HOW FUNCTIONAL DIFFERENTIATION ORIGINATED IN PREBIOTIC EVOLUTION

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ABSTRACT. Even the simplest cell exhibits a high degree of functional differentiation (FD) realized through several mechanisms and devices contributing differently to its maintenance. Searching for the origin of FD, we briefly argue that the emergence of the respective organizational complexity cannot be the result of either natural selection (NS) or solely of the dynamics of simple self-maintaining (SM) systems. Accordingly, a highly gradual and cumulative process should have been necessary for the transition from either simple self-assembled or self-maintaining systems of functionless structural components to systems with FD. We follow results of recent *in vitro* experiments with respect to competition among protocells, where a primitive type of selection begins to operate among them accompanied by a parallel evolution of their functional domain. We argue that minimal forms of FD should be established within the evolution of SM processes in protocells as they undergo a simpler selection process for stability and persistence in a prebiotic environment. We then suggest the concept of *closure of constraints* (CoC) as a way to identify and describe minimal FD in a far-from-equilibrium SM organization. We show in detail how the concept of CoC together with the conditions for its fulfillment can be applied in the case of a simple protocellular system that begins to couple internal chemical reactions with the formation of its membrane components. Finally, we discuss how such SM systems can evolve towards significantly higher levels of FD, suggesting this is mainly the result of functional recombination (formation of mechanisms) in the context of a modular SM organization.

KEY WORDS. Functional differentiation, self-maintenance, natural selection, constraints, closure, prebiotic evolution, organizational complexity, protocells.

INTRODUCTION

Biological systems are highly complex organizations consisting in many different sophisticated molecules (e.g., DNA, RNA and proteins) and other supra-molecular structures (ranging from, e.g., lipid and phospholipid membranes to tissues, organs, etc.). Those molecules and structural aggregates either based on their properties or, perhaps more importantly, on the organizational properties emerging out of the specific and intricate relationships they establish and manage to maintain among them, are

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considered as playing different functional roles in the dynamic behavior of the organizations in which they participate. As such, biological systems and, actually, even the simplest cell, exhibit a high degree of *functional differentiation* (FD) realized through mechanisms and devices that ensure their survival and adaptation.

However, it is sensible to think that even the simplest present-day cell organization is much more complex than what would be required for harboring a minimal form of FD. So, where did this FD come from and how was it developed? Several approaches resort to the mechanism of natural selection (NS) to ground the concept of function (Millikan 1989; Neander 1991; Godfrey-Smith 1994). But as it explained in Fox Keller (2007, 2010) and in Moreno and Ruiz-Mirazo (2009), the way in which NS is conceived within Darwinian theory requires an open, sufficiently wide phenotypic domain where selective forces may act. 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. This functional domain is already implicit in a minimal, but sufficiently rich concept of a cell.

This was not the case in prebiotic evolution. It is widely accepted that the organizational complexity and the respective functional domain of the simplest present-day cells should have been preceded by several infrabiological forms of organization. As it is argued in Moreno and Ruiz-Mirazo (2009), there are several experimental cases (e.g., Walde, et al. 1994; Segre and Lancet 2000, and Chen, et al. 2004), which have shown that multiplication, variation and heredity would constitute a necessary but not sufficient set of conditions for what is commonly understood as evolution by NS. There was for sure a time when the types of evolution were not based on the Darwinian NS, when NS was not fully operating (Godfrey Smith 2009).

Considering that there were systems that fulfill those conditions but do not evolve as present-day biological systems do, it is sensible to think that NS is the result of an already evolved functionality. As it shall be developed in section 3 (and also as has been argued in Moreno and Ruiz-Mirazo 2009), NS starts with a system able to exhibit some sort of structural as well as functional variability (i.e., an already functionally differentiated system). Therefore, NS cannot account for the emergence of minimal FD; on the contrary, NS needs FD in order to be operative. Accordingly, we have to conclude that a more or less long cumulative process has been necessary for the emergence of FD in the cells.

The structure of this paper is as follows: In section 1 we argue that a certain type of far-from-equilibrium self-maintaining (FFE SM) systems, based on chemical autocatalysis associated with self-assembly, seem to have some properties that can support the gradual process needed for the emergence of FD. Then, we argue that the concept of function before the appearance of NS should be considered in conditions that favor a simpler selection process for stability and persistence of self-maintenance organizations. In section 2, based on *in vitro* experiments, together with relevant *in silico* results, we suggest that the organizational complexity necessary for minimal FD emerges in cases where simple protocells begin to couple internal chemical reactions with the formation of their membrane components, in a cascading of primitive competitive-evolutionary selection dynamics. In section 3, we introduce the notion of closure of constraints (CoC) in order to present a detailed account of the conditions and requirements for minimal FD, and to provide a way to identify and describe minimal FD in a system. In section 4, we discuss how FFE SM systems with FD can evolve towards significantly higher levels of FD in an open way.

1. FUNCTION PRIOR TO NATURAL SELECTION

1.1. SIMPLE SELF-MAINTAINING SYSTEMS ARE NOT ENOUGH FOR MINIMAL F.D.

The main scientific alternative to evolution through NS for the explanation of the evolution of FD, are the dynamics of a self-maintaining (SM) system emerging out of self-organizing processes. Self-maintenance is a widespread phenomenon exhibiting different levels of organizational complexity, but its *minimal expression* consists of the so-called ‘dissipative structures’ (Glansdorff and Prigogine 1971; Nicolis and Prigogine 1977; Nicolis 1989). These are *forms of self-organization* that result in the production of systems that are maintained in a steady state through a flow of available energy. In such systems and in the presence of a specific flow of energy and matter in far-from-equilibrium (FFE) thermodynamic conditions, a huge number of microscopic elements adopt a global, macroscopic ordered pattern (a ‘structure’), which in turn contributes to the maintenance of the FFE flow of energy and matter enabling the persistence of the microscopic dynamics.

