2000). However, modern enzymes are highly complex molecules, and one must suppose that much simpler (and, of course, much less efficient) catalysts were used: as we have noted previously, ornithine is capable of catalysing the urea cycle in the absence of any protein (Cornish-Bowden and Cárdenas, 2007). Some reactions that are possible today because suitable enzymes exist could not have occurred at useful rates in prebiotic conditions, whereas others that are too slow to contribute to modern metabolism would have been fast enough to be part of a system with no competitors. It follows that there cannot be a one-to-one correspondence between the reactions in the KEGG database and those that were important in prebiotic conditions. Even the "RNA world" considered by many specialists to have preceded the present protein-dominated biosphere must have been far more complex than what existed in the first organisms, which probably depended on catalysts chemically more similar to present-day coenzymes than to either protein or RNA molecules. Note also that the first primitive systems would probably not have been closed in Rosen's sense, but closure could occur when some compounds were able to perform multiple functions, as illustrated in Fig. 1, multifunctionality of components being apparently essential for obtaining an (M, R) system (Cornish-Bowden and Cárdenas, 2007).

7. Discussion

A major principle that has emerged from studies of the circular organization of metabolism is that the circle of efficient causation can only be closed if some (and in reality probably many) of the catalysts used by organisms fulfil multiple functions. Multifunctionality, or "moonlighting", is increasingly observed (Tipton et al., 2003; Park et al., 2005; Sriram et al., 2005), but it is much more than just an interesting observation about living organisms, because it is essential to their survival (Cornish-Bowden and Cárdenas, 2007). The use of a small number of nucleic acid molecules to encode the sequences of all the proteins needed by an organism is itself an example of multifunctionality: just as the usual distinction between enzymes and metabolites is arbitrary, the usual distinction between enzymes and informational macromolecules such as DNA is also arbitrary, because a DNA molecule satisfies the definition of an enzyme, being a biological macromolecule that acts as a catalyst, capable of participating in many different chemical reactions and of being regenerated unchanged at the end of each.

Despite the importance of metabolic closure (or metabolic circularity), it is largely absent from current representations of metabolism. For example, in studies of scale-free topology metabolism is represented as a graph, but without the notion of closure, and such studies do not exploit closure but focus on the connectivity of nodes (Fell and Wagner, 2000). As another example, the "bow-tie" structure of metabolism (Csete and Doyle, 2004), derived from cybernetics, involves a bottleneck design of metabolism with many feed-back loops, but again without metabolic closure. It treats metabolism as an essentially linear process.

In this paper we have been searching for a general principle for formalizing our understanding not only of how an organism maintains its organization, but also of how two different organisms living in the same environment and fulfilling the same chemistry (as seen by an outside observer) can maintain distinct identities that can be transmitted to their descendants. It would be absurd to claim that the simple model illustrated in Fig. 3 provides a full answer to this question, but we believe it may represent a step towards an answer, and in any case we hope we have drawn attention to some problems that a theory of life will need to be able to explain.

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