A Self-referential Equation, $f(f) = f$, Obtained by Using the Theory of *(M,R)* Systems: Overview and Applications.

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Abstract

The notion of metabolic closure is presented and analyzed in terms of Robert Rosen's theory of *(M,R)* systems. Recent results concerning *(M,R)* systems are reviewed, specially those defining self-referential equations like $f(f) = f$. We related *(M,R)* systems to *Autopoiesis*, another theory centered on metabolic closure, and we speculate how an algebraic view of metabolism could be utilized in the analysis of metabolic pathways. As a helping metaphor we consider necessary to build, in the context of *(M,R)* systems, analogous to Tellegen's theorem, but based purely on closure arguments, to metabolic networks.

1. Introduction: Two theories of Metabolic Closure

The 20^{th} century saw many theories concerned with the "real" nature of living systems. They include, more or less in chronological order, the *Osmotic Forest* of Stephane Leduc, the *General System Theory* of Von Bertalanffy, *Cybernetics* by Norbert Wiener, the *Self-reproducing automata* of Von Neuman, the notions of *Hypercyles* by Eigen and Schuster, and *Autocatalytic sets* by Kauffman, and the metaphor of information which identifies "life" with DNA. To this list we should add the *Artificial Life* movement, an active, eclectic and interesting field lacking a central conceptual core [2]. In this crowded field, two theories stand out for their focus on the *circular organization* of cellular metabolism. These two theories are: *(M,R) systems* created by Robert Rosen (1958) [12] and the notion of *Autopoietic systems* set forth by Maturana and Varela in the early 1970s [4]. These two

theories reject the computer metaphor as valid to understand cellular organization or brain function and do not claim that reproduction or evolvability are defining traits of living systems; instead they consider central the undeniable fact that metabolism produces metabolism, which produces metabolism (*metabolic closure*).

In this manuscript we first introduce the theory of *(M,R)* systems. Second, we show how, from this theory, it is possible to find self-referential objects defined by the puzzling property of $f(f) = f$. Third, we introduce *Autopoiesis*, another theory centered on closure and we explain the relationship between these two theoretical views of living systems. Fourth, we explain why metabolic closure is an idea that must be incorporated in current theoretical models, especially in the new field of Systems Biology. Finally we speculate on the directions that mathematical viewpoints must follow to apply the idea of closure to cellular dynamics.

2. Overview of *(M,R) Systems*

First it is necessary to state that Rosen's work, which spans 40 years (1958-1998), cannot be summarized in a few pages. His model begins with a very simple observation: every biochemical reaction is catalyzed by an enzyme M_i that acts as a kind of operator as it transforms some *inputs* (the reactants on the left-hand side) into *outputs* (the reactants on the right-hand side) . But enzymes M_i are physical entities suffering some degree of wear and tear, and in any case many proteins are degraded functionally, to ensure that they are inactive when they are not needed. Thus, in the cell, every enzyme would eventually cease to exist if it were not continuously replaced by a set of processes R_i that have the specific function of maintaining M_i in a given concentration range. A system organized according to this logic is an *(M,R)* system. [Note: In Rosen's writings M refers to *metabolism* and R to *repair*, but as in the cell enzymes are not repaired like cars but rather continuously replaced by the processes of transcription and translation, we have changed his nomenclature and call R_i replacement]. But the same argument of wear and tear applied to enzymes can also be applied to every subsystem R_i as they are also made of molecules. Thus which are the entities that keep an adequate amount of R_i? , who are the repairers of the repairers? Rosen's solution, to avoid the problem of infinite regress, was to realize that in *(M,R)* systems with a sufficient degree of complexity the system itself, acting as a coherent totality, will continuously produce every R_i. These systems are the *(M,R) systems with organizational invariance*, which (for Rosen) are equivalent to living systems (Figure 1.). His intuition is an easy idea to follow, but the mathematical framework that he used to prove it is particularly difficult to understand, and even professional mathematicians have problems understanding his basic construction. In essence Rosen's mathematical view of metabolism consisted in identifying enzymes with *mappings* and the biological functions of *replacement* or *organizational invariance* with *procedures to select* mappings. In presenting his ideas Rosen used some basic elements of the Category Theory and also he never gave biological or mathematical examples. Thus it is not surprising that his writings have been ignored by practicing biologists. Recently some of us have clarified some important aspects of *(M,R)* systems that reveal the deep algebraic viewpoint contained in them [7].