The interest of the concept of self-maintenance is that it relates function to the self-maintaining system by interpreting it as a specific causal effect of a component or trait which contributes to the maintenance of the system and, consequently, of the component itself in an organizationally closed way. Then, in this context appears a basic idea of *functionality* understood as the contribution of a component to the self-maintenance of the system (Schlosser 1998; Collier 1999, 2000, 2004; Bickhard 2000, 2004; McLaughlin 2001; Christensen and Bickhard 2002; Delancey 2006; Edin 2008 and Mossio, et al. 2009). However, is the concept of simple self-maintenance enough for an explanation of the emergence of FD in biological systems?

Let us consider a candle flame as an example of a relatively simple SM system. In a candle flame the microscopic reactions of combustion generate a macroscopic pattern (the flame itself) that contributes to maintain the conditions for its own existence. Specifically, the flame makes a crucial contribution to maintaining the microscopic chemical reactions by keeping the temperature above the combustion threshold, vaporizing wax, and inducing convection (which pulls in oxygen and removes combustion products). In turn, the chemical reactions keep generating the combustion and thus the flame itself (Bickhard 2000). In particular, the emerging configuration (i.e., the flame) constrains the surroundings (temperature, wax, oxygen) by turning them into appropriate boundary conditions required for its own maintenance. As mentioned elsewhere (Mossio and Moreno 2010), the candle flame is a simple SM system, whose maintenance *depends on the work of a single constraint acting on its own boundary conditions* (see figure 1). The candle flame works to maintain the boundary conditions that enable it to keep on working. In all, a candle flame can do only one thing, which is equal to saying that a candle flame has only one function. Indeed, a candle flame has no options to select between alternative ways to enable its continuous maintenance, and this result in its incapability to correct the threat of getting extinct when the boundary conditions are not the appropriate ones (i.e., running out of wax, decrease of oxygen, etc.).

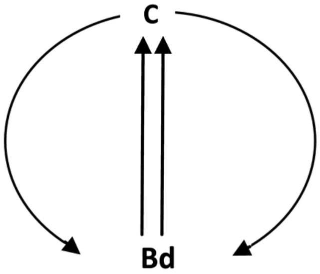

FIGURE 1.
Abstract depiction of a simple SM system whose maintenance depends on the work of a single constraint (C) acting on its own boundary conditions (Bd_C). It can be the case of a candle flame that constrains its surroundings by turning them into appropriate boundary conditions for its maintenance.

Anyhow, certain SM systems (as we shall discuss later) can reproduce themselves and therefore support some form of continuity allowing, at least in principle, an accumulation of novelties. These systems are potentially capable of producing and preserving new components with increasing structural complexity. Interestingly, these systems may recruit some of the new components to act as constraints to modify their own organization, which is precisely the scenario we are looking for the origin of FD. However, in order to explain the emergence of a minimal form of FD, it would be necessary that these systems show differentiated paths in the contribution to their self-maintenance in such ways that NS can operate.

How then can we explain the primitive cumulative process of organizational complexification up to the level of minimal FD, from chemical evolution to systems able to evolve by the mechanism of NS?

1.2. FUNCTION PRIOR TO NATURAL SELECTION THROUGH 'SELECTION FOR PERSISTENCE'

In several papers analyzing the origin of functions in prebiotic evolution, Fox Keller (2007, 2010) lays out what she considers as a minimalist conception of the notion of function. She specifically argues, as we also mentioned in the introduction, for the need of an already evolved functionality for NS to be operative. Additionally, she characteristically argues that in this context of pre-NS competitive-evolutionary dynamics, one should speak, in terms of *persistence*—rather than survival—of the system and capacity for *robust self-maintenance*—instead of fitness.

Fox Keller suggests that the origin of functionality could be traced down to systems that pose some simple form of self-regulation. According to her thesis, once such 'mechanisms' are observed, we could legitimately speak of function in a system. What Fox Keller is trying to stress is that, for a concept of function that preceded NS, what is needed is not a notion of "usefulness of an entity to a system" as strong as implied by the conditions that make NS operative, but rather the existence of a property that contributes to the *persistence* of the system to which that property belongs, which will be enough to trigger a different (simpler) *selection process for stability and persistence*. In other words, the application of feedback mechanisms, which result in the generation of stabilities in a system, is enough for the operation of simpler evolutionary-competitive dynamics that will result in different degrees of maintenance to those stabilities. She then ascribes functionality to these primitive 'regulatory' mechanisms, since they contribute to the maintenance of the stabilities.

Further, the capacity for regulation requires the modulation of an underlying range of functional operations on the structure of each trait, thus implying a higher degree of organizational complexity than what is likely to be found in prebiotic systems. Actually, Fox Keller's account is not very minimalist and this is evident in the examples she introduces in order to demonstrate her concept of minimal function. Indeed, the chemotaxis of *E. coli* and the regulation of oxygen in a termite's nest, due to the dynamic construction of a mount by the termites themselves, may be examples of several feedback mechanisms added and nested together into highly complex systems in the course of evolution, where the respective functionality presupposes a high degree of organizational complexity. Therefore, we should look for much more simple SM systems that may show a minimal form of 'selection' for persistence.

