

# Basic Autonomy as a Fundamental Step in the Synthesis of Life

**Abstract** In the search for the primary roots of autonomy (a pivotal concept in Varela's comprehensive understanding of living beings), the theory of autopoiesis provided an explicit criterion to define minimal life in universal terms, and was taken as a guideline in the research program for the artificial synthesis of biological systems. Acknowledging the invaluable contribution of the autopoietic school to present biological thinking, we offer an alternative way of conceiving the most basic forms of autonomy. We give a bottom-up account of the origins of "self-production" (or *self-construction*, as we propose to call it), pointing out which are the minimal material and energetic requirements for the constitution of basic autonomous systems. This account is, indeed, committed to the project of developing a general theory of biology, but well grounded in the universal laws of physics and chemistry. We consider that the autopoietic theory was formulated in highly abstract terms and, in order to advance in the implementation of minimal autonomous systems (and, at the same time, make major progress in exploring the origins of life), a more specific characterization of minimal autonomous systems is required. Such a characterization will not be drawn from a review of the autopoietic criteria and terminology (à la Fleischaker) but demands a whole reformulation of the question: a proper *naturalization* of the concept of autonomy. Finally, we also discuss why basic autonomy, according to our account, is necessary but not sufficient for life, in contrast with Varela's idea that autopoiesis was a necessary and sufficient condition for it.

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## **I Introduction: General and Historical Background of the Problem**

Three decades ago, Maturana and Varela proposed the idea that life, in its most fundamental core, is a certain form of organization, characterized by a recursive production dynamics. They invented the term autopoiesis (from Greek: *autos* "self" and *poiein* "creation") precisely to express the idea that the essence of the phenomenon of life is the individual organization by which each living entity recursively produces itself [38, 39, 78]. More specifically, according to the theory of autopoiesis, what defines life is a global network of relations establishing a self-maintaining dynamics in which *action* and *constitution* are one and the same thing for the system: that is, the activity of the system consists in the continuous (re)generation of all the processes and components that put it together as an operational unit.

Although the merit of developing this idea is to be given to Maturana and Varela, in one of Varela's last papers [80] he acknowledges his intellectual debt to Kant, who in the *Critique of Judgment* (§65, 66) conceives organisms as systems in which parts are formed for and from the others, constituting a whole dynamic unit outside which their very existence is meaningless. And he also acknowledges the importance and deepness of thought in this respect of Hans Jonas, a philosopher who—several years before the autopoietic theory was born—stated that the essence of life is expressed in the idea of metabolism, namely, in the continuous constitution of an identity through recursive internal processes and interactions with the environment [23]. Although the work of these authors did not have a direct influence on the elaboration of the autopoietic theory, the notion that living systems are guided by an internal principle, which arises from the recursive processes that constitute themselves, already appears there (as Varela himself points out).

Outside the philosophical domain, during the sixties and seventies, certain leading researchers in the field of theoretical biology also put forward similar ideas, claiming that the most basic feature of living systems is a network property by which a set of component production processes recurrently generates its own starting conditions. This central concept, with different shades and degrees of elaboration, is present in Rashevsky's *relational biology* [61], in Rosen's *metabolic and repair (M, R) systems* [63], and in Pattee's notion of *statistical closure* [55], for instance. Thus, instead of regarding life as a phenomenon that stems from the particular properties of various types of macromolecules and supramolecular structures (DNA, RNA, proteins, lipid aggregates, etc.), some authors already share the view that life is, rather, a *system property*. And so do the founders of the autopoietic theory.

Probably, the main contribution of Maturana and Varela was to articulate a full-fledged and coherent formulation of all these boiling thoughts and ideas. From their conception of autopoiesis as the nuclear principle underlying the phenomenon of life (and, to a good extent, also of cognition [39]), they created a new, clear, and far-reaching interdisciplinary and philosophical view. As Varela himself explains in his book *The phenomenon of life* [77, pp. 429–438], the idea of autopoiesis took shape from the concepts of self-referentiality and closure developed in the second cybernetics (especially by von Förster) during the sixties. So it was from that abstract and, to some extent, top-down theoretical perspective that Maturana and Varela, at the beginning of the seventies, started to construct the idea of autopoiesis, grounding it in the notion of organizational closure,<sup>1</sup> and foreseeing it as the definitional principle of a minimal living system.

Together with the notion of organizational closure, two other important concepts were crucial to mature the idea of autopoiesis: the concept of component production network (abstraction of the idea of metabolism) and that of individuality, or spatial-topological unity (the physical border, possibly as an abstraction of the idea of cell membrane). Their central claim is, then, that the closure or recursivity in the organization is generated by the way in which the components and production processes of the system get intertwined in the context of a complementary relationship between the network and the physical border (which are, at the same time, *condition for* and *result of* each other). Thus, the internal relations of production collectively acquire a coherent, operational meaning, since they contribute to the global maintenance of the system.<sup>2</sup> And the relations of the system with its environment, being necessary extensions of this primary internal organization, are conceived as *actions* that the system performs on its own behalf. So, starting from autopoiesis, Maturana and Varela build a theory

<sup>1</sup> The term “operational”—instead of “organizational”—closure was adopted later.

<sup>2</sup> Although the autopoietic authors avoid any reference to the idea of “function” or “functionality” in this context (for reasons that we will not go into), in this article the terms “operational,” “organizational,” and “functional” are used interchangeably.

that aims to cover both the *internal* aspect of the basic organization of living systems (metabolism) and the *external* aspect (agency), showing that they are two sides of the same coin. In other words, the idea of autopoiesis should already hold, according to its authors, all that is necessary for life and meaning.

Therefore, the concept of autopoiesis crystallizes from a very generic received idea of closure and recursivity, rather than from a theoretical scheme concerned with the natural processes of self-organization leading from the inert physico-chemical world to more robust and complex forms of (adaptive) self-maintenance. Actually, from Maturana and Varela's point of view, the formation of an autopoietic system is essentially a relational-constructive issue, a formal (or computational) problem whose solution should determine the set of abstract primitives (components) and rules of its interactions that achieve an operational closure [78]. Accordingly, their approach makes no consideration whatsoever of thermodynamic requirements or other energetic-material constraints, because (as they explicitly acknowledge [39, p. 89]) it does not affect the organization—the self-productive logic—of the system. In this way, claiming that life is founded on a purely relational property, the authors of the autopoietic theory contributed, among others, to opening up the road for ALife and the challenge to universalize biology.

However, the level of abstraction of the theory of autopoiesis (not only present in its original formulation, but maintained in most later contributions and ideas developed from it, such as Varela's conception of autonomy [75, 76]) is too high for an adequate naturalization of the problem, as Fleischaker already pointed out [20, 21]. Although Varela was interested in the chemical realizations of autopoietic systems [35], he was not really concerned about the way in which the basic organization of living beings could be influenced or conditioned by the laws of physics and chemistry (probably because he believed, all through his career, that it was not in any relevant way). Neither did he get much involved in the problem of the origins of life, because the discussions on whether proteins or nucleic acids had to come first (and similar ones) were too far from the point he was trying to make (namely, that life is a systemic property, characterized by the autopoietic way of organization, regardless of the components that come to realize it).

Here we are going to deal with the same problem that the autopoietic theory was posing (determining the nature of the minimal self-productive organization), with a similar insight (in the sense that systems with this type of organization are a prerequisite for a biological evolutionary process to begin [77; see also 64]) but a quite different approach, since our aim is to show the physico-chemical roots of the problem and their important general implications (i.e., the material-energetic requirements involved in the implementation of any system with that characteristic organization). In order to do so, it is not enough to make some amendment to the autopoietic criteria and terminology (as Fleischaker did [20, 21]), but the whole issue of the nature and origins of autonomous, self-constructing systems has to be reconsidered from scratch, retackled from a different standpoint [67, 68]. This new way to look into the problem will hopefully contribute to develop a more realistic and precise characterization of minimal metabolic systems, which can be used to focus with a different lens the research on the origins of life, or guide attempts to synthesize artificially the most basic autonomous systems.

Despite our deep criticism of the autopoietic theory (also regarding a possible complete definition of the phenomenon of life—see the last sections of this article), we think that the main message of Varela is still valid, and the attempt is to give it new life through a different interpretation of the issues involved. This message could help to articulate a shift of focus in the field of origins of life (certainly needed) and the ALife research projects that try to contribute to it. The shift would imply giving up our fixation on the specific molecular components of present living beings on Earth, with

the aim of thinking more in terms of the characteristic way in which they integrate an operational unit, a dynamic self-productive organization. Hence the importance of the autopoietic theory, and its fundamental claim that life is a property of a whole system, rather than a property of individual molecules (or populations of these molecules).

Nevertheless, this *system thinking* does not imply forgetting about the material mechanisms that are crucial to trigger off a biological type of phenomenon/behavior; rather, it means putting the emphasis on the interactive processes that make it up, that is, on the dynamic organization in which biomolecules (or, rather, their precursors) actually get integrated. According to this view, research should be directed to implement those—or very similar—interactive processes with molecular components and tools that are alternative to (and, perhaps, less complex than) the biochemical machinery present in known living beings. And always with the aim of showing a natural connection with other complex physico-chemical forms of organization.

The concept of autonomy is probably crucial to understanding how life could appear on the Earth, as well as for the project of developing a general theory of biology, such as Varela envisioned. However, this tentatively universal concept should also contribute to bridging the gap between the living and the inert worlds, and thus the level of abstraction in its formulation should not preclude its naturalization. That is why the account we offer in the following pages is very much concerned with the material-energetic-thermodynamic aspects of the problem of establishing a minimal autonomous organization.

