The problem of the emergence of functional diversity in prebiotic evolution

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Abstract   Since Darwin it is widely accepted that natural selection (NS) is the most important mechanism to explain how biological organisms—in their amazing variety—evolve and, therefore, also how the complexity of certain natural systems can increase over time, creating ever new functions or functional structures/relationships. Nevertheless, the way in which NS is conceived within Darwinian Theory already requires an open, wide enough, functional domain where selective forces may act. And, as the present paper will try to show, this becomes even more evident if one looks into the problem of origins. If there was a time when NS was not operating (as it is quite reasonable to assume), where did that initial functional diversity, necessary to trigger off the process, come from? Self-organization processes may be part of the answer, as many authors have claimed in recent years, but surely not the complete one. We will argue here that a special type of self-maintaining organization, arising from the interplay among a set of different endogenously produced constraints (pre-enzymatic catalysts and primitive compartments included), is required for the appearance of functional diversity in the first place. Starting from that point, NS can progressively lead to new (and, at times, also more complex) organizations that, in turn, provide wider functional variety to be selected for, enlarging in this way the range of action and consequences of the mechanism of NS, in a kind of mutually enhancing effect.

Keywords   Natural selection · Functional diversity · Prebiotic evolution · Self-organization · Self-assembly

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Introduction

Biological systems constitute highly complex organizations, whose dynamic behavior is based on the properties of sophisticated molecules (biopolymers: like DNA, RNA or proteins) and other supramolecular structures (e.g., lipid membranes) but also, and perhaps more importantly, on the specific and intricate relationships that these molecules and structural aggregates establish and manage to maintain among them. Since Darwin, all this complexity has been interpreted as a consequence of a long-term historical and collective process that goes far beyond the ontogenic trajectories of individual living beings. In that framework, in which the biological phenomenon conveys a large-scale spatial and temporal dimension (though taking place in a physically/materially limited environment), natural selection (NS) would be the main mechanism to explain how evolutionary pathways develop and, thereby, how the complexity of certain natural systems can increase over time. Nevertheless, the way in which NS is conceived within Darwinian Theory requires an open, sufficiently wide phenotypic domain where selective forces may act, which is implicit in a minimal, but sufficiently rich concept of organism. In turn, underlying the idea of phenotypic domain one can find an implicit, usually not very well defined idea of 'functional domain', which refers to the variety of ways in which the different (structural) components of a system interact and complement with each other, in their continuous contribution to the global maintenance of that system.[1]

In the case of full-fledged organisms there is little doubt that this functional domain exists. But where does it come from? How was it developed? Different approaches to the naturalization of the concept of biological function resort to the mechanism of NS to ground it (Millikan 1989; Neander 1991; Godfrey-Smith 1994). However, if one looks into the problem of origins, an indirect and rather elaborate mechanism like NS cannot be taken for granted: there was for sure a time when NS was not operating, so one should go step by step and try to determine what kind of systems and processes triggered off and fixed that type of evolutionary

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[1] What does this actually mean? Compare a frozen, inactive cell (like a spore) with an active, metabolizing one. The former entity has a high degree of structural diversity (many different macromolecules, made of many different atoms, other supramolecular aggregates, etc.) but lacks proper functionality, because those complex parts are not really operating, interacting through catalytic, transport or chemical conversion processes within the system. In contrast, the latter (the living cell) is also a functionally diverse entity because its structural components are playing differentiated roles in the dynamic behavior or activity of the system. To give another example, consider a modular type of molecule (e.g., a polynucleotide) consisting of a large number of different monomers (e.g., a long sequence of four different nucleotides, like in real life), which undergoes template replication in an in vitro selection-competition experiment (as will be described in more detail in "Lessons from Spiegelman's experiment"). This type of macromolecular entities are structurally very complex, but functionally speaking their diversity is rather poor: the only distinguishable roles they can play, as such molecules (i.e., without a cellular organization around) consist in enhancing their own replication rate or their resistance to hydrolysis. In the context of populations of molecules just competing with each other (not supporting each other), the idea of system-organization is very weak, and this clearly hinders our speaking in terms of a 'functional space'.

dynamics. According to Maynard Smith (1986), and to many other authors after him, the main conditions for a population of systems to start a process of evolution by NS are (1) that they multiply, (2) that they have heredity, (3) that this heredity is not totally accurate (variability), and (4) that some of the inherited traits affect the chance of reproduction or survival of individual systems.[2] These terms/properties—multiplication, variation and heredity—have the advantage (or disadvantage, depending on the perspective adopted) of being general, i.e., not necessarily characteristic of—or only applicable to—living entities. Hence the important conceptual difference between the idea of 'units of evolution/selection' and 'units of life' (Szathmary et al. 2005).

Nevertheless, one has to be very careful when dealing with systems that are not full-fledged living organisms: i.e., that are not, at the same time, units of life and units of evolution. The reason is that an oversimplification of any of those three properties may turn out to be tricky, since it can lead to artifacts, bottlenecks or counter-intuitive situations. For instance, in the classical experiments with 'self-replicating' micelles or vesicles carried out in Luisi's lab (Bachmann et al. 1992; Walde et al. 1994) there is multiplication, variation, and—one could argue—also some sort of heredity, even if it is just 'compositional heredity', like Segré and Lancet's (2000). But most researchers in the field of origins of life (including probably the authors of those experiments themselves) would be reluctant to accept that there is a proper process of evolution by NS happening there. Likewise in the more recent 'competition experiments' between different populations of fatty acid vesicles carried out by the group of Szostak (Chen et al. 2004), in which osmotically tense vesicles uptake freshly added lipid—and, therefore, grow and reproduce—much faster than deflated ones. Or, in a completely different context, as we will describe in more detail below, when the system under analysis is a population of macromolecules that can replicate very accurately (so there is a sophisticated form of multiplication and heredity) but not a sufficiently ample space for 'phenotypic variation' (see also footnote 1).