As we discussed in section 1.1., the minimal expression of an organization that persists is realized through a dynamically FFE, self-constitutive and dissipative kind of system, where a set of randomly interactive structural components bring forth a global self-maintaining arrangement. The components of such organization must allow a variety and plasticity of interactive relations. In a prebiotic environment such organization can produce new structural components (in an open way, and based on recombinations), generating this way an increase of different interactive relations. In addition, some components (catalysts) can selectively modify the composition 'rules' between the other structural components, thus generating internal structural variety. Provided a long and wide scenario, where adequate boundary conditions can be fulfilled, this organization has the capacity to explore and retain a wide variety of this type of self-(re)producing SM systems.

This brings up an important issue. Under suitable boundary conditions those systems could *persist* and *produce new structural components*. Hence, what we should look for is how new, more complex components can contribute to the maintenance and persistence of the organization they belong to.

Recapitulating, in this section we commented on the implications for the capability of simple self-(re)producing SM systems to persist in a prebiotic environment with respect to their functionality. We suggested that in principle, simple self-(re)producing SM systems might achieve a robust persistence and evolve towards systems with greater organizational complexity by recruiting and combining structural complexity for new and different organizational tasks.

Yet how can prebiotic systems evolve towards increasingly complex SM systems exhibiting a minimal form of FD? As we will see in section 2, this can be achieved by combining processes of self-assembly and self-organization in certain forms of self-reproducing SM systems.

2. EXPERIMENTAL RESULTS AND MODELS OF SELF-MAINTAINING PERSISTENCE IN PREBIOTIC EVOLUTION

In an attempt to understand the emergence of Darwinian evolution, Budin and Szostak (2011) try to identify mechanisms that enabled protocells to compete with one another. Considering the low levels of membrane phospholipids in the early stages of prebiotic evolution, they inquire on the selective advantage that could have driven the evolution from self-assembled simple single-chain lipids membranes to phospholipid membranes. They argue that according to their research results "phospholipid-driven competition could have led early protocells into an evolutionary arms race leading to steadily increasing diacyl lipid (e.g., phospholipid) content in their membranes" (ibid, p. 5252).

What is important concerning the quest for minimal FD, is that they suggest that the resulting decrease of membrane permeability—due to the increase in membrane phospholipid content during the passage from self-assembling to self-produced membranes—would impose the cascading of new selective pressures on early protocells, allowing the internal evolution of a metabolic and transport machinery that would overcome the reduced permeability. Specifically, they propose that in order to overcome the reduced permeability, protocells could have started to evolve membrane transporters along with proto-metabolic networks for synthesizing their own building blocks, as well as exploring new environmental niches compatible with compounds that were otherwise rapidly decaying in fatty acid membranes. They concluded that at this point of transition, from highly permeable vesicles to less permeable and more stable protocells, *a primitive type of selection begins to operate accompanied by a parallel evolution of the functional domain of the protocells.*

Additionally, as also Budin and Szostak mention, the transition from single-chain lipids to phospholipids had to be gradual. This would allow for the co-evolution of the metabolic and transport machinery, and would also permit the overcoming of the initial inefficiency of primitive catalysts. Accordingly, the primitive operation of the selective dynamics associated with phospholipid synthesis would correspond to small differences in the phospholipid content that gradually drive the transition. Considering the gradual alteration in the degree of the membrane's permeability, and that the respective competitive-evolutionary selective dynamics mirror the emergence of internal metabolic and transport machinery, there should had been a point where the underlying proto-cellular organization would had started to produce (though still partly) the main phospholipid components of its membrane. What should be noted now is that since the transition was gradual, the primitive operation of the competitive-evolutionary selective dynamics would had corresponded to small differences in phospholipid content. Accordingly, the beginning of the internal production of the phospholipid parts of the boundary in protocells is a case where internally generated structural complexity begins to get related to the dynamics of its production. This point is reached in a developing protocell only when its membrane permeability starts to decrease and a proto-metabolic SM network begins to be internally formed and sets about to operate, thus producing the main phospholipid components of the membrane. In the evolutionary transition from a self-assembled vesicle to a self-maintaining protocell, certain structural components and related processes (the membrane and the self-maintaining chemical proto-metabolic network) begin to play the role of parts in a whole (the self-maintaining organization of the protocell).

Moreover, Budin and Szostak clearly state: "We show that the resulting increase in membrane phospholipid content would have led to a cascade of new selective pressures for the evolution of metabolic and transport machinery to overcome the reduced membrane permeability of diacyl lipid membranes" (ibid, p. 5249). Accordingly, the resulting increase in membrane phospholipid content is a kind of 'control' of the boundary conditions, which in turn increases the internal synthesis of phospholipids residing in the membrane. All this process results in the growth and consequent division of the protocell (i.e., a primitive mechanism of self-reproduction). Primitive selection dynamics (in this case a phospholipid-driven competition for single-chain lipids) could thus start operating on the evolved population of such organizations (a population of protocells) resulting in a cascading of new selective pressures for several gradual evolutionary transitions (e.g., emergence of various material structures constraining different processes, such as efficient transportation of raw materials, the synthesis of proto-metabolic networks, and so forth). Consequently, the organizational differentiation of the protocells is increased by recruiting the newly generated (and/or by combining the already existing) components for new or different organizational tasks (i.e., importation of material resources, exportation of waste products, exploration of other niches compatible with structural complexity of a different material basis, and so on). Overall, this results in an even greater capability for self-maintenance, hence for persistence.