2.1. Algebraic formulation of (M,R) systems

The algebraic formulation of *(M,R)* systems starts with a very simple model, in effect every biochemical reaction such as the one catalyzed by the enzyme *valyl-tRNA synthetase* (with the identification number EC 6.1.1.9)

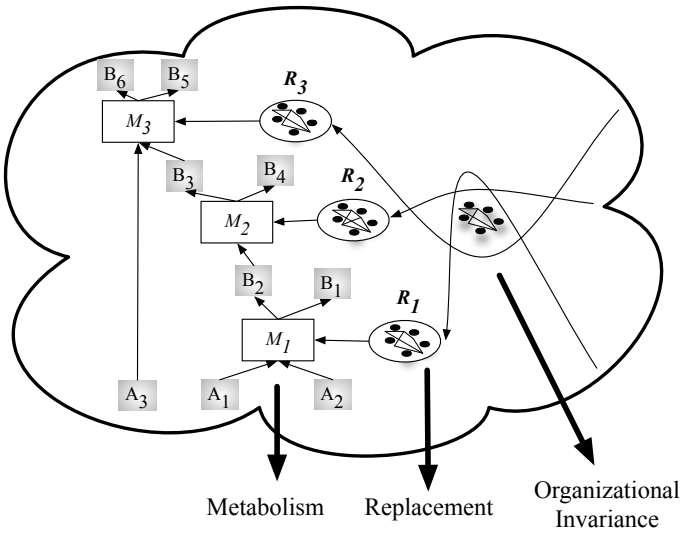

Fig. 1. Organizationally invariant *(M,R)* system. In this type of system three processes are intertwined. *Metabolism* represented by M_i transforms metabolites and is embodied by enzymes. *Replacement* represented by R_i maintains (by synthesis) a workable amount of each M_i . *Organizational invariance* replaces all R_i. These last two functions are, themselves embodied in subnetworks containing enzymes with their corresponding R_i, represented by a small network with dots. *Organizational invariance* (termed β by Rosen) depends on the totality of the *(M,R)* system. Superficially this machinery seems a nested chain of systems and subsystems, but as the replacement and organizational invariance biological functions also contain enzymes M_j, which also have to be replaced, the fundamental property of *circular organization* arises.

$$\texttt{ATP + L-valine + tRNA}^{\texttt{Val}} \overset{Enzyme=6.1.1.6}{\longrightarrow} \texttt{AMP + pyrophosphate + L-valyl-tRNA}^{\texttt{Val}}$$

can be viewed as the following transformation

$$a_1 + a_2 + a_3 \overset{Enzyme=6.1.1.9}{\longrightarrow} \mathrm{b}_1 + b_2 + b_3$$

As enzymes are not totally specific because other molecules similar to L-valine can participate in the reaction it is possible to define A_1 as the set of molecules resembling L-valine (a_1) that could participate in the above reaction. For example, another aminoacid, threonine, is sufficiently similar to valine to be mischarged by *valyl-tRNA synthetase* at a sufficiently high frequency to require a separate enzyme to correct the error. Thus the enzyme (6.1.1.9) can be viewed as a specific mapping M between two sets defined by Cartesian products:

$$\begin{aligned} M : A_1 \times A_2 \times A_3 &\longrightarrow B_1 \times B_2 \times B_3 \\ (a_1, a_2, a_3) &\mapsto M((a_1, a_2, a_3)) = (b_1, b_2, b_3) \end{aligned} \tag{1}$$

This formalization can be generalized to embrace the complete metabolic network made of thousands of coupled biochemical reactions as:

$$\begin{aligned} M_{met} : A = (A_1 \times A_2 \times A_3 \times \cdots \times A_p) &\longrightarrow B = (B_1 \times B_2 \times \cdots \times B_q) \\ \mathbf{a} = (a_1, a_2, a_3, \ldots, a_p) &\mapsto M_{met}(\mathbf{a}) = (b_1, b_2, \ldots, b_q) = \mathbf{b} \end{aligned} \tag{2}$$

or in more compact notation between the (huge) sets A and B.