Therefore, the triad (multiplication, variation, heredity—as expressed in Maynard Smith's general terms) would constitute a necessary but not sufficient set of conditions for what is commonly understood by Darwinian evolution. If there are entities (e.g., 'infrabiological' systems, as Szathmary et al. (2005) call them) that fulfill those conditions but do not evolve like biological systems do, one has to turn

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[2] In Maynard Smith's (1986) own words: «Given a population of entities having the properties of multiplication, variation and heredity, and given that some of the variation affects the success of these entities in surviving and multiplying, then, that population will evolve, that is, the nature of its constituent entities will change in time. Of the three essential properties multiplication means that one entity can give rise to two, and variation that not all entities are identical. Heredity means that like begets like.» (p. 4). More recently, Godfrey-Smith (2009), for instance, has reinterpreted this by saying that Darwinian Evolution requires «a collection of causally connected individual things in which there is variation in character, which leads to differences in reproductive output (differences in how much or how quickly individuals reproduce), and which is inherited to some extent. Inheritance is understood as similarity between parent and offspring, due to the causal role of the parents.» (p. 39).

more demanding. In other words, Darwinian evolution itself is the result of an already evolved mechanism; therefore this type of evolution has most likely been preceded by other "pre-Darwinian" competitive-evolutionary dynamics. For instance, Fox Keller (2007) suggests that, initially, other naturally occurring complex systems must undergo selection processes that have little to do with (proper Darwinian) NS. In those cases one should speak, according to this author, in terms of stability or persistence—rather than survival—of the system and capacity for robust self-maintenance—instead of fitness. What is important to highlight, in this context, is that reflecting about the problem of origins helps us realize: (1) first, that the conditions for evolution have themselves evolved; and (2) second, that the degree in which those conditions are fulfilled is also variable, as Godfrey-Smith has recently pointed out (2009).

In this paper we will focus particularly on the issue of variation, or variability, understood as phenotypic variation/variability, being faithful to the Darwinian spirit (Lewontin 1970) but analyzing it in a prebiotic context. Although, of course, each property of the aforementioned triad is not independent from the others, here we would like to address especially the question of *what type of variability was required to set up a selection mechanism* (the most primitive form of NS) that made possible a pre-Darwinian evolutionary process as a result of which living systems eventually originated. For that initial or precursory form of NS is structural variability enough or some sort of *functional* variability must be included, too? How can that type of variability be identified in a system? And where/what are the natural sources of it? Questions like these constitute the main target of the present article.

The line of reasoning will be constructed as follows: first, we will provide an empirical argument against certain simplified versions of the variability requirement, showing the need to overcome a preliminary threshold of functional—not just structural—diversity. Then, we will try to show that the most probable scenario for the emergence of this minimal space of precursor functional diversity is at the crossroads between self-organization and self-assembly processes. However, that is not enough: a particular type of self-maintaining organization, including an endogenous boundary (semipermeable membrane) and a cyclic network of catalytic interactions, is required. We will describe why in those conditions, in which a mixture of global/unspecific and local/specific constraints achieve some sort of closure or mutually reinforcing relationship, robust enough self-reproducing organizations may form, in a way that selective forces—beyond bare physical/chemical stability or efficiency—can start operating and ruling the evolution of the population. Finally, in the last part of the paper, we will briefly discuss the main transitions (related more directly to the development of hereditary mechanisms) by which NS progressively takes over as a selection principle and increases its importance in relation to other rules or mechanisms of evolution (like self-organization principles). This eventually leads to a complementary, more balanced situation in which one could say, roughly, that «NS disposes what self-organization proposes (Batten et al. 2008)».

Lessons from Spiegelman's experiment

Many scientists assume that life started as a self-replicating molecule, the first "gene".[3] According to this view, once the early "gene" or "replicator" had appeared in adequate environmental conditions, it would have rapidly generated a whole population of replicators, leading to a process of evolution by NS. Although a replicator is any entity that produces copies of itself, what the advocates of this view (the so-called "replication first" approach) have in mind when talking about primitive replicators as the condition for the beginning of evolution by NS are *modular templates* (Maynard Smith and Szathmary 1995; Szathmary 2006),[4] namely, relatively complex oligomers which, by their structure, tend to catalyze their own copies. Since the specific order and number of the building blocks ("modules") of these self-replicating oligomers are not directly involved in its template capacity, sequential changes can occur during its replication and those changes somehow become "memorized" or recorded (i.e., hereditarily transmitted), thus leading to different types of "populations" (different in terms of their sequences of building blocks). Then, it is assumed that those differences may provide advantages or disadvantages in the context of competing populations of replicators. Accordingly, in this process, we may ask whether a longer and therefore more complex replicating entity would have a selective advantage. Contrary to what might be expected, the answer is negative. Let us see why.

In 1967, Sol Spiegelman conducted an interesting set of experiments. He inserted RNA from a simple virus ($Q\beta$) into a solution that contained the enzyme RNA replicase from the $Q\beta$ virus, some free nucleotides and some salts. In this environment, the RNA started to replicate. After a while, Spiegelman took some RNA and moved it to another tube containing a fresh solution. This process was subsequently repeated. Shorter RNA chains were able to replicate faster, so the RNA became shorter and shorter. After 74 generations, the original strand with 4.500 nucleotide bases ended up as a dwarf genome, given the moniker of "Spiegelman's Monster", with only 218 bases. Such a short RNA had been able to replicate very quickly in these circumstances (Kacian et al. 1972). Thirty years later, Oehlenschläger and Eigen (1997) showed that the Spiegelman monster eventually becomes even shorter, containing only 48 or 54 nucleotides, which are simply the binding sites for the enzyme RNA replicase.

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[3] As it is well known, there are two basic views in the current research into the origin of life (Pereto 2005; Anet 2004), called respectively, "metabolism-first" and "replication-first". The former considers that the beginning of biogenesis should be based on chemical self-maintaining networks (driven towards higher levels of complexity by principles of self-organization), whereas the latter defends that life began with the appearance of self-replicating structures (i.e., molecules), driven towards higher levels of complexity by NS.