The evidences from the *in vitro* experiments of Budin and Szostak, together with the results of related *in silico* experiments (see e.g., Mavelli and Ruiz-Mirazo 2007; Ruiz-Mirazo and Mavelli 2007; Piedrafita, et al. 2009), suggest that the integration of the internally generated (at least partially) structural complexity in a dynamic organizational frame that exhibits self-maintenance creates an active separation between system and environment. This is the point where primitive competitive-evolutionary selection dynamics can begin to operate.

Initially, the molecular structure of these systems was totally separated from any form of functionality. Later, the reformed and less permeable compartment becomes relevant because it makes a contribution to the maintenance of a proto-cellular organization, in which proto-metabolic reaction networks are coupled with trans-membrane processes. Indeed, it is the alteration and the beginning of an internal control in the boundary conditions that favors the emergence of organizational complexity, which is explicitly related to the passage from a self-assembled vesicle to a self-maintaining protocell. It is in this frame where new molecules, produced within the protocell, can contribute differently in the maintenance of the protocell's organization and then one could speak of FD.

In the next section, we attempt to provide a detailed account of the characteristics, requirements and conditions for the emergence of minimal FD.

3. AN ACCOUNT OF THE ORIGIN OF FUNCTIONAL DIFFERENTIATION

The *in vitro* experiment analyzed in the previous section shows that certain SM systems could *generate distinct structures contributing in different ways to their self-maintenance*. The system produces different and localizable processes or structures, each making a specific and distinct contribution to the conditions of existence of the whole organization. Then, according to Mossio, et al. (2009), these components become candidates for functional ascriptions because, in addition to this differentiated contribution to the SM of the system they belong to, they have been generated (at least partially) and they are maintained within and by the organization of the system.

As we have seen, it is likely that in certain environments, chemical evolution lead to the appearance of populations of protocells with a significantly lower organizational complexity than the present-day cell, but which exhibit *minimal* FD and enable a primitive selection process. Simple protocellular systems may begin to couple internal chemical reactions with the formation of their membrane components, resulting thus in the alteration of their boundary conditions, which in turn will allow them to persist. In that case, the competitive-evolutionary dynamics of a primitive type of selection can begin to operate giving rise to a mutual reinforcement between FD and selection for self-maintenance. What is required for this primitive selection process to be operative is a population of SM self-reproducing systems that will contribute differently (at least in a minimal way) to the underlying self-maintenance in which they are operationally integrated.

Let us discuss the logic leading to the appearance of FD. As mentioned in section 1.1., in certain SM systems there are components that can operate as constraints, i.e., their causal role is mainly to modify the underlying dynamics of the system. For example, certain molecules can catalyze specific reactions, thus speeding the rates of chemical processes; or they can self-assemble constituting vesicles, which harness the process they enclose by ensuring high concentrations. In the scenario described so far, these structures harness certain processes. Now, in turn, what happens is that the persistence of these constraints depends on the processes they harness in an organizationally closed manner. In this way, there is a SM organization that ensures their repeatable effect, and which results in the persistence of the system. In a more formal description, we consider a SM organization as constituted by a set of structures ($C_1,...,C_n$) operating as constraints. Since in this context each constraint is not able to achieve self-maintenance on its own, the persistence of a constraint depends on the process it harnesses, and hence, there should be a form of mutual dependence between the set of constraints operating in the organization. In other words, ($C_1,...,C_n$) should constitute "a web of structures exerting mutual constraining actions on their boundary conditions, such that the whole web is collectively self-maintaining" (Mossio and Moreno 2010, p. 276). As such, each C_i (as well as any group of constraints) contributes to the maintenance of the web of the rest of the constraints which, in turn, maintains in a recursive way (at least some of) the boundary conditions necessary for the re-appearance and/or maintenance of C_i. We will call this form of SM organization a *closure of constraints* (SM_{CoC}) because it is maintained due to the action of these constraints and, in turn, these very constraints are recursively re-produced and/or maintained by the system. Since the constrained processes generated and maintained in the system make *a distinguishable contribution to its maintenance*, they become candidates for differentiated functional ascriptions. It is therefore *the operation of material components as parts of a web of constraints that contribute differently to the self-maintenance of the system by mutually contributing to the maintenance (of the boundary conditions) of other constraints in the web that will eventually classify them as functionally different* [1].

In more detail, and according to the arguments for the persistence of protocells presented in section 2, in order for a system with an organization of type SM_{CoC} to be able to interact [2] with the prebiotic environment and simultaneously to further maintain (i.e., to persist) itself under the operations of primitive selection dynamics, the following conditions should be met (see also figure 2):

Cond_1. The operational availability of a subset $\{C_i\}$ of the set of constraints (C): $\{C_1,...,C_n\}$ operating in SM_{CoC} should go on the constant formation and maintenance of the boundary conditions (Bd) that will ensure the continuous operation of the subset $\{C_j\}$ of the initial set (C), where $i \neq j$. Then, $\{C_i\}$ acts so as to determine (at least some of) the Bd_$\{C_j\}$.