$$\begin{aligned} M_{met} : A &\longrightarrow B \\ \mathbf{a} &\longmapsto M_{met}(\mathbf{a}) = \mathbf{b} \end{aligned} \tag{3}$$

where M_{met} represents the action of the overall metabolism and can be interpreted as a type of *generalized enzyme* that transforms one instance of molecules ($\mathbf{a} = (a_1, a_2, a_3, \ldots, a_p) \in A$) into an instance of molecules ($(b_1, b_2, \ldots, b_q) = \mathbf{b} \in B$). As many metabolisms are theoretically possible between sets A and B we define $\mathcal{M}$ as the set of all possible metabolisms (mappings) between A and B, to stress the fact that metabolism is a mapping we write f instead of M_{met}). In all his writings, Rosen assumed that:

$$\mathcal{M} = Map(A, B) = \text{set of all mappings between sets } A \text{ and } B \tag{4}$$

Thus, if metabolism (i.e. the collective action of thousands of enzymes) can be interpreted as a mapping, how one should interpret, the action of R_i, the function of replacement? We can formalize such a notion by considering that what it is needed is an operator, called a *selector* and denoted by Φ, that, using as input the *metabolic state* of the organism (which can be identified as the collective result of all the biochemical reactions thus with a certain b) generates f (which represents R). Thus immediately we have that:

$$\Phi(b) = f, \text{ with the condition } b = f(a) \text{ (for some } a \in A) \tag{5}$$

This condition immediately defines the Domain (B) and Range ($Map(A, B)$) of Φ. Then an *(M,R)* system has at least the following mathematical structure, which is based on the concurrent action of mappings f and Φ acting in synergy:

$$\begin{aligned} &\Phi : B \longrightarrow Map(A, B) \\ &b \longmapsto \Phi(b) = f \text{ (with } b = f(a) \text{ for some } a \in A) \\ &\Phi \in Map(B, Map(A, B)) = \text{set of possible selectors} \end{aligned} \tag{6}$$

In this scheme b is produced by f, which is produced by Φ. In order to have a fully organizationally invariant *(M,R)* system Φ must also be generated (Rosen uses the word entailed) from within the mathematical structure. In other words we need to find an entity that, in the strict boundaries of this formalism, produces Φ, using b (metabolic states) or f (metabolism) as variables. The solution to this problem constitutes the kernel of Rosen's work [7]. Formally what is needed is a function β with a property like $\beta(f) = \Phi$. Thus β is a procedure that, given a metabolism f produces the corresponding selector Φ. For β to exist it is required that the equation $\Phi(b) = f$, for every Φ must have *one and only one solution*, a most demanding condition if M=$Map(A, B)$. Thus the operation of an organizationally invariant *(M,R)* system corresponds to the following three mappings acting in synergy (f, Φ, β):

$$A \xrightarrow{f} B \xrightarrow{\Phi} Map(A, B) \xrightarrow{\beta} Map(B, Map(A, B)) \tag{7}$$

$$f(a) = b, \Phi(b) = f, \beta(f) = \Phi \tag{8}$$

Unfortunately Rosen never clarified, either with examples or by clear theoretical analysis, the nature of β. He only showed that its existence was mathematically consistent, and sometimes he even

recognized that its existence was mathematically difficult [13]. Other authors have either introduced its existence as a specific postulate [1] or, confused by the apparent chaotic situation, declared the complete approach of Rosen "invalid" [5]. Recently some of us have succeeded in clarifying the nature of β as the inverse of an evaluation map, also providing the first clear, and simple, mathematical examples of *(M,R)* systems. The crucial context where Rosen's construction could make sense is if the sets of Metabolisms ($\mathcal{M}$) and of Selectors ($\mathcal{S}$) are heavily restricted. It was found [7] that Rosen's scheme can only work if:

$$|A| \approx |B| \approx \mathcal{M}| \approx |\mathcal{S}|$$

which means that the the cardinality (i.e. "size") of $\mathcal{M}$ must be commensurable with B and not with the cardinality of $Map(A, B)$ (this is an enormous simplification for networks with thousands of metabolites). In this scheme β (which is an element of $Map(\mathcal{M}, \mathcal{S})$) can be interpreted as equivalent to some some b. This is an absolutely crucial element in Rosen's construction as it shows that a procedure to select mappings is equivalent to a set of molecules. This identification (see Figure 2.) is the core of metabolic closure as it avoids the infinite regress apparently implied by the "simple" (box and arrows) definition of *(M,R)* systems (see Figure 1.). Also, implicit in $|B| \approx |\mathcal{M}|$, we have that the set of permissible metabolisms should be a set of mappings that preserve some type of hidden mathematical structure in B. Thus instead of $\mathcal{M} = Map(A, B)$ we must impose restrictions like $\mathcal{M} = H(A, B)$, where $H(A, B) \subset Map(A, B)$, is a set of "structure-preserving mappings" (such as endomorphisms).

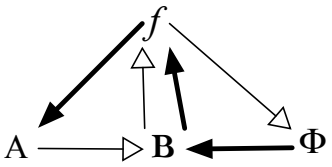

Fig. 2. Generative diagram. Rosen summarized his research with this diagram, which shows the generative relations between maps f, Φ and β. If an element acts as a function, a solid arrow starts from it, if an element acts a variable, it generates a blank arrow. As β is equivalent to some b, the diagram shows f, Φ and β to be mutually entailed (see [11], chap10). Rosen summarized the diagram in the elegant phrase: "Organisms are closed to efficient causes".

2.2. Closure in an infinite chain of (M,R) systems

The mathematical structure of an organizationally invariant *(M,R)* system is given by:

$$A \xrightarrow{f} B \xrightarrow{\Phi} H(A, B) \xrightarrow{\beta} H(B, H(A, B))$$

with the following boundary conditions:

$$\begin{aligned} &f(a) = b, \text{ with } f \in H(A, B) = C_0 \\ &\Phi(b) = f, \text{ with } \Phi \in H(B, H(A, B)) = C_1 \\ &\beta(f) = \Phi, \text{ with } \beta \in \ H(H(A, B), H(B, H(A, B)) = C_2 \end{aligned}$$

β being the inverse of the (putatively invertible) mapping Ev_b for "evaluation at b" .

What happens if we extend the above construction "beyond-β" with the same logic? First let us consolidate notation with $C_0 = A$, $C_1 = B, C_2 = H(C_0, C_1)$, and in general $C_n = H(C_{n-2}, C_{n-1})$. With respect to the mappings, we define $\Phi_0 = f, \Phi_1 = \Phi, \Phi_2 = \beta$, and for the transformed elements we define $c_0 = a$, $c_1 = b$, $c_2 = f$. The three building relations of an *(M,R)* system become, in this new notation,

$$c_1 = \Phi_0(c_0) \text{ (equivalent to } f(a) = b)$$
$$c_2 = \Phi_1(c_1) = \Phi_0(\text{equivalent to } \Phi(b) = f)$$
$$c_3 = \Phi_2(c_2) = \Phi_1(\text{equivalent to } \beta(f) = \Phi)$$

We may extend these recurrence relations as:

$$c_{n+1} = \Phi_n(c_n) = \Phi_{n-1}, \text{so that in fact } \Phi_n(\Phi_{n-2}) = \Phi_{n-1}$$

Thus an infinite *Rosen's chain* has the following structure

$$\begin{array}{rcccl} C_0 & \xrightarrow{\Phi_0} C_1 & \xrightarrow{\Phi_1} C_2 & \xrightarrow{\Phi_2} \cdots & C_n \xrightarrow{\Phi_n} C_{n+1} = H(C_{n-1}, C_n) \\ c_o & \longmapsto c_1 & \longmapsto c_2 & \longmapsto \cdots & c_n \longmapsto c_{n+1} = \Phi_n(c_n) = \Phi_{n-1} \end{array}$$