## 2 The Origins of Autonomous Systems: Introducing the Idea of Basic Autonomy

The problem of the origin of autonomous systems is a difficult one, for two important reasons. First, the most elementary examples of self-constructing systems that we know of today (e.g., some primitive bacteria) constitute too complex forms of organization, that is, are too distant from self-organizing systems that may come about spontaneously in far-from-equilibrium conditions, such as those appearing in nonlinear convection or reaction-diffusion phenomena (typical examples of the so-called *dissipative structures* [52]). Second, we do not have a clear or universally accepted criterion to characterize other possible—more primitive and rudimentary—forms of autonomy (although the present article is an attempt to advance in that direction). In spite of that, and even though it is impossible to know a priori what those systems could be like, it seems quite natural—from a bottom-up approach—to look for their origins in the context of physico-chemical dissipative systems with potential to go through processes of progressive growth in complexity.

Now, although the phenomenon of self-organization always involves the generation and maintenance of a global (or high-level) pattern or correlation that constrains the (low-level) dynamics of the components of the system (see [52] or [10]), in standard dissipative structures this occurs only provided that the system is put under the appropriate boundary conditions. If those (externally controlled) conditions are changed (in particular, if the input of matter or energy is outside a certain range), the self-organizing dynamics vanishes. Therefore, there is an important difference between the typical examples of “spontaneous” dissipative structures and real autonomous systems: in the former case, the flow of energy and/or matter that keeps the system away from equilibrium is not controlled by the organization of the system (the key boundary conditions are externally established, either by the scientist in the lab or by some natural phenomenon that is not causally dependent on the self-organizing one), whereas in the latter case, the constraints that actually guide energy/matter flows from the environment through the constitutive processes of the system are endogenously created and maintained.

If one considers the type of self-organizing systems that could evolve in the direction just indicated (i.e., towards a truly self-building organization), it soon becomes manifest—see below—that their internal dynamics should be capable of generating constraints (control mechanisms) that are not only global, like the macroscopic spatio-temporal patterns of typical dissipative structures, but also local and molecularly selective. This means that purely *physical* self-organizing phenomena have to be disregarded in favor of *chemical* ones, since only the latter would be able to turn into component production systems. And only in that context can a diverse enough set of control mechanisms and boundary conditions be internally generated and regulated, for it is possible that certain components of the system act as local and specific constraints on the production processes of some others.

Accordingly, the problem of the origins of autonomy must be tackled in a scenario where chemical reaction networks (component production systems) in far-from-equilibrium, dissipative conditions may thrive and achieve some robust self-maintaining dynamics. However, as Bickhard [6] points out, self-maintenance—the way it is usually understood—is not enough. In order to illustrate the point, he discusses an example of a dissipative system “in between” physics and chemistry: a candle. In his view, the burning of a candle is a “self-maintaining” phenomenon, for the flame itself contributes to keeping the system away from equilibrium, in the conditions that make it continuously realizable (in particular, a high temperature and a constant oxygen uptake). Even the shape it takes is very stable in the face of disturbances (of course, provided they are not too strong). Nevertheless, this system shows great limitations compared to what the author calls “recursive self-maintaining systems.” The latter would be capable of modifying their own self-maintenance processes in order to adjust to environmental changes that seriously threaten their continuity. This would be the case of a bacteria swimming up a glucose gradient, or a (fictitious) candle capable of fetching wax from its surroundings when it begins to run out of it.

What Bickhard here brings to the fore is a very important point, usually disregarded in models and experiments that try to go beyond phenomena of chemical “self-organization” or “self-maintenance”: the fact that this kind of system must develop its own mechanisms to stay away from thermodynamic equilibrium and, in particular, has to channel and modulate the interaction with the environment so as to keep its characteristic dynamics (the network of chemical transformations) running. The general tendency to overlook these aspects of the problem comes partly from the widespread idea that autocatalysis (or perhaps some other basic chemical mechanism) can bring about all that is required to constitute this kind of component production network. However, despite all the experimental and theoretical work carried out in this direction (following, among others, the pioneer model of *autocatalytic sets* [18, 25, 26]), there is little real evidence that this may actually be so. Rather, it seems that autocatalysis is a very important mechanism underlying many different types of complex chemical phenomena (from Belousov-Zhabotinskii oscillatory reactions to metabolic pathways in living organisms), but not the crucial one that marks out the transition from self-organization to self-producing or self-constructing systems, ensuring the viability of robust self-maintaining chemical networks.<sup>3</sup>

<sup>3</sup> Several experiments prove that certain biopolymers (e.g., oligonucleotides [28] or oligopeptides [31]) are, by themselves, also capable of autocatalytic behavior (even claimed to be the most elementary examples of molecular self-replication). Nevertheless, neither the diversity of chemical species nor the complexity of the catalytic loops present in those experiments can support the idea of an autocatalytic component production network in that context. Thus, we can find real examples of autocatalytic phenomena in systems with very simple components and very low organizational complexity (chemical oscillations), in systems with very complex components and organization (natural metabolisms), or in systems with fairly complex components (such as peptide or nucleotide chains) but poor organization (the just-mentioned experiments). However, we miss intermediate examples that, with diverse but relatively simple molecular compounds, show a higher organizational complexity than the traditional chemical dissipative structures. And that is the heart of the question.

The problem is more intricate: As we already mentioned and will show better below, it involves the articulation of various types of control mechanisms, both local and global. And it requires elaborating an account of minimal autonomous systems that is naturally embedded in—and consistent with—the general framework of thermodynamics. This leads to the conception of *basic autonomy* [67, 68] as the capacity of a system to *manage* the flow of matter and energy through it so that it can, at the same time, regulate, modify, and control: (i) internal self-constructive processes and (ii) processes of exchange with the environment. Thus, the system must be able to generate and regenerate all the constraints—including part of its boundary conditions—that define it as such, together with its own particular way of interacting with the environment. In other words, the constructive processes that actually put the system together have to be directly involved in the continuous exchange of material-energetic resources with the environment, and, conversely, the achievement of these resources is indispensable for the appearance and maintenance of those processes. Such an interdependence—on which we further elaborate next—between the constructive and interactive aspects of the phenomenon is precisely the cornerstone for the constitution of any real metabolic organization.<sup>4</sup>

## 2.1 Thermodynamic Issues Are Relevant for Basic Autonomy

Thermodynamics matters. However limited it may be in providing a detailed, complete, satisfactory explanation of the appearance of autonomous systems, its far-reaching implications cannot be disregarded. For instance, of all the processes that could—in principle—take place in the context of component production networks, only some will happen to be thermodynamically (let alone kinetically) feasible. This point is crucial because some processes (such as the generation of most polymer chains, or the transport of a substance against its gradient), are not spontaneous (i.e., they are “up-hill,” or endergonic, processes), yet are very important for achieving self-construction. And they can only take place if coupled to some other spontaneous (“downhill,” or exergonic) ones. Therefore, a whole set of endergonic-exergonic couplings is required, both to take advantage of a far-from-equilibrium thermodynamic situation in a constructive way, and to actually persist in that situation. This set of coupling mechanisms (both internal and with the environment, which is always the final source and sink of matter and energy) would put together some sort of cyclic proto-metabolic organization [46, 47]. Now, such coupling mechanisms (or, alternatively, the set of constraints that put them together) have to be built up by the system itself (otherwise, we could not be speaking about autonomy in minimally rigorous terms), and energy resources are required for the task.

This problem leads us to search for a fundamental connection between constraints and useful energy (or work)—a connection that, as a founding principle for basic autonomy, would constitute an important step in integrating the two different approaches to the analysis of minimal biological organization (the relational-constructive approach and the energetic-thermodynamic one [69]). Work can be regarded as a constrained release of energy [1, 27], as opposed to heat, which is energy that escapes all control. Work is usually defined in engineering terms as a form of energy that is “useful,” for it allows the performance of some type of function (typically an external function). However, the concept of work may be applied more generally, both to heteronomous systems (like classical machines) and to autonomous systems (like living beings), even if the conditions differ quite a lot from one to the other. In the latter case, work cannot be defined from the perspective of an external (intelligent) agent; rather, it should

<sup>4</sup> This is the reason why we prefer to use the term “self-construction” instead of “self-production,” highlighting the idea that (basic) autonomy cannot be just the result of an internal set of processes that achieve some closure (operational [75], catalytic [27], etc.), but always involves a material and energetic “effort,” linked to the establishment of a new way of interacting with the environment.

be conceived as any energetic contribution to the construction and maintenance of a certain cohesive, self-organizing system away from thermodynamic equilibrium. *Functional* actions in this context are those that ensure the self-maintenance and autonomy of the organization.<sup>5</sup> Hence the intrinsic functional character of the processes that are carried out by (and at the same time constitute) autonomous systems.

Thus, autonomous organization is only possible if it generates constraints that modulate the flows of energy so that those constraints are regenerated and contribute in this way to the recursive maintenance of the organization. In the simple heteronomous case of a thermal machine, the walls of the cylinder, the piston, and so on, are the constraints required to extract work from the expansion of a gas. In an autonomous system, things occur quite differently: constraints (as part of the ongoing self-constructing dynamics) are continuously being generated and regenerated, coming together to establish couplings of endergonic and exergonic processes, which constitute the fundamental way to produce work in the system.<sup>6</sup> So work involves constraints, but, as we said before, the generation of constraints in turn involves having some energy ready for use (i.e., energy in the form of work) in the first place.

On similar lines, Kauffman [27] claims that in an autonomous system “work begets constraints begets work.” In other words, he points out that a recursive relationship between work and constraints must be established if a system is to achieve autonomy; namely, “the work-constraint (W-C) cycle.” The W-C cycle triggers a new dynamics in a system, a constructive and recursive (though never fully closed) dynamics, as a result of which a web of constraints is progressively generated, hand in hand with a continuous process of propagation of work.