Cond_2. The operational availability of the second subset of constraints (C_j) operating in SM_{CoC} under Bd_$\{C_j\}$ should act so as to determine, (at least partially) the boundary conditions needed for *Cond_1* to be *recursively* [3] met (Bd_Cond_1).

Cond_3. $\{C_1,...,C_n\}$ should be, at least partially, internally produced.

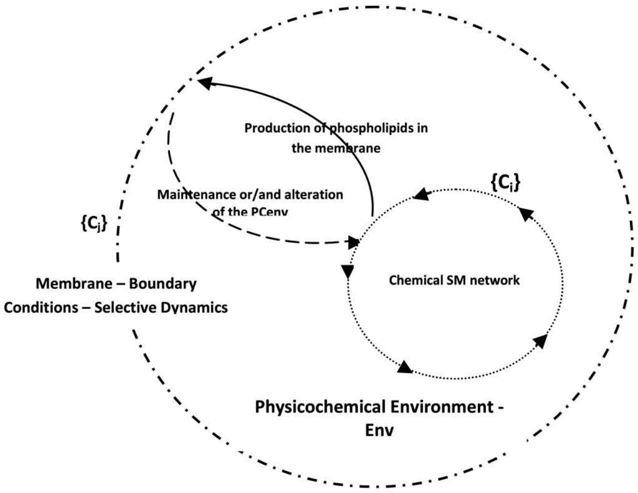

FIGURE 2. Abstract representation of a system exhibiting minimal FD. A protocell whose organization is maintained by the dynamic coupling of a *chemical* SM *network*, constrained by locally active constraints of the subset {C_i} (e.g. enzymes, energetic intermediaries, etc.), to the membrane encapsulating the SM network and acting as another subset of globally active constraints ({C_j}—e.g. playing the role of global *boundary conditions* that interface the *selective dynamics of the environment* and thus alters the *physicochemical environment* in (i.e., the Bd_{C_i} under) which the chemical SM network operates and produces, at least partially, the phospholipid component of the membrane. This is a special type of SM organization formed on the minimum closure of constraints ($SM_{min[CoC]}$).

Thus, in a system that operates by simultaneously satisfying all three conditions there are *at least two* distinct contributions (realized through {C_i} and {C_j}), the respective processes of which:

i. participate in the organization of the system that interacts with the environment, and they contribute to the maintenance of that organization and consequently, of the respective constraining components themselves (i.e., there is a SM organization formed on a closure of constraints - SMCoC);

ii. the system itself generates (partially, at the beginning) those two subsets of components constraining two processes that contribute differently to the maintenance of the organization SMCoC (i.e., the organization of type SMCoC can be minimally differentiated [4]).

Therefore, this system exhibits *minimal* FD and has a SM organization formed on the *minimum closure of constraints* ($SM_{min[CoC]}$). These conditions can be realized in a protocell that begins to internally generate the basic components of its membrane, thus altering the boundary conditions of its internal chemical network in a way that facilitates its self-maintenance. As suggested elsewhere (Piedrafita, et al. 2009), this may be the result of the coupling of two formerly independent systems (self-maintaining chemical networks such as relatively simple autocatalytic cycles, and self-assembling vesicles that could have been the result of the formation of supramolecular structures in a prebiotic environment), that when coupled together get transformed, becoming strongly inter-dependent operating under Cond_1, Cond_2 and Cond_3.

The determinative point of the emergence of minimal FD is the beginning of the partial internal generation and coupling of the processes constrained by $\{C_i\}$ and $\{C_j\}$. As explained in section 2, this should have been a gradual transition, where the operation of primitive selective dynamics associated with phospholipid synthesis (e.g., constrained by the operation of $\{C_i\}$) would correspond to small differences in the increase of the phospholipid content on the membrane of the protocell (e.g., $\{C_j\}$). It is difficult to tell which kind of constraints are required for a minimal $\mathrm{SM}_{\mathrm{CoC}}$, although recent studies in the evolution of protocells show as more probable the case where sets of structures of different nature have been integrated by exerting constraining actions on a SM organization. As the experimental *in vitro* and *in silico* results presented and discussed in section 2 show, at least for the moment, the persistence of any SM organization in a highly changing prebiotic environment seems unrealizable without the existence of a compartment. Therefore, in the protocells case discussed in section 2, we could say that $\{C_i\}$ and $\{C_j\}$ are two groups of material constraints of a different type (e.g., membranes playing the role of compartments and catalysts).

In particular, the operation of $\{C_j\}$ interfaces the selection dynamics of the environment (SEL_env) and thus alters the environment (Env) or, in other words, partially determines the boundary conditions in which $\{C_i\}$ operates. On the other hand, the operation of $\{C_j\}$ is based on the operation of $\{C_i\}$ on the dynamics of a chemical SM network (CHEM_sm) in $\mathrm{SM}_{\mathrm{min[CoC]}}$. $\{C_i\}$ partially determines $\{C_j\}$ and thus affects the way the latter interfaces the SEL_env, which results in the alteration of the Env in $\mathrm{SM}_{\mathrm{min[CoC]}}$ (see also figure 1).

The subset of constraints $\{C_i\}$ and $\{C_j\}$ are operating on two different aspects with respect to the maintenance of $\mathrm{SM}_{\mathrm{min[CoC]}}$. Specifically:

— $\{C_i\}$ operates on the dynamics of CHEM_sm and as such it (at least) partially determines Bd_$\{C_j\}$.