If we assume that these sequences converge to limits Φ_∞ and C_∞, (VARELA84), then passing to the limit in the above equations, we have

$$\begin{array}{rcl} C_n = H(C_{n-2}, C_{n-1}) & \xrightarrow{n\to\infty} & C_\infty = H(C_\infty, C_\infty) \\ \Phi_{n-1} = \Phi_n(\Phi_{n-2}) & \xrightarrow{n\to\infty} & \Phi_\infty(\Phi_\infty) = \Phi_\infty \end{array}$$

These two limits exhibit interesting properties:

- $C_\infty = H(C_\infty, C_\infty)$, which means that C_∞ is a reflexive domain, a structured set that is equal to the set of its endomorphisms;
- $\Phi_\infty(\Phi_\infty) = \Phi_\infty$ and so Φ_∞ is a self-referential object, i.e. a solution to $f(f) = f$.

Thus Rosen's basic construction behind his central result could be extended to an infinite chain to define limiting objects with interesting properties for the study of circularity. Surprisingly, it seems that Rosen, starting from a biological insight, was the first to notice how to define this type of mathematical object. A question not addressed here is to find the relation between Φ_∞ and Rosen's β (the inverse to the evaluation at b map Ev_b).

3. Autopoiesis: another theory centered on closure

In 1973 the Chilean Neurobiologists Humberto Maturana and Francisco Varela introduced the concept of Autopoietic systems (auto= self and poiesis = producing) as a theoretical construction on the nature of living systems centered on the circular organization of metabolism. This notion is a given in autopoiesis, and is immediately clarified by the definition of an autopoietic system:

> **An autopoietic system is organized as a bounded network of processes of production, transformation and destruction of components which: (1) through their interactions and transformations continuously regenerate and realize the network of processes that produced them, (2) constitute the system as a concrete entity in the space in which the components exist by specifying the topological realization of the system as such a network**.

Thus in an Autopoietic system the result of any given process is the production of components that are eventually transformed by the rest of the network into the components transformed by this process. This property is a complementary view of the idea of metabolic closure in *(M,R)* systems. In effect instead of focusing of how enzymes are replaced (closure in Rosen's sense) in Autopoietic systems all molecules, metabolites and enzymes are treated equal, and are viewed as participating in a complex web of transformations that continuously destroys and produces them (closure in the sense of autopoietic systems).

Autopoietic systems are not mere algebraic devices based on closure. They must also self-produce the boundary that defines them as entities separate from the non-system. This apparently trivial clause has profound implications as it touches upon the problem of autonomy and also serves to weed out from the Autopoietic forest some pure formal systems. Many attempts have been made to formalize and simulate Autopoiesis. From tessellation computer models, initially done in an IBM 360 [15]to the current effort termed Computational Autopoiesis (done in *Swarm*). Also the unusual tool of *Indicational Calculus*, developed by Spencer-Brown[14] was tried by Varela [16]) to give an algebraic framework to Autopoietic systems. But none of these simulations or formal frameworks have generated clear-cut, satisfactory results as it seems that a fundamental tool, to work with closure in Autopoietic systems, is missing.

4. Why closure is important

As expected, we claim that the notion of metabolic closure is an important missing actor in current models of metabolism. It is by itself a small mystery why closure, an obvious property of cellular organization, has not been recognized as a relevant aspect. Perhaps this neglect reflects that closure is also another enunciation of the classic problem of the chicken and egg, and self-referential problems (like the liar paradox) are notoriously difficult to disentangle. Another reason could be thermodynamics which, correctly, interprets living systems as open systems. But metabolic closure is a *functional* notion that does not deny thermodynamics. In other words, living systems are thermodynamically open systems but organized under the framework of metabolic closure, the two notions do not contradict each other; they belong to different domains.

If we want to understand metabolism as a coherent working totality, we must face the unique property that almost all system components are themselves produced by the system. This condition demands a reassessment of the notions of causality and reductionism in metabolic networks.