Without contradicting the laws of thermodynamics, this establishes a new energetic “logic” in the system. The cycle is open, and it certainly involves irreversibility and dissipation, but as it drives the system further and further away from equilibrium, it also sets up a way to counterbalance and keep under control the typically increasing dissipative effects of that situation through the establishment of a more and more complex web of endergonic-exergonic couplings. In sum, the W-C cycle expresses the deep interrelation between self-construction and autonomy, for it shows how and why the latter, in its minimal form, is only possible through component production systems that are at the same time operationally closed and thermodynamically open.

### 3 Basic Autonomy as Self-Construction: Fundamental Problems

The conception of basic autonomy as the self-constructing capacity of a system (stemming from the recursive consecution of the W-C cycle and through the establishment of a set of endergonic-exergonic coupling mechanisms) provides a theoretical framework that will allow us to articulate a more precise and explicit account of the origins of natural autonomous systems and, hopefully, also contribute to design the synthesis of artificial ones. The Varelian top-down and rather abstract conception did not make it easy to collect all the ingredients involved, whereas Kauffman’s recent approach to the problem, though sharp and enlightening, does not go far enough in the direction required. One has to step forward and get wet, specifying what kind of constraints—and associated types of work—are needed to effectively achieve minimal self-constructing systems. Such will be the objective of the next pages: determining which are the nec-

<sup>5</sup> Herein we shall use the term “functional” in its most elementary sense, as the quality of an action through which the components of a network can alter the probability of other components’ production [11]. This means, considering the network as a whole, that all those processes that contribute to the self-maintenance of the global system are to be regarded as functional [45, 7]. Hence, a function cannot be explained except by an argument that resorts to the coherence or persistence of the system as a whole.

<sup>6</sup> Kauffman draws an interesting parallel between an endergonic-exergonic coupling and a “cycle of work,” understood in the traditional terms of Carnot thermal engines [27].

essary and sufficient conditions (components, control mechanisms, etc.) to establish the minimal set of endergonic-exergonic couplings that brings about basic autonomy. In order to do so, we will briefly analyze a series of interconnected problems to be faced by any component production system aiming to become an autonomous chemical entity (for a more detailed account see [66]).

### 3.1 The Problems of Space-Time Coordination and Efficient Energy Transfer between Constitutive Processes

If the couplings between endo and exergonic processes were to happen just in pairs, in an isolated way, they would not lead anywhere. A properly sequenced and interconnected set of couplings is required in order to get a coherent operational dynamics. In this regard, it is crucial that the system generates some local constraining mechanisms with which the reaction speeds of many different processes can be modified and properly regulated.

In general, if a process, a chain of reactions, or a coupling is favorable from a thermodynamic point of view (i.e., if it is globally exergonic), that means that it is actually possible. However, that does not say anything about when or how it will take place. For example, the degradation of glucose is, in principle, spontaneous at physiological temperature ( $\Delta G^\circ = -686$  kcal/mol), but it would take years in any living being if it were not for the presence of enzymes that act as catalysts of the process and help energy be quickly released from it (triggering other metabolic processes). Anyway, that is only part of the story. The capacity of enzymes to change activation energies per se would lead the system to crazy autocatalytic behavior if it were not for the sophisticated regulation mechanisms that control their action. These mechanisms (based on several kinds of inhibitors—competitive, allosteric, etc.—for a very good review on metabolic control see [19]) guarantee the homeostasis of the whole system (its global metabolic consistency and even its specific responses to certain external disturbances).

The problem is that enzymes (or other biomolecules with catalytic activity, like RNA) are too complex to be present at the first stages of the origin of living beings. But the job could possibly be done at the beginning by a few types of more rudimentary catalysts (perhaps oligopeptides or smaller multimers, as de Duve [16] suggests), whose formation would be favored in the context of lipidic or fatty acid self-assembled structures, such as primitive vesicles. These catalysts, instead of being substrate-specific, would carry out more generic tasks (possibly grouped into several reaction types: reduction-oxidation, polymerization, transport, etc.), and take part in reaction pathways that would have little resemblance to those present in the simplest living beings today (i.e., to genetically instructed metabolic pathways).

In addition to all this, it is important to recall that the coupling of endergonic and exergonic processes always involves an exchange or transfer of energy, which in practical terms (i.e., in terms of mechanical and thermodynamic efficiency) demands some sort of intermediary component.<sup>7</sup> Furthermore, the maintenance of a whole network of coupled processes requires establishing some way to distribute energy resources (captured from the environment) across the entire system, so as to satisfy the energetic needs of the processes occurring in it. This is impossible to achieve if each pair of processes has its own intermediary component. Thus, a set of “common energy tokens” or “currencies” turns out to be necessary to deal with this problem from the beginning, from the very appearance of autonomous systems.

Living beings on Earth (i.e., cellular metabolisms as we know them at present) are far too complex and evolved systems to be directly relevant for our analysis here, but

<sup>7</sup> One might argue that the exchange of energy between two coupling processes (e.g., two chemical reactions) could, in principle, be achieved through a direct transfer of heat. However, in practice this is not feasible because an efficient (minimally reliable) coupling mechanism cannot be articulated if such high levels of dissipation are involved.



it is interesting to recall that all of them share some very basic features in this sense, which apparently have been there from their actual origins. They use a common set of energetic intermediaries, or energy currencies (as the remarkable work carried out in bioenergetics during this century has shown [32, 41, 22]). It is particularly significant that at least two different types of energy currencies, one soluble in water (typically ATP, but we could think of other precursor molecules, like pyrophosphate [4]) and one linked to transport processes (a gradient of the electrochemical potential—of protons or sodium ions), are necessary in all unicellular living beings, which continuously transform the energy gathered from whatever external source into either of those convertible currencies. The universality of this feature in our biological world is quite clear [71], but we have to analyze to what extent this is necessarily so in the context of basic autonomous systems of any kind. In order to do so, we need to consider first another fundamental problem related to it.

### 3.2 Building its Own Boundaries

The construction of the boundaries of a system, of a *border* defining it as a distinguished unit from the environment, stands out as one of the major challenges in reaching basic autonomy. Some researchers in the field of origins of life have speculated about the possibility that proto-metabolisms could form in free solution (this would be the case of de Duve's [16] "thioester world," for example) or on inorganic surfaces (e.g., the "bi-dimensional proto-metabolisms" suggested by Wächtershäuser [79]), presuming that the compartmentation or self-encapsulation, of the system would take place after the constitution of a relatively stable and robust component production network. These authors offer a theoretical scheme in which the energetic-material viability of that kind of network is, in principle, seriously taken into account, and that does not include the endogenous production of a topologically closed interface (i.e., a global constraint that establishes the spatial boundaries of the system).

We will not judge here the adequacy of these *acellular* models as representatives of primitive, prebiotic chemical networks (in fact, we regard them only as interesting proposals in that connection). However, we have to be critical in so far as they are also presented as models of some sort of ancestral "metabolism" (i.e., models of fully self-producing networks or of hypothetical prebiotic organisms). The generation of a physical border is a crucial step toward autonomy, because that is the only way, on the one hand, (i) to assure the control of energy flow required for the robust maintenance of the network, and on the other, (ii) to solve the problem of diffusion and dilution (control of concentrations). Furthermore, if the precursor reaction network does not become self-enclosed, it will not be able to create a particular—and minimally stable—chemical micro-environment, being directly exposed to all changes taking place in the milieu. In other words, the system will not have any control over the boundary conditions that bring about its distinctive, far-from-equilibrium dynamics (and, thus, it will be extremely fragile).<sup>8</sup>

So, in tune with several other authors (from Oparin himself [53] to—more recently—Harold [22], Morowitz [49, 47, 48], Deamer [12, 13, 15], Bro [8], Pohorille & New [60], or Cemin & Smolin [9]), we claim that membranes or cellular boundaries play a fundamental role in the constitution and maintenance of any (proto-)metabolic system. In any case, it seems much easier to tackle the question of compartmentation while the molecular complexity of the prebiotic system is still low (and the inside-outside distinc-

<sup>8</sup> Neither a free chemical reaction network (à la de Duve) nor a layer of active organic matter on a mineral surface (à la Wächtershäuser) can overcome these problems. In the second case, precipitation on the mineral surface could temporarily serve as a mechanism to get around the problem of diffusion, but it carries along with it some other difficulties, such as the high dependence of the network on such a static external structure. Wächtershäuser himself acknowledges [79] that the characteristic holism of metabolic systems comes about with cellularity.

tion involved is not so marked). Even though a global enclosure may be considered initially as an additional source of difficulties in getting a chemical network running (for instance, concerning the accessibility of certain compounds to the system), things only get more complicated if its appearance is postponed. In fact, it is quite clear that the more complex the network, the more complex the boundary required to hold it together in a sustainable way. Thus, the development of (proto-)metabolic systems towards higher and higher degrees of chemical and organizational complexity can be more easily understood through the idea of a network-boundary complementary relationship, which allows for a continuous process of coevolution between the two.

### 3.3 The Osmotic Problem

Due to the gradual establishment of a different chemical environment within the system (presumably more elaborate than the surrounding milieu), some mechanisms to compensate and adjust for these changes and disparities are required. In particular, any cellular system with a minimal internal complexity (or potential to grow in complexity) has to face a serious problem related to the semipermeability of its boundary: an eventual *osmotic crisis*. This seems to be an *ab initio* problem [58; see also 50] for any real cellular proto-metabolic organization, and will determine the type of boundary that this kind of system has to create.