— $\{C_j\}$ interfaces the SEL_env thus making $\{C_i\}$ operative by (at least) partially determining Bd_$\{C_i\}$.

Therefore, the two subsets of constraints are dynamically coupled but contribute differently to the maintenance of $\mathrm{SM}_{\mathrm{min[CoC]}}$. We suggest that this is a genuine case of a minimal closure of constraints. Accordingly, a system with an organization of type $\mathrm{SM}_{\mathrm{min[CoC]}}$ exhibits minimal FD.

As explained in Budin and Szostak's experiment, the organization of type $\mathrm{SM}_{\mathrm{min[CoC]}}$ is considered sufficient to initiate a cascading of gradual evolutionary transitions (emergence of efficient transport mechanisms and proto-metabolic networks) that would had mirrored the gradual transition from a partial generation and control of the global boundary conditions to the almost total internal generation and control of the diverse ways the system can regulate the exchange of matter and energy with the environment (e.g., as is the case in modern cells). This will result in the increase of FD. Let us see how this idea applies to the protocell example we have analyzed so far (see also figure 3). Let us consider:

a. that with respect to $\mathrm{SM}_{\mathrm{min[CoC]}}$, the dynamics of the membrane are, at least partially, operationally coupled to the chemical dynamics of the SM network,
b. the gradual alteration in the degree and the type of membrane's permeability and
c. that the respective competitive-evolutionary selective dynamics mirror the emergence of internal metabolic and transport machinery.

There could had been a point where certain building blocks inserted in the membrane (i.e., lipids and peptides) would start to open channels when the osmotic pressure reaches a certain threshold, thus constituting precursor channels to control matter inflow/outflow and the release of waste products. In that case, the organization of the system begins to evolve from type $\mathrm{SM}_{\mathrm{min[CoC]}}$ to type $\mathrm{SM}_{\mathrm{[CoC]}}$. Now, the following conditions are met:

Cond_1. The operational availability of a subset $\{C_i\}$ of the set of constraints $(C_1,\ldots,C_n)$ operating in $\mathrm{SM}_{\mathrm{CoC}}$ should act so as to determine (at least some of) the Bd_$\{C_j\}$ and Bd_$\{C_k\}$ (i.e. the structures operating on the formation of primitive channels/transport mechanism) (where $i \neq j \neq k$).
Cond_2. The operational availability of the second subset of constraints of constraints $\{C_j\}$ should operate so as to (at least partially) determine Bd_$\{C_i\}$ and Bd_$\{C_k\}$.
Cond_3. The operational availability of the third subset of constraints $\{C_k\}$ should operate so as to (at least partially) determine Bd_$\{C_i\}$ and Bd_$\{C_j\}$.
Cond_4. $\{C_i\}$, $\{C_j\}$ and $\{C_k\}$ should be, at least partially, internally produced.

In other words, the new higher level of organizational complexity of the system would allow, through its interaction with a dynamic environment, the generation of *distinguishable structural components or processes* (e.g., primitive channels/transport mechanism) to be *differently* linked to some *specific results* affecting the global operational dynamics and architecture of that system, with respect to its capacity for even more robust self-maintenance in that environment.

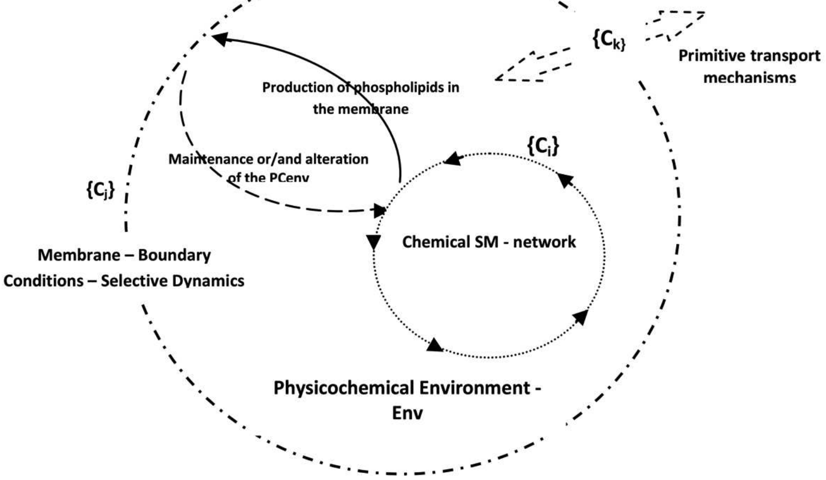

FIGURE 3. Abstract depiction of an SM organization exhibiting FD formed on a closure of constraints SM$_{[CoC]}$.
A protocell whose organization is maintained by the dynamic coupling of a *chemical SM network*, constrained by locally active constraints of the subset $\{C_i\}$ (e.g., enzymes, energetic intermediaries, etc.), to the membrane encapsulating the SM network and acting as another subset of globally active constraints ($\{C_j\}$ —e.g., playing the role of global *boundary conditions* that interface the *selective dynamics of the environment* and thus alters the *physicochemical environment* in (i.e., the Bd_$\{C_i\}$ under) which the chemical SM network operates and produces, at least partially, the phospholipid component of the membrane. Now, the continuous production and residing of more phospholipid components in the membrane of the protocell will make it much less permeable, which in turn will implicitly apply new selective pressures that could result in the formation of primitive transport mechanisms (e.g. the group of constraints $\{C_k\}$) operating on the release of osmotic pressure, which will at least partially determine the maintenance of the boundary conditions needed for the continuous operation of the processes constrained by $\{C_i\}$ and $\{C_j\}$ (e.g. avoiding osmotic burst in the protocell).