[4] Even very simple template replicators may show "hereditary" variations. Think, for instance of the case of a self-replicating crystal, which by chance incorporates a screw-dislocation. Since this dislocation speeds up the binding of ions, it preserves its screw structure as the crystal grows. But in order to display an evolutionary process, advocates of the so-called "Replication First" hypothesis require modular self-replicating templates, namely, replicators possessing sequences of different building blocks, whose hereditary modifications will be considered the key element for displaying an evolutionary process (Szathmary 2006).

What lessons can we learn from this experiment? Removed from the organization of the host cell, and put into a simplified environment, the only "useful" possibility for the populations of replicators is to replicate faster (as well to become more resistant to hydrolysis). Any kind of random walk leading to an increase in the structural complexity[5] of replicators will be selectively discarded, because it is not (and, apparently, cannot be either) linked to some novel "useful" result. Clearly, we are faced with a serious problem: since the linear (i.e., merely structural) variations of the replicators have no other phenotypic meaning *per se*, evolution would only lead to local optima with minimal or very low complexity levels (because the way to improve fitness is mere structural variation). What this experiment shows is that in the absence of some organization providing a sufficiently rich phenotypic domain, selective forces cannot perform beyond a minimal space of action. Thus, a scenario that provides enough phenotypic variety to be selected for is required in the first place (Wicken 1987).[6]

Advocates of the "replication-first" approach may object that, given adequate environmental variety, a process which increases the structural complexity of the replicators may occur. For example, Pross (2003) has argued that:

«If we consider the primal replicating molecule to be some biopolymer of limited length—say 10 units long, its process of imperfect replication might randomly lead to 9-unit and 11-unit sequences, in addition to 10-unit entities. Now, if we arbitrarily assume that the shortest sequence length capable of replication is 10 units, then this would mean that the 9-unit sequence plays no further role, other than to provide a source of building blocks for active replicators. Thus, the 10-unit replicator represents the minimum complexity wall in the Gould (1996) metaphor. If we now consider the possible reactions of the 11-unit replicator, formed by genetic drift from the 10-unit one, we could by further acts of imperfect replication lead to the formation of both 10- and 12-unit sequences. (…) Of course, the emergence of more complex replicators would not be kinetically sustainable if the added complexity were unable to provide some kinetic advantage—complexity must provide some existential advantage. It now seems clear that the kinetic advantage that longer sequences could provide would not have stemmed from any inherently greater replicating ability associated with the longer sequences (Spiegelman's experiment demonstrated that) but, rather, *through a variety of catalytic effects that some particular sequences might have afforded*.» (ours italics, p. 401).

Therefore, Pross implicitly admits that the functional domain required by selective mechanisms to drive systems towards higher levels of complexity is linked

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[5] As explained in the next sections, within the framework of competing populations of self-replicating molecules (namely, in the absence of proto-metabolic organizations), the term "variation" can only mean structural variation.

[6] Following parallel lines of reasoning, we find Bedau's (1991) claim against etiological accounts of function (in particular, against the so-called 'selected effect theories'): he argues that even clay crystals could fulfill what is usually supposed to be required for evolution by NS but, nevertheless, they do not perform functions.

to what he calls a «variety of catalytic effects».[7] However, the concept of catalytic networks or—the more abstract—'catalytic task space' (Kauffman 2000) corresponds to a rather different scenario. As explained in more detail below, it involves a framework in which populations of molecules, instead of competing for faster replication, have diverse catalytic effects on each other, as a way of coordinating the particular locations, times and speeds at which their chemical transformations occur. This implies gathering together different reactions, i.e., embedding the processes of synthesis of new structures—and degradation of other ones—in a self-maintaining *organization*. Thus, in the end, many advocates of the primacy of replication and selection accept the almost immediate inclusion of organizational features in the system, if the latter is really to make any progress in complexity.[8] But why should an organizational framework (implicit in this concept of 'variety of catalytic effects') change things and provide functional diversity? To answer this question we should first consider what is meant by functional diversity in the context of prebiotic evolution, and how it can be initially generated.

On the idea of 'minimal functional diversity'

When looking for the origin of functional diversity, the first problem that we face is the very understanding of what we mean by diversified parts in a natural, possibly prebiotic system. As we have already seen, systems endowed with a remarkable level of structural diversity do not necessarily show functional diversity. Nevertheless, in the literature of origins of life the term "function" is widely used (unfortunately, most of the times in a purely intuitive sense, without precise justification) to convey the idea of an increase in the complexity of a part—or subset of parts—*playing a certain role* in the organization of a system. Now, what does it mean that something is playing a role in a system? In chemistry it is common to describe what happens in a reactive system in terms of molecules (or atomic substructures) "performing operations" (e.g., creating or destroying particular bonds). But in order to find distinguishable functions in a prebiotic chemical system it is necessary to "naturalize" the very idea of function, namely, to formulate it independently of any intentional agent.

  Given the difficulties explained in the previous sections, a possible alternative to grounding the idea of function on the mechanism of NS (which would imply etiological accounts of functionality spanning through a rather long and indirect historical sequence of events), is to conceive of it as something directly linked to the

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[7] Something similar happens with the recent approach proposed by Rosenberg and Kaplan (2005). These authors propose a definition of NS in which fitness is almost trivial. And to avoid this risk, to widen the concept of fitness to encompass something more than mere tautology, it is necessary to let reproductive self-maintaining networks be capable of introducing variations in their catalytic tasks to compete with each other.