As we have discussed elsewhere [8] numerous characteristics of classical biochemistry make more sense when the parts are explained in terms of the requirements of the whole system rather than the much more usual reverse. For example, many biosynthetic pathways are inhibited after a branch point by what is usually regarded as the end-product of the branch (in reality it is not the end of anything, but just the link metabolite at the boundary with another part of metabolism), a textbook case being provided by the inhibition of aspartokinase by lysine. However, there is nothing in the chemistry of converting aspartate into phosphoaspartate that would lead one to expect an enzyme that catalyses the reaction to be inhibited by lysine. The inhibition, in short, cannot be explained solely in terms of the components concerned, aspartokinase and lysine, but requires consideration of the system as a whole, and in particular the fact that lysine is not synthesized as an end in itself, but because it is used for protein synthesis.

In effect, for us it is a given that the "parts" of metabolism (enzymes, metabolites, macro-molecular assemblies) have intrinsic properties which are independent of the system that contains these

parts. But the interconnectedness of a system with metabolic closure could make this reductionism illusory

For example, genes and their products cannot be understood without reference to the systems in which they occur. Perturbing the expression level of any gene typically affects the levels of hundreds of different mRNAs [19], and as measurement techniques improve we may eventually need to replace "hundreds" by "thousands", or even by "all". In any case there is a general pleiotropy, predicted many years ago by Kacser [20], but long ignored, as all genes act in concert with one another and with their environments.

5. Closure, possible theoretical pathways

5.1. Thermodynamics and closure

Closure arguments could be powerful, elegant and unexpected. As an important example let us consider *Tellegen's theorem* for electrical networks, which is virtually unknown outside its original field the little-known for electrical networks (resistors, capacitors, diodes, batteries) (Figure 3.). In this type of network Kirchhoff's laws **and** the close connectivity demanded by current to flow imply that the sum over all branches of the power `(voltage x current)` consumed or produced is zero ($\sum_{k=1}^{n} V_k{}^1.I_k{}^1 = 0$). But the generalization known as *Quasi-power theorem* is almost magic. In effect, if we consider two circuits with identical topology (like circuits 1 and 2), but different components, the so-called co-product ($\sum_{k=1}^{n} V_k{}^1.I_k{}^2 = 0$) is also zero, even when no physical connection exists between the voltages in one circuit and the currents in the other! As Tellegen's theorem relates, in many contexts (electrical, mechanical, chemical), fluxes (I) with driving forces (V), it is considered a cornerstone of network thermodynamics[9].

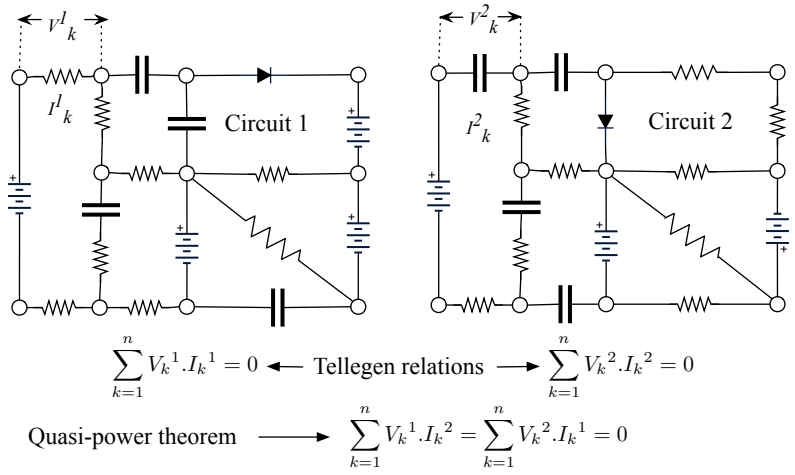

Fig. 3. Tellegen's and Quasi-power theorems. In any closed circuit (like circuit 1) the sum over all the branches of the power is zero, independently of the topology or the components. In circuits with the same topology (like circuits 1 2) the co-product (voltage in one branch multiplied by the current in the equivalent branch of the other circuit) also gives zero, even when no causal relation can exist between the voltages and currents in the two circuits. The "quasi-power" denomination comes from the product IV (electrical power) when I and V belong to the same branch, when these two variables are not causally connected IV becomes "quasi power".