By osmotic crisis we mean a critical situation of imbalance that results from concentration differences between the inside and the outside of the cellular compartment. Such is the case of the so-called *Donnan effect*, where an excess in anionic charges associated with the polymer chains built within a biological system is not adequately compensated and, given the necessary semipermeability of the membrane, the spontaneous entrance of water into the compartment is unavoidable, leading to its bursting. The fundamental mechanisms that (all) living beings use to face this problem are *pumps*, which help to keep ionic concentration gradients below a threshold that would turn out to be fatal for the system. One could imagine some other kind of mechanism to hold the structure up, such as a *cellular wall*, which is quite common in the known living world, too (though not ubiquitous).<sup>9</sup> In fact, the cellular wall typically allows for the plasmatic membrane to withstand a much higher osmotic pressure than that under normal conditions. However, it seems rather unlikely that the cellular wall precedes the invention of ion pumps, since (being a highly porous structure) it cannot contribute to control the material flow across the cell, which is of paramount importance for the basic organization of the system (and always relies on the semipermeability and transport capacities of the membrane).

So, what kind of physical border is required for the constitution of a minimal autonomous system? Just any boundary will not do. For example, a vesicle made exclusively of structural amphiphilic<sup>10</sup> compounds is not enough, even if these compounds are endogenously generated. The physical border must, of course, be tightly linked to the component production network (being at the same time a condition and a result of it, as the autopoietic school strongly emphasizes), preventing the diffusion of the components, preserving spatial cohesion of the system, and providing a chemical habitat of its own, where the system comes to exist. Nevertheless, it must also play an active role, fundamental to establishing a new, autonomous way of interaction with the environment and avoiding problems such as the osmotic crisis. This is precisely what makes the self-construction and robust maintenance of the system possible.

<sup>9</sup> Other possibilities, like the development of a cytoskeleton, seem too complicated at this preliminary stage.

<sup>10</sup> *Amphiphiles* are molecules made of two distinct parts: a polar group (typically called the *head*) attached to a nonpolar chain (typically called the *tail*). They are well known for their collective properties of self-assembly and the diverse types of supramolecular aggregates (micelles, vesicles, etc.) that they form depending on experimental conditions.

Therefore, rather than a boundary produced—and reproduced—by means of some autocatalytic reaction happening within it (according to the autopoietic approach to research on minimum autonomous systems), we should pursue a physico-chemical interface capable of regulating interactions with the environment and controlling matter and energy exchanges with it. Such an interface cannot be a mere physical border, but must be a semipermeable chemical envelope where coupling mechanisms (in particular: energy transduction and active transport mechanisms) are anchored. The membranes of all known cellular living beings have this kind of mechanism, making clear that their ongoing activity is fundamental for the constitution and maintenance of all natural metabolisms.

A boundary with selective permeability (facilitating the transit of some substances, like small organic compounds, and preventing the diffusion of others, like polymer chains) and with channels of interaction with the environment must be an aggregate of global and local constraints, since it involves a topologically closed surface (e.g., a micellar or vesicular self-assembly of amphiphilic molecules) where various other components with more specific tasks are inserted: some devoted to carrying out catalysis or mediated transport, and others to capturing energy from external sources and transforming it into the system's own means.

So, at this point, we can recapitulate and say that autonomy involves couplings between reactions and transport processes through the membrane. As a consequence, there have to be, at least, two types of interconvertible energy currencies: a *chemical* one and a *chemiosmotic* one (i.e., a gradient of electrochemical potential). This is the only possibility for the system to gain control over the flow of energy and solve all the problems related to its material implementation as a cellular self-constructing organization. The use of component currencies and gradient currencies in a complementary way precisely reflects the two dimensions of the problem of generating an encapsulated network of component production processes. On the one hand, as has been pointed out already, the capture of external energy and its conversion into chemically useful compounds is critical for the system to keep a nonlinear, cyclic, far-from-equilibrium transformation dynamics; on the other hand, it is also indispensable for establishing regulatory mechanisms of material flow (such as channels, carriers, or ion pumps) to be able to overcome obstacles related to the inherent cellular nature of the system (problem of accessibility, osmotic crisis, etc.), opening the way to develop a much higher chemical complexity inside than outside its boundaries. This is such a fundamental feature that all later forms of autonomy will need to keep it (as Harold [22] so rightly points out: all cellular living beings are examples of “vectorial metabolisms,” based on a very tight interweaving between chemical reactions and membrane processes, made possible through bioenergetic mechanisms).

#### 4 Basic Autonomy as Self-Construction: Universal Material Requirements

According to the issues discussed above, membrane, catalysts, and energy currencies constitute the minimal set of components required to constitute and maintain the network of endergonic-exergonic couplings underlying basic autonomy (see Figure 1). Currencies prevent energetic dispersion and facilitate the coupling of chemical and transport processes—one of the keys to implementing a real metabolic system. Catalysts, in turn, are in charge of the temporal and spatial coordination of the set of processes to be coupled, modifying activation energies, self-regulating their own action, and making phenomena such as mediated and active transport<sup>11</sup> possible. And

<sup>11</sup> A mediated transport process is said to be *active* when, by itself, it is endergonic (that is to say, it can only occur in so far as it is coupled with an exergonic process).

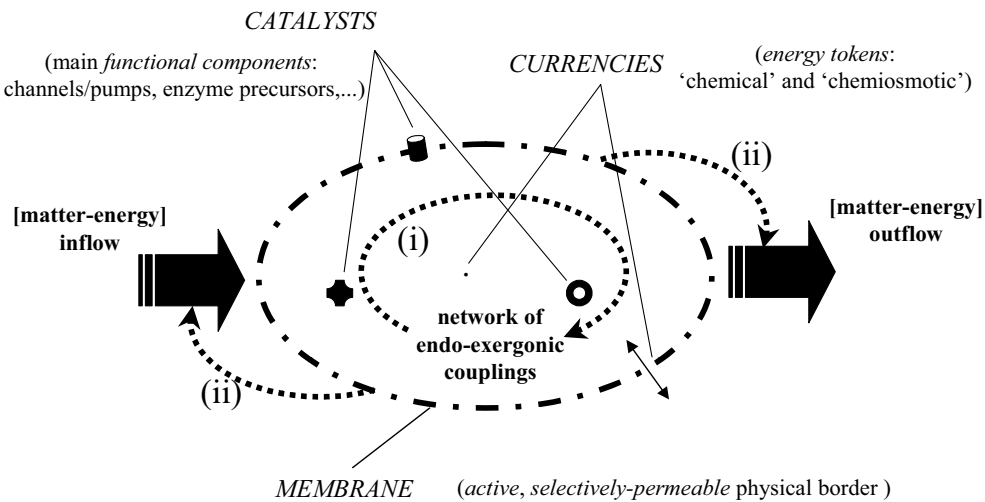


Figure 1. Fundamental components of a self-constructing (basic autonomous) system. The endergonic-exergonic couplings involve both internal processes and interactions with the environment. Hence the two types of arrow: (i) to depict the functional and organizational integration of inner constitutive processes, and (ii) to show that this cannot happen without some control of the boundary conditions (i.e., active regulation of the flow of matter and energy through the system). The most obvious peculiarity of self-productive (autopoietic) systems is reflected in the nature of the boundary, which includes here channeling mechanisms that are necessary to develop *selective permeability* and *agential* behavior (capture of energy resources, control of concentration gradients by active transport, etc.).

last, but not least, the membrane provides a special chemical habitat for all these processes to take place, including those involved in its own constitution (as a border that separates and, at the same time, connects the outside and inside of the system). In other words, the membrane leads to an asymmetry, a qualitative difference between inside and outside, shaping the interactions between system and environment so as to ensure autonomous management of the flow of energy and matter through it.

These three ingredients are strongly interdependent. If one fails, all of them fail. The relationship between the membrane and the catalysts is complementary, since the former contributes to the production of the latter (because of the minimal concentration thresholds required, and also because polymer synthesis could, indeed, be promoted on the inner surface of the boundary) and, at the same time, without the latter the properties of the membrane as an active border would vanish (especially as regards the transport of compounds into and out of the system). The system of currencies and the membrane are also mutually necessary. The membrane requires both chemical and chemiosmotic currencies (for its construction and osmotic viability, respectively). In turn, although it is not so difficult to imagine chemical currencies in the absence of cells (think of a scenario à la de Duve), what is important is the whole set, gradients included, and this makes compartmentation necessary. Finally, without good entanglement and coordination between catalysts and currencies, the role played by both of them as facilitators of the couplings would not be possible (recall the role of ATP as a cofactor of many enzymes, for example).

In sum, in order to establish a minimal autonomous organization, a deeply interwoven set of components (including local and global constraints) is required, together with the production and interconversion of different types of work (at least, chemical and osmotic work). Chemical work is directly linked to the creation of molecular bonds, where energy is stored and kept for subsequent transformations. Osmotic work is related to the creation of concentration gradients (or, more precisely, electrochemical

potential gradients) of several compounds for which the boundary of the system shows selective permeability.<sup>12</sup>

The way in which a chemical system manages to create this initial tapestry of work and constraints is not easy to determine. According to our account, the ingredients of the triangle of basic autonomy must be jointly developed in the context of self-organized cellular systems in far-from-equilibrium conditions, but the issue requires further, experimentally supported research. Thus, in the next section we briefly review how the question of (basic) autonomy has been tackled up to now in the field of ALife and point out which would be the most promising avenues of research to yield more satisfactory results.

## 5 Some Considerations for the Design of Artificial Basic Autonomous Systems

During the last decade, many efforts have been made to artificially reproduce a truly autonomous system. In addition to a research program in computational simulations, in the field of realizations there are two main lines of research: autonomous robotics and artificial synthesis of chemical or in vitro systems. As we are concerned in this article with minimal forms of autonomy, in the following we will only discuss the in vitro research, which is at present the most promising program in that respect. This decision is also based on the fact that the in vitro research program is more coherent with the approach to autonomy that we develop in this article (i.e., basic autonomy as a chemical process, and the importance of thermodynamic requirements). Thus, we will analyze next the efforts to artificially reproduce basic autonomous systems (BASs) using chemical compounds as building blocks.