[8] As an example to illustrate this change of focus we can quote Albert Eschenmosser (2007: 12837), an expert in the so-called 'RNA world' view, who has recently pointed out that what really matters in the chemical process of the origin of life is finding «a chemistry that may have the potential to mediate, or to be part of, a process in which a library of chemical reactions moves toward becoming a network of potentially metabolic reactions».

current organization of a system: i.e., to what the system or different parts of it are doing in a precise time window. Bickhard (2000) and Christensen (Christensen and Bickhard 2002), for instance, have proposed a way to naturalize the idea of function in the framework of far from equilibrium (FFE) self-maintaining systems. According to this view, functions are contributions that the constitutive parts of a FFE system make to its self-maintenance (SM). As Bickhard (2000: 116) expresses it, "the contributions that a self-maintaining system make to its own continued existence are, in that sense, *functional* for that system: they serve the general function of helping to maintain the existence of the far-from-equilibrium system. Derivatively, any components or parts of a system (…) serve such a function insofar as they make such a contribution. Serving a function, in this sense, is necessarily relative to the system whose maintenance is being contributed to."[9]

This approach is very interesting for our purposes, because it situates the emergence of functions in a prebiotically plausible form of organization. Actually, spontaneous dissipative structures, like hurricanes, thermal convection systems (e.g., Bénard cells) or chemical oscillations (e.g., B-Z reactions) are examples of FFE self-maintaining systems. As described by different authors (Prigogine and Stengers 1979; Nicolis 1989), in these cases a collective pattern of spatial and/or temporal order, spontaneously triggered off by fluctuations and the establishment of long-range non-linear correlations among the components of the system, once formed, acts as a constraint on those very components. The relevance of this type of "self-organizing" processes lies in the fact that it explains how, in certain specific scenarios, a set of chemical reactions could have come spontaneously together, and maintained together, under FFE conditions. Thus, if FFE self-maintenance provides a minimal naturalized account for understanding what is "functional", some form of functional diversification could have appeared very early in prebiotic evolution.

However, there is caveat. Although it surely covers an important aspect of the problem, this standpoint is not really satisfactory, for one main reason: as recently argued by Mossio et al. (2009), in order to speak about functions in a system *differentiation* (both in terms of the parts of the system and their specific contribution to its global self-maintaining dynamics) turns out to be crucial, and standard dissipative structures do not show organizationally distinguishable parts. This is quite obvious in the case of hurricanes or Bénard cells, for example, which can hardly be considered serious candidates as systems with functional parts because their components are, molecularly speaking, very homogeneous. Things become more difficult to discern in chemical dissipative systems, like BZ-reactions

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[9] Bickhard explains this idea with a very simple example: a candle flame. The flame is a macroscopic pattern of billions of molecules, which exhibits a global coherence in far from equilibrium conditions, therefore, requiring a continuous flow of energy (and matter) for its maintenance. However, besides the external conditions necessary for its maintenance, the candle flame makes several active contributions to its own persistence: it maintains a temperature above the combustion threshold, it vaporizes wax into a continuing supply of fuel, and, in a standard atmosphere and gravitational field, it induces convection, which pulls in continuing oxygen and removes combustion products. A candle flame, in other words, exhibits self-maintenance (Bickhard 2000). According to this view, the activity of a self-maintaining system has an intrinsic relevance for the system itself, to the extent that its very existence *depends on* the effects of its own activity. It is this causal loop that justifies the fact of explaining the existence of a self-maintaining system by referring to its effects.

or the example of the candle that Bickard uses to illustrate his ideas (see footnote 9). Despite the fact that these systems are molecularly more heterogeneous and, in principle, one could say that different compounds are involved in different reaction loops or steps of the network, it is still not possible to distinguish, strictly, functional parts. Like in physical dissipative systems, here again the generation of a global pattern with its reinforcing effect (constraining action on the lower level dynamics) is a bare phenomenon of collective self-maintenance: i.e., there is no distinguishable contribution of a part to the maintenance of the whole. It is an 'all or nothing' situation: a compound reacts or does not react. Its absence may destroy the global pattern, but it does not modulate or shift it in any specific way. In the example of the candle flame, to take that particular case, it is really hard to say in what sense a given (type of) component does something different from another in so far as the maintenance of the flame pattern is involved.

But, what does it actually mean that a self-maintaining (SM) system has to show also organizationally relevant *differentiated* parts? Trying to explain which is the minimal degree of organizational complexity that a "self-re-producing" system has to present in order to harbor functions, Schlosser (1998) has defended that it should involve "alternative state cycles depending on environmental conditions" (p. 350). We can interpret the requirement of including "alternative state cycles depending on environmental conditions" as follows: suppose we have a scenario populated by SM systems, including differences in their global dynamics (say, SM(1), SM(2)…); then environmental differences would lead to differences in viability (i.e., some forms of self-maintenance will be more viable in certain environmental conditions than others). This seems to imply (in our reading of Schlosser)[10] that the system should be capable to switch its internal regime according to the external environment, namely, that it should be an adaptive system (even if it is just a primitive type of it).

Now, we consider that this goes too far; i.e., such a condition appears too demanding. It is possible—and reasonable—to think of simpler systems that might keep its dynamic organization running, within a certain range of environmental perturbations, just through continual or immediate feed-backs between their coupled cycles of reactions (that is to say, in a non-controlled/regulated/adaptive way). It is not even necessary that they present a modular-type of internal organization (in the sense that a change in one part would leave the other parts essentially unaltered, so that the effects of internal changes and reconfigurations do not spread systemically). Yet, those hypothetical prebiotic systems (still relatively simple as compared to living organisms) could provide a minimal domain for functional diversity, provided that various localizable processes or components in them (more precisely, processes or components *produced* by them) make a specifiable contribution to the maintenance of the whole organization. In other words, the candidate for a functionally distinguishable part or process should be linked to some meaningful outcome, namely, to some specific result affecting the survival/self-maintenance of the system to which it belongs. This means that one should be able to tell how each

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[10] We should acknowledge that Schlosser's "minimal" conditions for functional diversity are not thought for a prebiotic scenario.

part *specifically* affects the global operational dynamics and architecture of the system (or, rather, the different global operational dynamics and architectures it may take). But let us explain in more detail what such a system should be and look like.