This is a system with a SM organization exhibiting FD formed on a closure of at least three subsets of constraints $SM_{[CoC]}$. However, as we shall discuss in the next section, there is a limit in this process of creation of new FD. How, then, the organization of a system of type $SM_{[CoC]}$ can evolve towards significantly higher levels of complexity?

4. TOWARDS HIGHER[5] LEVELS OF F.D.

In our analysis, two structures could be functionally different in a system if the processes that they constrain make different types of contributions to the maintenance of the system. We have considered that a minimal form of FD appears in certain SM prebiotic systems, based on a closure of constraints. These constraints are certain types of molecular structures. Presumably, this would require, as mentioned, relatively complex molecular components; yet if compared with present-day biological macromolecules, like DNA or proteins, these components should have been much simpler. Now, for achieving wider repertoires of functional diversity [6], increasingly higher levels of structural complexity are necessary, since in chemical SM networks the development of more precise and diverse functions is directly related to the development of stereo-specificity (namely, the particular spatial configuration of the components of a big molecule, which in turn requires the increase of size and composite variety).

The generation of structural complexity is a spontaneous process of combination of simpler building blocks as the chemical domain allows the recombination-based production of structures. Macromolecules and macromolecular aggregates (supra-molecular structures) are very large structures (in terms of molecular sizes) held together by intermolecular forces rather than covalent bonds. Macromolecular aggregates can be achieved by self-assembly of the molecules that made up the aggregates. Self-assembly has allowed the construction of a variety of different shapes and sizes, due to the formation of the aggregates that is directed through non-covalent interactions. The big difference in the strength of these two types of bond—covalent bonds hold together material structures by energies around 1.70 *ev*, whereas non-covalent bonds are around 0′5 *mev*—explains that macromolecular aggregates can be seen as quasi-combinatorial entities. By this we mean a scenario where basic units can be combined as building blocks in constructive operations, namely, that parts can be associated in different ways forming a great variety of stable aggregates, instead of being driven deterministically towards unique aggregates. Since the forces that hold together these parts are relatively weak, the presence of constraints can easily drive the formation of, so to speak, almost free combinations of building blocks.

Nevertheless, in the conditions of a prebiotic environment the generation of structural complexity is based mainly in the formation of new composite molecules, whose building blocks are held together through covalent bonds [7]. This process is limited in size and variety since, among other reasons, the combinations between building blocks are driven by specific laws that forbid many possibilities. Now, for the building blocks to be amenable to combinatorial processes, it is necessary that the dispositions between the parts be undetermined by intrinsic, lawful interactions. This is the case of structures based on many weak interactions (van der Waals forces, hydrogen-bond formation, medium-range electrostatic/ionic forces, hydrophobic effect...) acting in parallel within the different building blocks, which allow easily to a considerable number of combinations among them.

These interesting macromolecular structures can be generated by self-assembly processes (Lehn 1995), although it is very unlikely that we could find such type of complex structures in abiotic conditions. Since these complex structures are formed using relatively weak chemical bonds, various physical forces can easily damage them. Macromolecular structures tend to be highly vulnerable to disruption by impinging signals and are constantly in need to replenishing their dissipating energy and order as their structural bonds have energies measured in electron volts, even fractions (Collier and Hooker 1999). Although some relatively complex structures could be maintained due to spontaneous processes of self-assembly compensating their decay, most of them (as we see in present day cells) can only be maintained through operations of repair and reproduction. Hence, though initially relatively complex structures could be formed in different environments, driven by geological or other types of abiotic processes, an accumulative production of complex structures leading to the appearance of interesting macromolecular structures is not likely to occur outside the frame of SM systems. The reason is that within SM systems these increasingly complex structures could be recruited to perform functional activities and, therefore, by entering in a SM organization of mutually dependent constraints, they ensure their maintenance. In other words, the formation of new and more complex structures can be ensured if they become functional parts within SM organizations. Thus, although a large part of biological organization depends on complex structures formed by self-assembly processes that do not involve energy dissipation (thermodynamic equilibrium), the variety of functional processes of the cell results from a combination of complex self-assembly and self-organizing processes (Karsenti 2008).

As we shall see, the consequences of this mutual interplay between self-organization and self-assembly is the emergence and maintenance of new larger structures, made of simpler functional components. The central point is that the increase of structural complexity leading to new functions in a system would be mainly the result of a compositional processes of the arrangement of the "primitive" functional parts, namely, the differentiated constraints involved in the organizational closure of the system.

Through several processes of self-assembly, the resulting structural complexity can be capable of triggering new structural changes, and new possibilities for a subsequent composition in an open way of recombination (see endnote 10). As E. Fox Keller (2009) has pointed out:

> The formation of the covalent and non-covalent bonds that hold such molecular complexes together can also sometimes change the structure of the components with which the process started. In so doing, they can also induce changes in the rules of engagement, thereby creating the possibility for new interactions, new binding sites, new hooks. The new binding sites are not simply the consequence of the new proximities created by molecular binding, but more interestingly, of the changes that have been triggered in the ways in which the component parts can interact. They might be thought of as Brownian motors in evolutionary space, feeding on chance events to build ever more complex configurations (...) The phenomenon I am trying to describe rests on two basic facts: first, that many complex macromolecular structures are capable of stabilizing in a variety of distinctive shapes or forms, and second, that the binding of new molecules can trigger a shift from one conformation to another, thereby exposing new binding sites, and new possibilities for subsequent composition (ibid, pp. 22-23).