From a general perspective, the present situation is quite good for our purposes, because within the scientific community there is growing interest in the problem of creating artificial cellular systems in the lab [74, 59, 15, 34, 36]. The reasons for this interest range from the enormous potential that cellular encapsulation has in the field of biotechnologies and pharmaceuticals, to the relevance of the *minimal-living-cell project* for basic research on the origins and artificial synthesis of life (which is the focus of this article).

In principle, there are two main ways to approach the design of minimal cellular systems in vitro [34]: *bottom-up* and *top-down*. The bottom-up approach attempts to create a self-organizing and self-maintaining cellular organization starting from the known basic components (amphiphilic molecules, amino acids, etc.), whereas the top-down approach attempts to decrease dramatically the complexity of an existing living cell (without killing it) by eliminating all that is accessory in it. However, as a matter of fact, most experimental work mixes these two approaches to some extent: typically, the cellular envelopes are produced in a bottom-up way (relying on the self-assembling properties of amphiphilic molecules), and the other molecular components included in the system are already formed (or previously added as part of a kit). It is important to be aware of this, especially in cases where the organic compounds involved (RNA, DNA, enzymes) are so complex that they have to be borrowed from already existing living cells.<sup>13</sup>

<sup>12</sup> Of course, autonomous cellular systems show nowadays a much wider capacity to generate work (not only chemical and osmotic, but also mechanical work), based on a sophisticated network of coupling mechanisms that reflects how the interaction with the environment and the management of resources taken from it have developed throughout biological evolution. This ranges from the coupling mechanisms required for the appearance of basic motility capacities (allowing phenomena like chemotaxis, more reliable reproduction by division, intercellular associations, free movement, etc.) to those articulating rapid ways of cellular communication necessary for cognitive processes.

<sup>13</sup> We are quite critical of recently proposed models of "RNA or DNA minimal cells" (see, for instance [74] or [36]), in that they take for granted the energetic requirements associated with the processes of synthesis of such macromolecules (which are strongly endergonic). If one takes those requirements into account, it becomes obvious that such complex components and their

In this context, we consider that the bottom-up strategy is the one to be pursued, forgetting—at least temporarily—about the macromolecules that constitute present living beings, and concentrating on the implementation of basic autonomous systems. Along the same lines that Bro [8] defends, the real challenge for a wet ALife project is to create *chemical automata* using components other than the ones found in biological systems known to us. This could also give the key to part of the problem of the origin of life on Earth, since the precursor molecules that made up basic autonomous systems (proposed here as necessary prebiological systems) could be quite different from the biomolecules that later came to integrate full-fledged living beings.

Therefore, our starting point must be a physico-chemical domain where cellular aggregates (micelles, vesicles, etc.) can form as a result of spontaneous self-assembly processes. These, by themselves, are equilibrium structures that do not show any interesting chemical-transformation dynamics. The first step would be, then, to take these systems away from thermodynamic equilibrium, trying to entangle the formation of cellular aggregates with some chemical reaction that takes place within each compartment. This was already achieved by Luisi and coworkers more than a decade ago [2, 3], and those experiments were claimed to be the minimal chemical realizations of autopoietic systems [35, 33]. In fact, as far as the theory of autopoiesis conceives the complementary relationship between reaction network and physical/topological boundary, they could very well be so (even if the chemical diversity of the network implemented seems too low in comparison with the original autopoietic idea).

Nevertheless, as we argued in the previous sections, that view involves a rather limited conception of the role of the boundary in the constitution of a basic autonomous organization. Of course, it is very important to establish a mutually dependent relation between a chemical reaction network and a compartment that encapsulates (and at the same time is the result of) it, but doing so does not bring about minimal autonomy, because the system has no control over the material and energetic flow through it. The cellular systems that appear in this scenario can change their shape, grow, even reproduce autocatalytically, but do so without any control. Besides, since they have no mechanism to modify or adjust critical boundary conditions (such as electrochemical potential differences), they are very fragile with regard to possible changes in external parameters (such as the pH).

According to our account on the origins of autonomy, the problem of transport across the boundary of the system is crucial from the very beginning, from the actual constitution of a cellular system with a minimal internal complexity and a changing environment. Among other researchers, Deamer is particularly sensitive to this problem, and has tackled it from different angles, always concerned about the prebiotic relevance of the organic molecules involved [54, 13, 14, 59, 15]. However, his empirical work has been mainly focused on the analysis of the permeability and accessibility of different types of compounds across plausible prebiotic membranes. Thus, the cellular systems he and his colleagues have dealt with in the lab do not get close to our idea of basic autonomy either, in particular because they lack energy transduction and active transport mechanisms (and hence they would not be able to overcome, by their own means, the problem of an eventual osmotic crisis).

The issue is quite tricky, for it involves the integration of several types of components (at least the three that we suggested in the previous section) and the coupling of chemical and transport processes. However, the bet is that it can be achieved with relatively low molecular complexity: macromolecules are not needed for the task. Of course, this is a working hypothesis that cannot be tested but through experiments. In

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processes of synthesis have to be embedded in the self-constructing dynamics of the whole system, because they are actually viable thanks to the network of endergonic-exergonic couplings that make it up and, ultimately, to the energy transducing mechanisms established on its boundaries.

principle, transport mechanisms (such as channels, carriers, and pumps) do not seem to require long—or very sophisticated—polymer chains. However, if they are involved in the coupling with a chemical reaction, this is not so clear. It brings us to the problem of the origins of biocatalysis: that is to say, the problem of what could be the missing link between inorganic and organic (enzymatic) catalysis. The catalytic capacity of short polymer chains is certainly important (especially in view of their difficulty in folding), but perhaps we should try to imagine a scenario where catalysis is not based on stereospecific molecular properties, and can still be metabolically relevant.

In any case, what is certainly the key requirement from our perspective is that the cellular system be treated as an integrated, functional organization in which energy and material requirements are of fundamental importance. Therefore, a more encompassing empirical approach is required, in the sense that it should try to connect chemical reactions with transport processes across the boundary of the system, articulating energy transduction mechanisms. The artificial mimicking and simplification of known bioenergetic devices (see, for instance, [73] or [30]) is a good starting point for an avenue of research that deserves much more attention.

## 6 Potential and Limitations of Basic Autonomous Systems

On these lines, BASs would be a key step not only in the origin of life on the Earth, but on any other planet, or in any attempt to create artificial life in vitro. Before higher levels of complexity (based on macromolecular mechanisms, e.g., genetic or enzymatic mechanisms) are achieved, there has to be some self-constructing organization (like the one we described in the previous sections) through which the material and energetic problems associated to the actual capacity to generate that complexity are solved. In other words, autonomous component production machinery is a requisite for life, or even a *prerequisite* (depending on how one defines life). From our perspective (see the next section) the phenomenon of life is deeply related to but qualitatively different from basic autonomy, and in this analysis of the potential and limitations of BASs we will start by giving an explanation for that conception.

BASs have a functionally integrated and homeostatic dynamics that, in principle, allows for their propagation and the exploration of new ways to operate (both internally and with regard to the environment). They can get reproduced, though not very reliably (by autocatalytic growth and statistical division), and they will naturally diversify, because they are open systems and their continuous production of components in a changing environment has the potential to yield ever new molecular species. The new components, processes, and coupling mechanisms that contribute to the constructive self-maintenance of the system (i.e., that reinforce and expand the W-C cycle) will be integrated and possibly kept within that dynamics. And so autonomous systems will develop and turn into more robust self-maintaining organizations. Thus, from the very beginning of autonomy, we can say that a potentially open scenario of systems with *self-modifying* [24] and interactive capacities is established.

However, is this enough to guarantee the *long-term sustainability* of this kind of systems in a changing environment with limited resources? And do BASs really make possible a process of open-ended increase in complexity? These are very important questions related to the evolutionary capacities of biological systems, and it is not clear if systems with just a bare self-constructing organization could realize them.<sup>14</sup> BASs are adaptive agents (in the sense that they can—and need to—exert actions on their

<sup>14</sup> These two questions are, in fact, tightly linked, because the long-term stability of a biological world crucially depends on how living beings change in time and evolve through generations. Similarly, the long-term maintenance of autonomous systems will not be guaranteed until they achieve high metabolic efficiency and robustness, reliable reproduction, and an open-ended capacity to adapt and grow in complexity.

immediate environment to ensure their own maintenance in the face of external perturbations), so, apart from each individual's homeostatic mechanisms, they can develop interactive (competitive or cooperative) strategies to make more extensive and efficient use of the material-energetic resources available in their surroundings. And, given their capacity to multiply and propagate, one could even speculate about the possibility that a population of BASs might form some proto-ecosystem, establishing primitive food webs, which would increase their chances of maintenance—as such a population—in a materially limited environment.

Nevertheless, the ways in which BASs could change and evolve are very different from the biological way of doing it. They lack mechanisms for reliable heredity, so, strictly speaking, they cannot start a Darwinian evolutionary process. They are also limited in that they have an upper bound of complexity (see below) that prevents them from changing over time in a really open-ended way (in the sense of von Neumann [51], later reinterpreted by authors like Pattee [56] or McMullin [37]).

Without special molecular mechanisms (which rely on the development of stereospecificity and, accordingly, on a quite significant growth of polymer chains [72]), BASs are able to reach only a very primitive level of efficiency, versatility, and reliability in the catalytic and transport functions necessary for their continuous self-construction. And, maybe more important for the issues we have just raised, this in itself constitutes a limitation on the possibility for possible metabolic innovations to be effectively fixed (“recorded” in each individual organization) or to be later spread to other members of the population.