5.2. Closure in current viewpoints about metabolism

Now let us consider various approaches used to study metabolism in the context of closure. The most common approach, for at least half a century, has been the study of biochemical processes in isolation (Figure 4.). This approach was valid and important as it revealed many kinetical details of biochemical reactions, however it does not contain any network thinking, though it has helped to create the necessary body of experimental data.

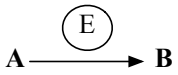

Fig. 4. A simple process, no network. Most biochemistry during the XX century was the study of simple reactions and the property of the enzyme E involved. In these studies the network disappears (conceptually and practically) as the reaction is studied in a test tube.

Second, we should consider the existence of allosteric modulation of an enzyme. This is an example of a feedback loop and it was considered to embody the systemic integration of the organism, mostly because of the influence of Cybernetics influence that considered negative loops to be central to the dynamics of complex systems (Figure 5.). But in this simple system neither the enzymes or reactant A are produced by the system.

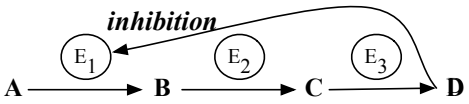

Fig. 5. A linear chain with negative feedback. Many enzymes have secondary (allosteric) sites where molecules can modulate their catalytic efficiency. Some examples shows that the end product of a metabolic pathway inhibits the first enzyme in the pathway. This negative-feedback would assure an efficient control of the complete pathway.

Third, is the case of an *metabolically embedded* linear chain. Imagine a linear chain of enzymes operating inside its normal cellular environment (Figure 6.). Now we somehow increase the amount of enzyme E_2. Is the flux through $B \to C$ going to increase? The field of metabolic control analysis responded to this very important question and the answer is "not necessarily" [17]. For the context of algebraic biology the relevant facts, are not the details of the demonstration, but the the hypothesis behind such demonstration. Two aspect must me underlined. First, metabolites (A and D especially) are produced and consumed by the network and second, the demonstration assumes a systemic property that the network always operates in steady state. In some sense we can say that these two conditions transform metabolic control analysis into a first example of systemic thinking related to the idea of closure.

A fourth, and rather new, type of analysis come from the description of the mathematical properties of the graphs that represent metabolism [18]. It has been argued that the connectivity of the metabolic graph is peculiar and follows a scale-free law in which the average connectivity is low and only a handful of metabolites, such as pyruvate, are "well connected" (i.e. participate in many reactions). It is not our intention to review here this rapidly growing literature, but rather to point to two aspects. First, cellular organization is arbitrary segmented in (at least) three relationship domains (metabolic, signaling and regulatory), but this segmentation is artificial. Second, we must

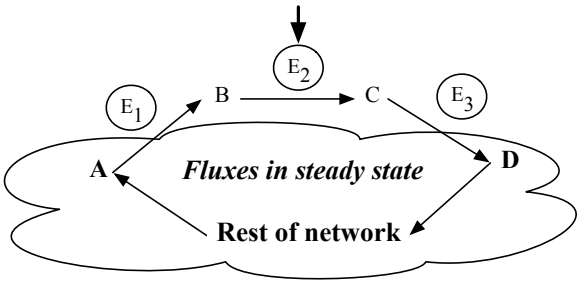

Fig. 6. A linear enzymatic chain embedded in a functional network (the approach of metabolic control analysis). Changes in enzyme concentration (like E_2) are not necessarily translated into higher fluxes. The demonstration depends on the assumption of steady state, a system-wide condition related, but not identical, with closure.

add that a "law" that applies equally well to metabolic pathways and the degree of interaction between actors is or very profound or (perhaps) trivial [3]. As this manner of studying connectivity bypasses completely the notion of closure, to focus more on static interconnectivity it misses a crucial property of metabolic networks (Figure 7.).