Beyond bare collective self-maintenance: the necessary interweaving of global-unspecific and local-specific constraints

Once clarified what we understand by a minimal form of functional diversity, we focus our attention on characterizing the type of self-maintaining organization that would incorporate or instantiate it. In order to do so it is important to realize, first, what kind of role or action could potential functional parts perform in the context of a physico-chemical (infrabiological) system. Let us assume, as a starting point, a relatively simple autocatalytic cycle, like the one involved in the Formose reaction, in which the self-maintaining dynamics is based on the structure and dynamic properties of a set of chemical compounds (formaldehyde, calcium hydroxide, various monosaccharides,…). As we said before, in this type of cyclic reaction networks it may be difficult to speak about functions yet. But the only way for such a type of system to increase in complexity and achieve that minimal functionality threshold is to produce further components that, acting like material constraints on other components and processes, modify its intrinsic dynamics and its global modes of operation.

Let us see why. Through the action of more complex constraints, local environments can be harnessed in specific ways. In turn, these constrained processes can lead to the production of new constraints, and so on, provided that all the steps involved satisfy the conditions of global self-maintenance. For example, different constraints can, either sequentially or concurrently, modulate in more and more specific ways local dynamical processes within the network and this, in turn, will make possible the synthesis of increasingly complex structures, acting as more sophisticated and accurate constraints. In fact, here lies the reason why biological systems have to be *chemical* (not merely physical): only this type of cyclic, self-modifiable reactive systems (Kampis 1991), seem to have the potential to evolve towards unlimited degrees of organizational complexity and versatility.

Now, for chemical evolution to become, in practice, truly open-ended things are not so straight forward: a long series of transitions have to occur (eventually leading to the origins of proper living systems) because the material and relational conditions that autonomously generated systems have to meet are quite demanding (Ruiz-Mirazo et al. 2008). Once at a *bio*-chemical stage, which involves genetically instructed cellular metabolisms, it is clear that there is ample functional diversity (made obvious by the large and varied amount of tasks that enzymes carry out in any living cell). But the key point is: how far behind did this functional diversity start thriving, and what was the initial level of structural complexity of the molecules or constraints involved?

Although there is no easy answer to these questions, we shall argue for a rather primitive form of functional diversity that does not require complex macromolecules (like biopolymers): instead, it could be founded on the association between

processes of assembly of relatively simple, energetically quasi-conservative structures and distributed, holistic patterns of organization. Namely, a scenario in which the global viability of the system is a consequence not only of the self-maintenance of a thermodynamically dissipative chemical network, but of the interplay between chemical reactions and other types of processes (e.g., self-assembly, diffusion, transport through the membrane,…).

In other words, functional distinguishability would emerge when a set of constraints *of different nature* come together, in a sort of mutually reinforcing effect, which lies at the core of a new, more robust self-maintaining dynamics. So, apart from the causal loop between 'macro' and 'micro' levels implied in any constraining action (as present also in the collective patterns of standard FFE dissipative systems), now an additional causal loop is required at the level of the different constraints, to ensure their integration. This integrated mixture of different types of constraints is, in fact, deeply rooted in biological organization: if we analyse biological organisms and their underlying molecular mechanisms, the amount and variety of inter-dependent constraints involved in their organization is really amazing; and the actual constraining action of—and among—those components is, in some cases, rather general and unspecific (think of a semi-permeable lipid bilayer) but, in some other cases, local and very precise (e.g., enzymatic catalysis, stereospecific regulation or selective transport).

So the idea is that a minimal functional domain, with potential to be progressively enhanced, involves far from equilibrium self-organizing processes and global dissipative patterns but, at the same time, other processes (e.g., of self-assembly) that produce molecular structures whose states are closer to equilibrium conditions (or at the edge between equilibrium and non-equilibrium conditions). This is the only way in which thermodynamically driven processes can progressively develop into kinetically self-controlled networks, what is fundamental to achieve a proper (minimally robust) interweaving of different types of constraints within the same system (heart of the problem of emergence of functional diversity, as we just said). In other words, the constraints involved cannot be of any kind: they need to be 'linkers' or 'coordinators' of processes, as well as of their own activities or actions as constraints. Otherwise, their necessary integration and cohesion to ensure a robust self-maintenance of the system would be missing.

What is not at all trivial to determine, in this context, is the precise amount of different constraints that have to be present simultaneously in a system for minimal functional distinguishability to emerge. Our proposal, stemming from a theoretical analysis of the concept of biological autonomy and its prebiotic roots (Ruiz-Mirazo and Moreno 2004), considers that, at least, three very different types of constraint are necessary at this initial stage: membrane, energetic intermediaries and (rudimentary) catalysts (see also Ruiz-Mirazo et al. 2004). The first defines the global boundary of the system (drawing a clear cut in/out distinction) and not only avoids the dilution problem, keeping the necessary molecular ingredients together, but provides a scaffolding to set up more sophisticated mechanisms of control of the matter/energy flow through the system. The second is crucial to establish couplings between exergonic (thermodynamically favorable) and endergonic (thermodynamically unfavorable) processes: that is, to make possible (not just kinetically, but

thermodynamically speaking) some processes that would never happen otherwise, at the expense of others. If we realize that most polymerization processes in aqueous solution are endergonic the relevance of this aspect becomes apparent. Finally, catalysts are absolutely unavoidable because the whole temporal and spatial coordination of chemical reactions and concentration profiles depends on them. As it is well known, a catalyst is a molecular compound that lowers the energy barrier of the transition state of a reaction, providing an alternative pathway for that chemical process to occur. Hence, catalysts actually *drive* reactions that would otherwise be blocked or significantly slowed down by a kinetic barrier, inducing millions of molecules to follow some particular chemical pathways and not others.[11] This job is exquisitely done by enzymes in present days metabolisms, but their presence in prebiotic scenarios is highly improbable. The working hypothesis here is that before substrate-stereospecific catalysis was developed (which required the synthesis of such complex macromolecules) there were other, less efficient types of catalytic processes, carried out on surfaces (Wächtershäuser 1988) or by simple oligomers (de Duve 1991).