Many of the molecular inventions and novelties arising in the hypothetical scenario of BAS will turn out to be ephemeral, especially if they involve an increase in complexity. This is due to the fact that, at this stage, growth in complexity means an important energetic and material cost for the system (most polymerization processes are highly endergonic), and the only way for such a system to maintain and extend its particular organization and components is through a highly distributed autocatalytic dynamics. That is to say, it needs to carry out recursively the whole network of processes in order to achieve self-maintenance. And, as Segré et al. [70] have pointed out, this is only possible if complexity levels are not too high (or, as they more specifically state, if the polymer chains are not too long).

Thus, for autonomous systems to be able to climb up in complexity, a new, safer way to evolve has to be developed: a way that enables them to keep *reliably*—not only through improving their global, distributed autocatalytic dynamics—those new components and features that can be crucial for their long-term sustenance. In some sense, we could say that BASs already have a certain capacity for “heredity” or some “chemical memory,”<sup>15</sup> but this is still very precarious: it is distributed over the whole organization and not really trustworthy because it is subject to unpredictable, random changes, as Rocha [62] rightly underlines.

All this leads to a first major bottleneck in the evolution of autonomy: the functional components of BASs need to grow in length to enhance their catalytic power and efficiency (size matters for stereospecificity [72]) but, at the same time, if the system generates higher levels of complexity (new molecular aggregates that can act as more sophisticated constraints), its brittleness also increases, since there are no mechanisms to store that complexity in the system (let alone to transmit it to other generations). In other words, standard autocatalytic mechanisms are not reliable or precise enough to ensure the continuous reconstruction and long-term maintenance of progressively more elaborate molecular components (whose functionality will depend on the particular

<sup>15</sup> This is what Segré et al. [70] call “compositional information,” although the name is not very fortunate, because at this stage it is far too early to start using the term information (see a bit further below, even if there will be no room in this article to discuss the issue in depth).



sequence of building blocks making each of them up) and the type of organizations that those components would build up.

The solution to this problem comes with the appearance of molecular replication and hereditary mechanisms, that is, when some functional components begin to show *template* activity. In other words, the system has to start producing macromolecular components capable of making copies of themselves (“hereditary replicators” in Maynard Smith and Szathmáry’s [40] terminology). This involves a completely new form of autocatalytic behavior in the system, which will bring about greater accuracy in its reproduction (as a whole organization),<sup>16</sup> and a high probability that its most relevant functional components (together with the new dynamic organizational properties) will be transmitted from one generation to the next. We conceive this stage as a *one-polymer world* [5], like an RNA world, but deeply embedded (it could not be otherwise) in a new type of metabolic organization: that of *hereditary autonomous systems* [65, 66].

There is, however, a second important bottleneck for autonomous systems in achieving open-ended evolutionary capacities and long-term sustainability. It is related to the impossibility of making fully compatible (i.e., attaining by means of the same type of macromolecular structure or polymer chain) two different sets of properties that come to be crucial in this new scenario [42, 5, 65]: on the one hand, plasticity, specificity, and efficiency in the performance of metabolic tasks (which requires the development of substrate-specific catalysts), and on the other hand, reliable storage, replication, and transmission of *records*, or information-carrying components (i.e., development of genetic mechanisms, or what Maynard Smith and Szathmáry [40] regard as “unlimited hereditary replicators”).

In order to overcome this problem, hereditary autonomous systems have no other possibility but to start producing two types of macromolecular components that will take up different but complementary functions in the organization of those systems. The two types of components (informational records and highly specific catalysts or, equivalently, genotype and phenotype) strongly depend on each other, and their (code-mediated) complex interrelation changes profoundly the organization of autonomous systems, at both the individual (metabolic) and the collective (ecological) level. In fact, the solution to the bottleneck can only be articulated in the context of a more global network, which involves many autonomous systems and many generations (i.e., synchronic, or ontogenic, relations, and diachronic, or phylogenic, ones). And the new type of autonomous system that results from this transition, characterized by a genetically instructed metabolism with *efficient* capacity for open-ended evolution and long-term sustainability, is to be called, properly, a *living system*.

## 7 Reviewing the Definition of Life

The statement concluding the last section clashes with the autopoietic conception of life, in that a rather elaborate form of autonomy (not just basic autonomy) is regarded in this article as minimal life, whereas autopoiesis is proposed as a necessary and *sufficient* condition for life [78]. This calls for a review of the problem of how to define the phenomenon of life, which we will briefly address here (focusing on three critical points on the autopoietic definition), but have recently tackled elsewhere [65] in a deeper and more encompassing way.

The main reasons why we claim that the autopoietic definition of life has to be reconsidered and better accomplished can be shown by analyzing three interrelated issues. The first one is concerned with the level of abstraction that a definition of life should have in order to be applied to living beings not only as we know them, but as

<sup>16</sup> Note the different use of the terms “replication” and “reproduction” (following Dyson [17]).

they could be (in Langton's famous phrasing of the challenge [29]); that is to say, the question of universality. The second has to do with the difficulty of discerning whether life must be conceived, essentially, from the perspective of an individual organism or as a collective phenomenon, that is, in the framework of a wider organization. And the third one, although tightly linked to the previous two, refers more specifically to the problem of whether basic autonomy is a sufficient criterion to decide if a concrete system is truly living. Special attention will be given to this last issue, since it is the most directly related to the topic and contents of the present article.

### 7.1 Universality

It is quite common, especially in a field like ALife, to argue that a definition of life that makes any explicit reference to particular types of molecular components would be "Earth-chauvinist." Hence the claim that it should be articulated in rather abstract and even formal terms. In this respect, the autopoietic definition of life is perfectly adequate. However, it is excessively abstract, in that it offers a conception of the living that is too detached from the physical—material and energetic—requirements that are crucial for its actual implementation (as Fleischaker [20, 21] already highlighted, and we have also remarked herein). This leads to a characterization of the *minimal organizational logic* of biological systems in which physics and chemistry (thermodynamics in particular) have nothing to say. Now, according to the arguments we have developed in previous sections, any real and complete autopoietic system (or BAS, as we prefer to call it) must be made of certain types of components: in particular, it must have a semipermeable active boundary (i.e., a membrane), an energy transduction/conversion apparatus (a set of energy currencies), and at least one type of functional component controlling and facilitating self-construction processes (catalysts).<sup>17</sup>

These are general types of components (note that there is no reference to their specific molecular composition) that come to the scene when a careful analysis of the material-energetic requirements for the implementation of this kind of system (organization) is carried out. And they contribute to characterizing it in a more comprehensive way, without losing universality. The fact that Varela and Maturana attempted to define life in even more abstract terms could be interpreted as a consequence of how they actually worked up the concept of autopoiesis (see Section 1). In any case, this excess of abstraction is probably one of the reasons why they understated the organizational changes linked to the appearance of macromolecular components (like nucleic acids and proteins) in the course of prebiotic evolution, as we shall see below.

### 7.2 Perspective

Varela is right in stressing the individual aspect of the phenomenon of life, for only individual living beings are autonomous agents endowed with a self-produced and active physical border, machinery for hereditary reproduction, and a degree of functional integration that is much higher than in any supraorganismic system or network. He claimed (like Rosen [64] and others), that the process of biological evolution (and the unfolding of all the hierarchical levels of organization in which the biological world is structured) must be a consequence of the—necessarily previous—process of constitution and development of systems that already show a characteristic individual organization: the self-productive, or metabolic, organization.

However, can we speak of minimal life without including evolution? In order to answer this question it is very important to realize that the individual organization of any living being both reflects and depends on the existence of a historical-collective

<sup>17</sup> To be coherent with what we said in last section, in order to have full-fledged living systems another type of macromolecular component should be included here (informational records), but that is not relevant to the argument at this point.

organization, which, through genetic components, *informs* (instructs) each metabolism. And this involves radical changes in the way metabolic processes are carried out by the system. Genes play a fundamental role in the operational dynamics of each living being because they are necessary for the synthesis of its proteins, its most valuable functional components. In fact, all the genetic machinery serves to articulate the link between the complex metabolic organization of living systems and the evolutionary process out of which they come (and of which they are, at the same time, cause and effect). Accordingly, the evolutionary capacity of life, on an individual scale, cannot be regarded as a mere potentiality, but as a feature that is manifested in a particular way of organization.

In other words, the insertion of autonomous, self-constructing systems in an open-ended process of evolution has strong implications for their individual organization. This is the reason why we make the distinction between bare and instructed metabolisms. And this is why (as we have argued more extensively elsewhere [65]), a definition of living beings (as individual entities) cannot be made without taking into account their ecological and evolutionary side. It is precisely through the entanglement between the individual-metabolic and the historical-collective dimensions of their behavior and organization that they ensure their robustness and long-term sustainability as complex autonomous systems. Therefore, our disagreement with the Varelian autopoietic conception does not lie in the idea that autonomy must precede evolution (in fact, we completely agree on that point), but in the conviction that the conditions for the long-term maintenance of autonomous systems with an effective capacity to grow in complexity have to be reflected in their organization, even at the most basic, individual-metabolic level of analysis.

### 7.3 Autonomy

According to Varela and the autopoietic school, self-production (or self-construction, as we prefer to call it here) is a sufficient condition for life because it logically precedes and includes the ability to reproduce, propagate, and generate diversity. Furthermore, the overall conception is that living systems will not alter their most basic and characteristic form of organization (the autopoietic organization), even if their components, homeostatic mechanisms, ways to interact, and so on, change and get increasingly more complex. From the autopoietic theorists' perspective, nucleic acids, for instance, could be admitted as necessary for Darwinian evolution; but that—they claim—would not modify the essential logic of the living organization, its self-productive dynamics. That is why the question of whether to include one or the other type of component in the definition of life is not really relevant for these authors. They just take it as an empirical challenge to determine what is the minimal degree of molecular complexity required to realize an autopoietic organization.