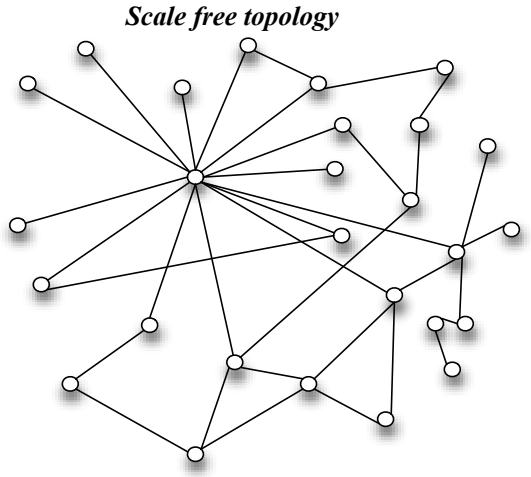

Fig. 7. Metabolism as a graph, but without the notion of closure. The analysis of connectivity of metabolic, signaling or genetic networks has become a very active field [3] [18]. The idea behind "scale-free" networks does not exploit closure as it focus on the connectivity of nodes.

We have given, in the context of closure, a brief analysis of the main current models trying to produce system-wide theories of metabolic networks. According to us what is needed is a global framework (Figure 8.) that does not see a process (like $A \rightarrow B$) in strict isolation, or only locally. Instead what is needed is a representation that (at least) does not forget that enzymes are produced by the network and that metabolites always participate in loops that (auto) generate them. Rosen's original viewpoint, enzymes as mappings and the overall stability depending on special methods to select mappings, as well as his interpretation of metabolism as a as a category and not a mere graph, must be analyzed and transformed in a workable method.

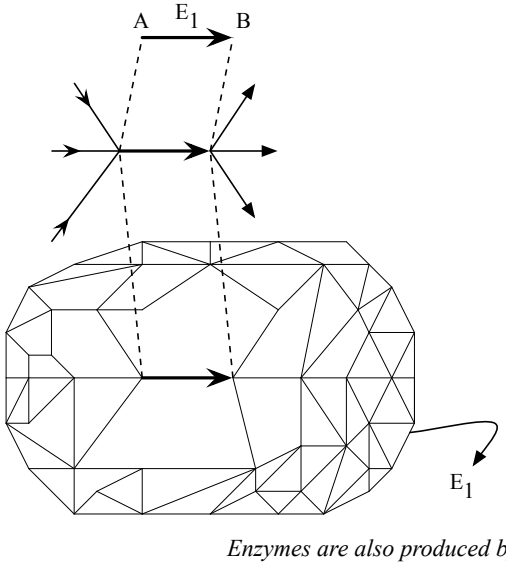

Fig. 8. A process and its many contexts. Every metabolic process can be viewed at (at least) three different levels: a) in isolation (top), b) with its local connectivity (middle) or c) embedded in the complete network, including all metabolic, signaling and regulatory processes. For example, the network must contain the processes fabricating enzymes (like E_1). The challenge is how to incorporate to this intuitive notion of closure the formal view of metabolic closure as an invertible evaluation mapping.

6. Conclusions

Closure, for metabolic networks, is an obvious idea that somehow, with the exceptions of Rosen, Maturana and Varela, has managed to remained unexplored. Perhaps this collective *blind spot* is due to our ingrained notion that every biological system must be interpreted as an input → output device or as an stimulus→ response system. In these viewpoints the internal state is specified by the stimulus or input. In contrast, as it has been shown for autopoietic and *(M,R)* systems [10], that autonomy (i.e. the property of defining the internal dynamics with very little influence of the external milieu), is a hallmark of metabolic closure. In summary metabolic closure and most current theories used to understand metabolism are fundamentally incompatible as they clash in the understanding of the proper role of autonomy (hence closure) and in the use/abuse of reductionist notions[21]. We claim that the framework opened by Rosen could be an important tool to understand metabolism. Although the framework is still in its infancy, it is surprising that Rosen's basic intuition generates so easily self-referential equations like $f(f) = f$. Perhaps the next step, in using pure algebraic ideas in biology, could be to obtain, *using only closure arguments*, an analogous of Tellegen's theorem for metabolic networks or, more immediately, to deduce some results from metabolic control analysis using closure instead of the hypothesis of steady state.

7. List of Symbols/Nomenclature

Set of mappings between sets A and $B = Map(A, B)$
Structure preserving mappings between sets A and $B = H(A, B)$
Cardinality of set $A = |A|$

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