In any case, this mixture of constraints, including both global-unspecific and local-specific ones, and involving both self-organization (far-from equilibrium) and self-assembly (closer to equilibrium) processes, would theoretically be enough to put together a minimal self-maintaining organization with functionally differentiated parts (less would seriously threaten the viability/stability of the system—although this will have to be eventually tested in the lab, of course). Nevertheless, what is important to stress is the fact that these diverse constraints should mutually enable their continuous regeneration, in a way that it is possible to start saying that the self-maintaining system is endogenously producing—and reproducing—all of them. This is crucial not only for robustness, but also for the very emergence of a functional domain: by internally synthesizing its own constraints the system becomes much more plastic; i.e., capable to perform a diversified modulation of its own self-maintaining dynamics. And it is in this organizational context where different constraints can make *distinguishable contributions* to the global self-maintenance of the system. Thus, the emergence of functional diversity goes hand in hand with the emergence of autonomy.

An example of a self-maintaining system harboring a minimal form of functional diversity is Ganti's (1975, 2003) chemoton model. Of course, this is a holistic system in the sense that its parts (i.e., each of the three coupled autocatalytic cycles) cannot be changed without changing the whole system (Maynard Smith and Szathmary 1999). Yet, this does not mean that there are not specific contributions by such parts: for example, the template cycle's contribution is specific and not identical to, say, that of the membrane production cycle. We can find a more recent example in Ruiz-Mirazo and Mavelli's (2007, 2008) 'minimal lipid-peptide protocell' system. This is a model of a self-reproducing vesicle whose membrane consists of both fatty acids and small peptides, taken to be precursors of present

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[11] Interestingly, catalysts are an example of what Juarrero (1999, 2008) calls *context-sensitive constraints*, namely, a type of constraints that «take the system away from *independence* by making the elements comprising the system interact in such a way that their behavior depends on one another's—and on what went on before and what is occurring around them in the environment.».
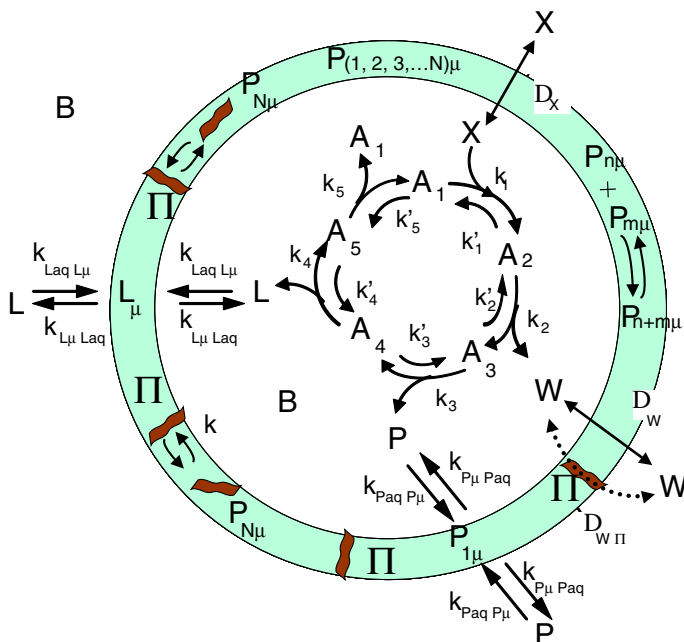
Fig. 1 Schematic graph (taken from Ruiz-Mirazo and Mavelli 2007) of a minimal self-maintaining 'lipid-peptide' cell. This type of proto-cellular organization would be based on the complementarity between an internal autocatalytic reaction cycle and the self-assembly processes that make up the membrane (from its lipidic and peptidic building blocks). Peptides inserted in the membrane in the adequate orientation would constitute precursor channels to control matter inflow/outflow

day's biomembrane main components (phospholipids and protein channels)[12] with the main objective of simulating realistically membrane processes coupled to chemical autocatalytic reactions. More precisely, some of the *in silico* experiments performed (Ruiz-Mirazo and Mavelli 2007) can help us illustrate the idea. In those experiments the "mechanical" dynamics of the membrane is operationally coupled to the chemical dynamics of the autocatalytic network in the following way: when the osmotic pressure reaches a certain threshold, peptides in the membrane open channels; and this happens because, due to the elastic tension (a mechanical process), polipeptides inserted in the membrane adopt the suitable conformation to become waste-transport channels (see Fig. 1), making possible a faster release of the waste molecules, and thus, a decrease in osmotic pressure differences. So peptides in the membrane have a clearly specifiable function: they keep the amount of internal compounds bellow a certain threshold, so that the system avoids an eventual osmotic burst. Whereas the autocatalytic network has the specifiable function of synthesizing fatty acids and aminoacids (so that the lipidic/hydrophobic

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[12] Further work by these authors (Mavelli and Ruiz-Mirazo 2007; Piedrafita et al. 2009) suggests that this type of systems had its origin in formerly independent systems (self-maintaining chemical networks, self-assembling vesicles), each with its own recognizable dynamics and requirements for stability, and when they coupled together they got transformed, becoming strongly inter-dependent.

boundary can be formed, including those self-assembling peptides within it…). And all together they contribute to the system's maintenance (as well as to its growth and possible reproduction).

In sum, in order for a system to harbor a minimal form of functional diversity, it has to show an operational distinction between a microscopic and an emergent macroscopic level where constraints appear and get entangled. But, interestingly, once we have a self-maintaining organization of this type, functional diversification can proceed, at least in principle, indefinitely: certain structures constrain selectively the low level microscopic behavior of different collections of elements in such a way as to enable them to generate more stable macroscopic structures which, in turn, constrain also selectively another collection of microscopic elements, leading to the production of another macroscopic constraint and so on…. provided that, all together, they end up depending on each other. So the synthesis of structural and organizational diversity can be, in principle, unlimited. In this way, the system can indefinitely explore what Kauffman called "the adjacent possible" (2000), and not only in the space of catalytic tasks, but in a more general space of functional constraining actions.