However, although BASs are characterized by a self-constructing dynamics that makes possible their development into living beings (otherwise they would not constitute a crucial step in prebiotic evolution) and, in turn, living beings naturally retain some of the features inherent in BASs (because they ultimately come from them), we must be aware that there are very important differences in their organization and effective capacities (both metabolic and evolutionary, as we already mentioned in the previous subsection). The transition from the origin of autonomy to the origin of life is marked out by several serious bottlenecks (see Section 6), and the ways to overcome those bottlenecks (e.g., the molecular mechanisms developed for the task) involve important, qualitative changes in the organization of autonomous systems, at both the individual and the collective level.

More specifically, in order to start a process of open-ended evolution, autonomous systems have to incorporate genetic machinery, where informational records are partly

decoupled from all the muddle of metabolic reactions [44]. This decoupling turns out to be fundamental from the organizational point of view, since it allows the recruitment for and by the individual systems of the results of a much more encompassing process of evolution (by natural selection), a process that takes place on a very different, much longer time scale. The changes in the genetic components are largely independent of (detached from) the dynamic, metabolic processes that those components instruct, and that is why, in practice, the former appear as quasi-inert molecular structures. And it is precisely when autonomous systems incorporate and integrate such quasi-inert components in their metabolic organization that it becomes possible for them to grow steadily in complexity and evolve in a truly open-ended way.

All this involves the establishment of a translation mechanism (a code) between the informational and catalytic components of the system (DNA and proteins, in known living systems), and a radically new form of organization, as Pattee [56, 57] has consistently argued and we have also put forward in some other articles [42, 43, 44, 65]. Accordingly, there must be a qualitative jump between basic autonomy and life: only living systems need to have a metabolic organization based on two types of complementary macromolecular components coupled through a code, because they have a form of autonomy with capacity for Darwinian evolution and long-term sustainability. Therefore, although the particular genetic code found in all living organisms on Earth could be, to a certain extent, arbitrary (i.e., a frozen accident), the actual existence of a code would be necessary both for the long-term persistence and for the open increase in complexity of any population of autonomous systems; thus, it should be considered as a universal feature of life.

One could still argue that autonomy and agency are enough to characterize life, regardless of whether they are sustainable in the long run. And actually, if we ever find (in the lab, or on another planet) true BASs, it will be quite difficult to refrain from calling them living systems, given their repertoire of biologically relevant features. The trouble is that this conception of life—apparently more universal—turns out to be inconsistent with a precise enough characterization of the fundamental organization of real living beings (which are, after all, genetically instructed metabolisms). In other words, we would have to deal with two distinct theoretical constructs claiming to grasp the basic organization of the living: one autopoietic and the other, so to speak, “autopoietic with a code.” Actually, if BASs are ever found, they will develop—and belong to—their own phenomenological domain; so if we decided to call that domain “biological” (not merely “proto-biological”), we would then have to elaborate a new (more specific and richer) definition for life as we know it on Earth.

## 8 Final Remarks

So far—probably due to the development and strong influence of molecular biology in the last decades—enormous efforts have been and are being put into the abiotic synthesis of the components of present living beings (of biomolecules, normally taken as separate chemical species), disregarding the fact that they actually occur together and their origins and nature may well be strongly interdependent. Nevertheless, at present there seems to be an increasing tendency towards more systemic approaches in biological research at large. It is of crucial importance, in our opinion, that this shift also take place in the field of the origins of life and contribute to refocusing its research program.

The importance of the idea of autonomy, in particular of the BAS, lies in its great potential to explain the origin of molecular complexity as a property of a whole system, rather than a property of individual molecules. The identity of the system only appears when a coherent set of couplings that link components, processes, and flows

of energy is established. Thus, the system is the result of a deep intertwining between constructive and energetic relations, between the organizational structure and the specific materiality of its components, which explains the holistic character of the processes that make it up. The emergence of an effective capacity for adaptive self-maintenance (agency) is the consequence of this holistic organization. Furthermore, inherent in the functional dynamics of BASs is their potential to produce and recruit ever new components, which brings about a changeable scenario where novelty and variety naturally emerge. In other words, the constructive and self-maintaining dynamics of these systems intrinsically pushes them to expand and explore new ways of operating, both internal and externally.

So the appearance of BASs gives way to the creation of a world of functionalities, as a consequence of their own machinery of continuous production and reproduction of components, out of which those that contribute to the maintenance of the system as a whole will be recruited. However, as we highlighted in Section 6, this capacity faces a dilemma: the higher the complexity of the components, the more fragile the system becomes. This apparent limitation can only be overcome with the invention of a new kind of organization, based on the development of hereditary mechanisms which are metabolically “off line.”

Nevertheless, the real value of BASs is in their creative nature as autonomous chemical machines (or chemical automata), which allows us to think of a possible transition between the inert and the living. In fact, only assuming the existence of BASs (or very similar systems), a realistic scenario for the origins of genetic information can be conceived, because they are the natural factory of all highly complex molecular mechanisms (i.e., macromolecular ingredients and constraints). Furthermore, without BASs it does not even make sense to conceive of informational systems, because (as Wicken notably points out [81, p. 104]) genetic sequences require an open world of functionalities in which these sequences are expressed.

Therefore, it is only in the context of metabolic agents like BASs that genetic machinery can be developed and—even more important—make sense. That is why both the phenotype-genotype distinction and the consequent open-ended evolution process are simply not viable without basic autonomy. In turn, genetic information plays an outstanding role in the unfolding of autonomous systems because it makes their basic self-constructive dynamics compatible with an evolutionary dynamics in which a steady increase in complexity is possible.

There is no doubt that the challenge to synthesize life artificially is still very far from our ability (will it ever be close?). However, the *in vitro* production of autonomous chemical systems is certainly at hand, and it should be seriously tackled because, apart from all the new knowledge and applications it would bring about, it would constitute the first major step in that huge challenge.

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### References

1. Atkins, P. W. (1984). *The second law*. New York: Freeman.
2. Bachmann, P. A., Luisi, P. L., & Lang, J. (1992). Autocatalytic self-replicating micelles as models for prebiotic structures. *Nature*, *357*, 57–59.

3. Bachmann, P. A., Walde, P., Luisi, P. L., & Lang, J. (1990). Self-replicating reverse micelles and chemical autopoiesis. *Journal of the American Chemical Society*, *112*, 8200–8201.
4. Baltscheffsky, H. (1996). Energy conversion leading to the origin and early evolution of life: Did inorganic pyrophosphate (PPi) precede adenosine triphosphate (ATP)? In H. Baltscheffsky (Ed.), *Origin and evolution of biological energy conversion* (pp. 1–9). New York: VCH Publishers.
5. Benner, S. A. (1999). How small can a microorganism be? In *Size Limits of Very Small Microorganisms (Proceedings of a Workshop)* (pp. 126–135). Washington, DC: National Academy Press.
6. Bickard, M. (1993). Representational content in humans and machines. *Journal of Experimental and Theoretical Artificial Intelligence*, *5*, 285–333.
7. Bickhard, M. H. (2000). Autonomy, function and representation. *Communication and Cognition—Artificial Intelligence*, *17*(3–4), 111–131.
8. Bro, P. (1997). Chemical reaction automata. *Complexity*, *2*(3), 38–44.
9. Cemin, S. C., & Smolin, L. (1997). Coevolution of membranes and channels: A possible step in the origin of life. Personal communication.
10. Collier, J. H., & Hooker, C. A. (1999). Complexly organized dynamical systems. *Open Systems and Information Dynamics*, *6*, 241–302.
11. Csanyi, V. (1989). *Evolutionary systems and society: A general theory of life, mind and culture*. Durham, NC: Duke University Press.
12. Deamer, D. W. (1994). Sources and syntheses of prebiotic amphiphiles. In G. R. Fleischaker, S. Colonia & P. L. Luisi (Eds.), *Self-production of supramolecular structures* (pp. 217–229). Dordrecht, The Netherlands: Kluwer Academic.
13. Deamer, D. W. (1997). The first living systems: A bioenergetic perspective. *Microbiology and Molecular Biology Reviews*, *61*(2), 239–261.
14. Deamer, D. W. (1998). Membrane compartments in prebiotic evolution. In A. Brack, (Ed.), *The molecular origins of life. Assembling the pieces of the puzzle* (pp. 189–205). Cambridge, UK: Cambridge University Press.
15. Deamer, D. W., Dworkin, J., Sandford, S. A., Bernstein, M. P., & Allamandola, L. J. (2002). The first cell membranes. *Astrobiology*, *2*(4), 371–381.
16. de Duve, C. (1991). *Blueprint for a cell: The nature and origin of life*. Burlington, NC: Neil Patterson Publishers.
17. Dyson, F. J. (1985). *Origins of life*. Cambridge, UK: Cambridge University Press.
18. Farmer, J., Kauffman, S., & Packard, N. (1986). Autocatalytic replication of polymers. *Physica D*, *22*, 50–67.
19. Fell, D. (1997). *Understanding the control of metabolism*. London: Portland Press.
20. Fleischaker, G. R. (1988). Autopoiesis: The status of its system logic. *BioSystems*, *22*, 37–49.
21. Fleischaker, G. R. (1990). Origins of life: An operational definition. *Origins of Life and Evolution of the Biosphere*, *20*, 127–137.
22. Harold, F. M. (1986). *The vital force: A study of bioenergetics*. New York: Freeman.
23. Jonas, H. (1963). *The phenomenon of life. Toward a philosophical biology*. New York: Harper and Row.
24. Kampis, G. (1991). *Self-modifying systems in biology and cognitive science: A new framework for dynamics, information and complexity*. Oxford, UK: Pergamon Press.
25. Kauffman, S. (1986). Autocatalytic sets of proteins. *Journal of Theoretical Biology*, *119*, 1–24.
26. Kauffman, S. (1993). *The origins of order: Self-organization and selection in evolution*. Oxford, UK: Oxford University Press.