A complex 'lipid world' as a plausible scenario for the appearance of functional diversity

Finally, let us analyze briefly the type of prebiotic scenario that we consider most probable for the appearance of this type of non-trivial self-maintaining/self-producing systems with minimal functional diversity. Current scientific knowledge gathered in the field of origins of life supports the hypothesis that some local environments of the primitive Earth (e.g., hydrothermal vents) could offer adequate and relatively stable conditions for interesting chemical evolution processes to happen: a constant flow of energy and micro-porous surfaces, for instance, would have favored the appearance of far from equilibrium chemical cycles leading to the formation of relatively complex organic compounds (Martin and Russell 2003). However, these precursor reaction networks, although very important to explain the availability of certain chemical species in prebiotic times, would still be unable to constitute functionally integrated self-maintaining systems, since they are too directly dependent on—or immediately exposed to—environmental conditions (thermodynamic flows, diffusion forces,…). So the level of organizational complexity they can achieve is strongly limited.

This initial threshold of complexity could be overcome with the assistance of self-assembled/self-generated boundaries, like vesicles, which are quite plausible supra-molecular structures on the primitive Earth (Deamer 1997; Luisi 2006; Mansy et al. 2008). In fact, it is important to recall at this point that all biochemical reactions, as we know them, occur in compartments and distributed domains that guarantee the specific internal conditions required for metabolism to run. And it might be the case that similarly complex reaction networks could only develop within compartments, under the suitable physico-chemical constraints which allow, for instance, high enough local concentrations, as well as a precise control of matter

and energy flow through the system (selective in-and-out permeation of certain metabolites, energy transduction mechanisms…).

As an interesting hypothesis to pursue we suggest that this, so to speak, 'dirty chemistry', happening in heterogeneous conditions (including colloids, like lipid membranes and aggregates—i.e., completely different from the traditional 'well-stirred tank flow reactor' conditions), was relevant right from the very beginning. Until recently most models that addressed the problem of the origins of NS and/or biological functionality did so in the context of populations of self-replicating molecular species, assuming a high level of molecular complexity (e.g., Eigen and Schuster 1979; Joyce 1989; Szathmary and Maynard Smith 1997) or considering abstract reaction networks, all supposedly happening in free solution (Rosen 1973, 1991; Kauffman 1986, 2000). At present, instead, the role of the compartment is being increasingly recognized as crucial in the organization of *proto-metabolic*[13] systems and, in fact, the challenge of reconstructing in the lab 'artificial minimal cells' by using a lipid vesicle as the container is becoming more and more feasible (Szostak et al. 2001; Luisi et al. 2006; Solé et al. 2007; Rassmussen et al. 2008).[14]

Some years ago, Segré, Lancet and co-workers (Segre and Lancet 2000; Segré et al. 2001) already introduced the 'lipid world' scenario as an alternative to the—nowadays still more popular—'RNA-world'. They developed a model of lipid auto- and hetero- catalytic reaction networks capable of a certain degree of heredity and variability, and therefore, of displaying a primitive form of evolution. However, as Szathmáry and coworkers recently pointed out (Santos and Szathmary 2009) the composome or 'GARD' model, based just on the aggregation and catalytic properties of lipids—even if a very diverse collection of them is involved—shows several evolutionary bottlenecks. The key lies, as we advanced before, in the combination of different kinds of components and processes to put together a self-maintaining system with distinguishable functional parts. Lipids, surfactants or amphiphilic compounds, by themselves, are not enough, either.

The central question to pose is in what ways can lipids and compartments be helpful for oligomerization processes (or other chemical reactions) to take place, and vice versa (i.e., in which ways could oligomers assist compartments). Fernando and Rowe (2007, 2008), for instance, take an interesting step in this direction, exploring networks of autocatalytic reactions that evolve through a NS type of algorithm and avoid the problem of 'side reactions' thanks to their being confined

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[13] A proto-metabolic entity is a chemical self-maintaining network driven by catalysts that, whatever their nature, cannot have displayed the exquisite specificity of present-day enzymes and must necessarily have produced some sort of "gemisch" (i.e., a heterogeneous collection of molecules, de Duve 2005, pp. 150–151).

[14] This scenario of protocells can be seen also from a populational perspective. As David Deamer (2008) has expressed it: «the result of this process would have been that vast numbers of microscopic assemblies of molecules appeared wherever organic compounds became concentrated at the interface between the atmosphere, water and mineral surfaces. In one scenario (…) these assemblies took on a cell-like form (…) each cell-like assembly had a different composition from the next. Most were inert, but a few might have contained a particular mixture of components that could be driven towards further complexity by capturing energy and small nutrient molecules from the environment (…) As the nutrient molecules were transported into the internal compartment, they became linked together into long chains in an energy-consuming process.».

within individual lipid containers. They even mention explicitly the problem of generation of functional diversity in the discussion of their results (Fernando and Rowe 2008, pp. 365–369). From a rather different approach, Ruiz-Mirazo and Mavelli (2007) work, already mentioned above, focuses more directly on the mimicking of real membrane processes coupled to chemical autocatalytic reactions, putting forward a protocellular model for a self-maintaining, 'basic autonomous system' that would already include a set of components in which functional differentiation—and integration—is more evident (see Fig. 1).

The advantage of these, so to speak, 'elaborate lipid-world scenarios' (as compared to the traditional ones of self-replicating biopolymer populations) is that, starting from very simple building blocks, whose synthesis is more plausible in prebiotic conditions, self-organization and self-assembly processes would naturally come together in the constitution of systems with a relatively complex organization (a proto-cellular organization where different functional parts can already start being identified). Since these systems would be capable of self-maintenance and multiplication (by fission, or statistical vesicle reproduction) a primitive form of NS could begin operating in that context, favouring those systems whose functional integration is more efficient, eliminating others. The variety of ways/combinations in which the—tentatively functional—components of such systems may contribute to their maintenance and reproductive success ensures a wide enough phenotypic space for NS to be actually selective as an evolutionary mechanism, without falling into 'dead ends' or too narrow complexity traps. Thus, from this point onwards, a new collective-interactive dynamics is established in the population of protocells: a dynamics in which NS, so to speak, eliminates less efficient individuals, leaving room for new, different ones. As a result, this process will provide more variety, wider functional diversity to be, in turn, selected for, enlarging the range of action and consequences of NS, in a kind of mutual enhancing effect.

Conceptual implications: complementarity between NS and functional diversification

At the beginning of this paper we formulated a dilemma: NS apparently needs an open functional domain in order to operate but, nevertheless, it is hard to conceive how this functional domain may actually develop without the action of NS. We have argued that a particular type of self-maintaining system, based on a minimal form of kinetic control (through the operational closure of catalytic and other type of constraints taking place in protocellular compartments), would provide that initial and minimal functional domain, with potential for further diversification. Then, we proposed that populations of such type of (self-re-producing) systems could appear before the rise of complex macro-molecules, like biopolymers (i.e., before something like an "RNA world" comes to stage). Of course, it should be acknowledged that the relative weight/strength of NS in this prebiotic (pre-macro-molecular) scenario would be limited, because the generation of complexity/functional diversity and, more specifically, its persistence in the system and its hereditary transmission to

subsequent generations are also limited.[15] In any case, the main message of this paper is that, from very early phases, one should think in terms of a collection of individual (proto-cellular) organizations, which generate (and, at the same time, are being generated by) a larger populational and trans-generational web of relations, even if such a collective-historical-populational level is still very far from what we understand nowadays as an evolving ecosystem/biosphere.

So, although these primitive infrabiological systems would lack reliable hereditary mechanisms and a clear genotype-phenotype distinction, primitive forms of NS might already start playing an important role in their evolutionary dynamics. When a population of systems keeps growing in an environment with limited resources, no matter the complexity of the individuals, a certain number of them (the relatively less efficient or less stable ones) is forced to disappear, as a consequence of competitive system-system interactions or direct exchanges with the environment. Notice that this involves a continuous reshaping of the "fitness landscape", and each time a certain type of individual is eliminated by selective forces the conditions for the appearance of another "species" increase. Therefore, the removal of a given type of individual would prompt the generation of new ones. Indirectly, this removal facilitates the exploration of the *adjacent possible* and thus, in the longer run, also facilitates the creation and retention of more complex functional components (with more efficient catalytic properties, for instance).[16] In these conditions, if the organization of the self-reproducing systems is such that allows for functional diversification, the complexity and variety of these systems in the population will increase as well. But, the more different and complex the individuals get (i.e., the wider functional/phenotypic space at reach), the more powerful the action of NS becomes.

From this perspective, as hereditary mechanisms develop and higher molecular and organizational complexity can be reliably transmitted from generation to generation, NS would progressively increase its relative importance (with regard to self-organizing principles—surely more critical at the initial phases) in determining the properties of infrabiological systems. This is so because there is a deep connection between two major problems in this context: namely, the synthesis of increasingly complex components, capable of performing highly specific catalytic/metabolic tasks, and the reliability in the transmission of variants through the reproduction of the individuals. Actually, both problems can be roughly solved with the same invention, an RNA-type of polymer. Once this type of modular macro-component appears, combining stereospecific template and catalytic activities, NS would become a much more powerful 'driving force', because (1) the potential for functional diversification is remarkably enhanced, and, at the same time, (2) the transmission of variants is also carried out with much higher reliability. As a result,

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[15] This limitation is due to the holistic means of reproduction and cannot be overcome until modular templates appear within the system (Maynard Smith and Szathmary 1995).

[16] The removal of less fit individuals facilitates the exploration of more complex organisations, provided that the latter, of course, are fitter than all other more simple individuals.

the opening of functional variability space happens to be complementary with an increase in the power of action of NS.[17]

One may wonder, however, whether in a relatively stable environment NS is doomed to end in a stable domain (rather than continually produce adaptive novelty). Actually, as Bedau (1996, p. 338) has pointed out, this problem appears in many computational models when the environmental conditions are stable: «When selection is made on the basis of a fixed fitness function, the resulting adaptive dynamics eventually stabilize rather than continually produce adaptive novelty. For example, Mitchell and Forrest (1994) explain that adaptation toward a fixed goal is the characteristic—and desired—outcome when natural selection is implemented in a so-called "genetic algorithm" and applied to engineering problems such as optimization (e.g., circuit design and job shop scheduling), automatic programming (evolving computer programs for specific tasks like sorting lists), and machine learning (e.g., predicting protein structure).» But, in our scenario, this would not be really a problem because, as the same author explains, «a significant aspect of the environment to which any given organism must adapt is all the other organisms with which it interacts. So, when a given organism adapts and changes, the evolutionary context of all the other organisms changes. Thus, even without an externally changing environment, adaptation can be a co-evolutionary process that internally changes the selection pressures which shape adaptation, thus making open-ended adaptive evolution an intrinsic property of the system (Packard 1989; Holland 1992).»

This point is of paramount importance, because it helps us understand that the source for functional diversity lies ultimately in the *organization* of (proto-) biological systems. And indirectly, this implies that the mechanism of NS is, after all, also endogenously constructed. In other words, NS should be conceived as a very complex, meta-level constraint on all what self-organization processes bring about. NS, therefore, requires the unfolding of a series of organizational conditions, among which the minimal requirements for systems that display and unfold functional diversity, as we have tried to explain here, should be included.

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[17] This is even more manifest at later stages, when proper 'genetically instructed metabolisms', based on the interplay between different types of polymer (proteins and polynucleotides), take over. The establishment of a stronger genotype-phenotype decoupling in these systems would open radically new possibilities for finding and retaining highly complex components. While in (pre-)RNA worlds the exploration of new combinations of building blocks is still somehow tied to the dynamical constraints of the self-maintenance process of the individuals, the actual segregation of genetic records (evolutionary logic) and catalytic units (metabolic logic), allows to explore new structures much more freely, transcending individual lifespans. For a more extended account of this problem, see: (Ruiz-Mirazo et al. 2008).

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