27. Kauffman, S. (2000). *Investigations*. Oxford, UK: Oxford University Press. [Provisional draft (1996). *Investigations*. Santa Fe Institute Working Paper, Chapters 3 and 4.]
28. von Kiedrowski, G. (1986). A self-replicating hexadeoxy nucleotide. *Angewandte Chemie. International Edition in English*, 25, 932–935.
29. Langton, C. G. (1989). Artificial life. In C. G. Langton, (Ed.), *Artificial life I (proceedings of the First Conference on Artificial Life, Los Alamos, September, 1987)* (pp. 1–47). Redwood City, CA: Addison-Wesley.
30. Lanyi, J. K., & Pohorille, A. (2001). Proton pumps: Mechanism of action and applications. *Trends in Biotechnology*, 19(4), 140–144.
31. Lee, D. H., Granja, J. R., Martínez, J. A., Severin, K., & Ghadiri, M. R. (1996). A self-replicating peptide. *Nature*, 382, 525–528.
32. Lipmann, F. (1941). Metabolic generation and utilization of phosphate bond energy. *Advances in Enzymology*, 1, 99–107.
33. Luisi, P. L. (1993). Defining the transition to life: Self-replicating bounded structures and chemical autopoiesis. In W. Stein & F. J. Varela (Eds.), *SFI studies in the sciences of complexity, Lecture note Volume III* (pp. 17–39). Reading, MA: Addison-Wesley.
34. Luisi, P. L. (2002). Toward the engineering of minimal living cells. *The Anatomical Record*, 268, 208–214.
35. Luisi, P. L., & Varela, F. J. (1989). Self-replicating micelles: A chemical version of a minimal autopoietic system. *Origins of Life & Evolution of the Biosphere*, 19, 633–643.
36. Luisi, P. L., Oberholzer, T., & Lazcano, A. (2002). The notion of a DNA minimal cell: A general discourse and some guidelines for an experimental approach. *Helvetica Chimica Acta*, 85, 1759–1777.
37. McMullin, B. (2000). John von Neumann and the evolutionary growth of complexity: Looking backward, looking forward. *Artificial Life*, 6, 347–361.
38. Maturana, H., & Varela, F. J. (1973). *De máquinas y seres vivos—una teoría sobre la organización biológica*. Santiago de Chile: Editorial Universitaria S.A.
39. Maturana, H., & Varela, F. J. (1980). *Autopoiesis and cognition. The realization of the living*. Dordrecht, The Netherlands: D. Riedel.
40. Maynard Smith, J., & Szathmáry, E. (1995). *The major transitions in evolution*. Oxford, UK: Freeman & Co.
41. Mitchell, P. (1961). Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. *Nature*, 191, 144–148.
42. Moreno, A., & Fernández, J. (1990). Structural limits for evolutive capacities in molecular complex systems. *Biology Forum*, 83(2/3), 335–347.
43. Moreno, A., & Fernández, J. (1992). From records to self-description: The role played by RNA in early evolutive systems. *Acta Biotheoretica*, 40, 1–9.
44. Moreno, A., & Ruiz Mirazo, K. (2002). Key issues regarding the origin, nature and evolution of complexity in nature: Information as a central concept to understand biological organization. *Emergence*, 4.1/4.2, 63–76.
45. Moreno, A., Umeréz, J., & Fernández, J. (1994). Definition of life and research program in artificial life. *Ludus Vitalis*, II(3), 15–33.
46. Morowitz, H. J. (1968). *Energy flow in biology*. New York: Academic Press.
47. Morowitz, H. J. (1992). *Beginnings of cellular life*. New Haven, CT: Yale University Press.
48. Morowitz, H. J. (1999). A theory of biochemical organization, metabolic pathways, and evolution. *Complexity*, 4(6), 39–53.
49. Morowitz, H. J., Heinz, B., & Deamer, D. W. (1988). The chemical logic of a minimum protocell. *Origins of Life & Evolution of the Biosphere*, 18, 281–287.

50. Morris, C. E. (2002). How did cells get their size? *The Anatomical Record*, 268, 239–251.
51. von Neumann, J. (1966). *Theory of self-reproducing automata*. [A. W. Burks (Ed.)] Urbana, IL: University of Illinois.
52. Nicolis, G., & Prigogine, Y. (1977). *Self-organization in non-equilibrium systems*. New York: Wiley.
53. Oparin, A. I. (1924). *Proiskhozhdienie zhidny*. Moscow: Moskovskii Rabochii. [The origin of life. English translation (1967), *The origins of life*, J. D. Bernal (Ed.) (pp. 199–234). London: Weidenfeld & Nicolson. Earlier English translation (1994), *Origins of life. The central concepts*, D. W. Deamer & G. R. Fleischaker (Eds.). (pp. 31–71). Boston: Jones and Barlett.]
54. Oliver, A. E., & Deamer, D. W. (1994). Alpha-helical hydrophobic polypeptides form proton-selective channels in lipid bilayers. *Biophysical Journal*, 66, 1364–1379.
55. Pattee, H. H. (1973). The physical basis and origin of hierarchical control. In H. H. Pattee (Ed.), *Hierarchy Theory* (pp. 73–108). New York: Braziller.
56. Pattee, H. H. (1977). Dynamic and linguistic modes of complex systems. *International Journal of General Systems*, 3, 259–266.
57. Pattee, H. H. (1982). Cell psychology: An evolutionary approach to the symbol-matter problem. *Cognition and Brain Theory*, 4, 325–341.
58. Peretó, J. (1994). *Orígenes de la evolución prebiótica*. Madrid: Eudema.
59. Pohorille, A., & Deamer, D. W. (2002). Artificial cells: Prospects for biotechnology. *Trends in Biotechnology*, 20(3), 123–128.
60. Pohorille, A., & New, M. H. (2000). Models of protocellular structures, functions and evolution. In *Proceedings of Rencontres de Blois "Frontiers of Life"*, Chateau de Blois, France.
61. Rashevsky, N. (1960) *Mathematical biophysics: Physico-mathematical foundations of biology*, 3rd ed. (1st ed., 1938; 2nd ed., 1948). New York: Dover.
62. Rocha, L. M. (1996). Eigenbehaviour and symbols. *Systems Research*, 13(3), 371–384.
63. Rosen, R. (1971). Some realizations of (M, R)-systems and their interpretation. *Bulletin of Mathematical Biophysics*, 33, 303–319.
64. Rosen, R. (1991). *Life itself: A comprehensive inquiry into the nature, origin and fabrication of life*. New York: Columbia University Press.
65. Ruiz Mirazo, K., Pereto, J., & Moreno, A. (2004). A universal definition of life: Autonomy and open-ended evolution. *Origins of life and evolution of the biosphere*, 34(3), 323–346.
66. Ruiz-Mirazo, K. (2001). *Physical conditions for the appearance of autonomous systems with open-ended evolutionary capacities*. PhD dissertation, University of the Basque Country. (Complete version only available in Spanish—<http://www.sc.ehu.es/PhBioGroup>.)
67. Ruiz-Mirazo, K., & Moreno, A. (1998). Autonomy and emergence: How systems become agents through the generation of functional constraints. *Acta Polytechnica Scandinavica*, Ma91, 273–282.
68. Ruiz-Mirazo, K., & Moreno, A. (2000). Searching for the roots of autonomy: The natural and artificial paradigms revisited. *Communication and Cognition—Artificial Intelligence*, 17(3–4), 209–228.
69. Ruiz-Mirazo, K., Moreno, A., & Morán, F. (1998). Merging the energetic and the relational-constructive logic of life. In C. Adami, R. Belew, H. Kitano, & C. Taylor (Eds.), *ALife VI (Proceedings of the Sixth International Conference on Artificial Life)* (pp. 448–451). Cambridge, MA: MIT/Bradford Books.
70. Segré, D., Ben-Eli, D., & Lancet, D. (2000). Compositional genomes: Prebiotic information transfer in mutually catalytic non-covalent assemblies. *Proceedings of the National Academy of Sciences of the U.S.A.*, 97, 4112–4117.
71. Skulachev, V. P. (1992). The laws of cell energetics. *European Journal of Biochemistry*, 208, 203–209.



72. Srere, P. A. (1984). Why are enzymes so big? *Trends in Biochemical Sciences*, 9, 387–390.
73. Steinber-Yfrach, G., Rigaud, J.-L., Durantini, E. N., Moore, A. L., Gust, D., & Moore, T. A. (1998). Light-driven production of ATP catalysed by FF-ATP synthase in an artificial photosynthetic membrane. *Nature*, 392, 479–482.
74. Szostak, J. W., Bartel, D. P., & Luisi, P. L. (2001). Synthesizing life. *Nature*, 409, 387–390.
75. Varela, F. (1979). *Principles of biological autonomy*. New York: Elsevier.
76. Varela, F. J. (1994). On defining life. In G. R. Fleischaker, S. Colonia, & P. L. Luisi (Eds.), *Self-production of supramolecular structures* (pp. 23–31). Dordrecht, The Netherlands: Kluwer Academic.
77. Varela, F. (2000) *El fenómeno de la vida*. Santiago: Ediciones Dolmen.
78. Varela, F. J., Maturana, H., & Uribe, R. (1974). Autopoiesis: The organization of living systems, its characterization and a model. *BioSystems*, 5, 187–196.
79. Wächtershäuser, W. (1988). Before enzymes and templates: Theory of surface metabolism. *Microbiological Reviews*, 52, 452–484.
80. Weber, A., & Varela, F. (2002). Life after Kant: Natural purpose and the autopoietic foundations of biological individuality. *Phenomenology and the Cognitive Sciences*, 1, 97–125.
81. Wicken, J. S. (1987). *Evolution, thermodynamics and information. Extending the Darwinian program*. Oxford, UK: Oxford University Press.