This initiates a new and almost indefinitely open domain of structural variety. Even so, in order to go in this direction, a certain form of modularity is required. A modular organization is one in which different functional components may be separated and recombined, such that allows an almost free compositional process. Of course, to achieve such result it is necessary to start with relatively stable entities amenable to a variety of physical forms of assembly or aggregation. Otherwise, they could not act as building blocks for the construction of new functional entities. Once this condition is fulfilled, these basic components can be treated as building blocks that can be re-combined through processes of arrangement, so that an open world of combinations can be achieved [8]. This way, new more complex structures can be generated through compositional processes of arrangement of similar core structures.

This process is interesting since it allows, in turn, the generation of composite devices able to perform highly specific types of work, namely, *machines*. A (mechanical) machine is a structure made of organized parts that, by its movements, is able to perform a type of work. For example, in the case of proteins, secondary structures are organized in rigid parts, whose relative movements can generate different effects, leading to the amplification of small displacements, or other mechanical effects described by words borrowed from the depiction of machines: lever and spring, ratchet and clamp and so forth (Morange, personal communication, 2008). To see them as machines performing work requires some input of energy, which, through a stable arrangement of a set of structures with specific shapes, is harnessed *to perform well specifiable* functional processes. In the case of the ATPase, for example, its chemical function involves a true mechanical action, as it transports solutes against a gradient. Actually, this protein works as a small motor, with a rotor and a stator, the first spinning around the second to generate ATP.

Actually, living cells are constituted by a host of molecular machines that participate reciprocally in their respective processes of fabrication, maintenance and operation. And this impressive diversity of functions is ultimately the support of further levels of functional complexity in living organisms. This leads us to the main point of this section. When components act as building blocks in constructive operations, namely, when parts can be combined in different ways instead of being deterministically driven towards unique aggregates, a new open functional domain can be explored. This is precisely what stands behind the concept of machine. Actually, the idea of machine is functionally linked to the idea of a mechanism, which could be defined as a set of "entities and activities organized such that they are productive of regular changes from start or set-up to finish or termination conditions", namely, an arrangement of parts (a structure) "performing a function in virtue of its components parts, component operations, and their organization. The orchestrated functioning of the mechanism is responsible for one or more phenomena" (Bechtel and Abrahamsen 2005). Hence, since machines are the result of *compositional* processes of arrangement of parts, these arrangements could be used to generate new ones, and this process can proceed indefinitely. A given "simple" machine can in turn be integrated in a more complex arrangement of parts, such that new functions could be achieved [9].

This implies that *through processes of arrangement of parts an open* [10] *world of functions can be achieved*. Since machines are the result of compositional processes of arrangement of parts, and that the output of these new, more encompassing arrangements could be also a new function, new functions could be created. In turn, these processes could be used to produce new and more complex molecular machines, in a recursive way, and these new machines can generate new functional tasks. Therefore, machines are on this perspective, the material support of mechanistic processes which in turn allow the creation of unlimited functional diversity.

5. CONCLUSIONS

As argued elsewhere (Moreno and Ruiz-Mirazo 2009), it now seems even more possible that the source for FD lies ultimately in the organization of (proto-) biological systems. For two reasons. First, because certain SM systems formed on a closure of constrains (SM_{CoC}) can reproduce themselves and, therefore, support some form of continuity allowing, at least in principle, an accumulation of novelties. Second, because SM_{CoC} systems are potentially capable of producing and preserving new components of increasing structural complexity. In other words, SM_{CoC} systems provide a framework to allow the production of a wide variety of new components, some of which could be recruited by the systems themselves for modifying their own organization. This is precisely the scenario we have suggested for the origin of FD. Interestingly, the exploration of new forms of structural complexity (new components) through assembling processes implies a certain degree of decoupling from the dynamics of the chemical SM_{CoC} organization. This allows a relatively free exploration of the domain of structural complexity, which ultimately can be recruited for increasing in an open way, in turn, the functional complexity of the overall SM organization. This scenario opens up an unlimitedly wide domain of harnessed processes. Matter and energy flows can be manipulated to achieve highly complex and specific tasks. Therefore, by recruiting these macromolecular machines SM systems can create new and more complex ways of viability and develop internal subsystems, for example, adaptive mechanisms.

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NOTES

1 This has some important implications with respect to many physical or mechanical systems that are mistakenly considered as fulfilling the condition of a closure of constraints (CoC), which in turn, contribute differently in the maintenance of the underlying organization. An example would be a billiard table where balls are arranged in a way that can hit the one another after striking on the sides of the table. Assuming a frictionless table, the whole setup can be maintained for a long period of time thus achieving CoC, and assuming different forces responsible for the initiation of the movement of each ball we can have different speeds thus achieving different contributions. However, it is obvious that we would be very reluctant to discuss about FD in such a system since the whole closure is achieved through the externally imposed boundary conditions (speed and space of movement), let alone the creation of the structural components of the system. Another, more interesting example is Ruse's (2003) rain-cloud-river water cycle, where a set of structures (clouds, rain, spring, river, sea, clouds) seems to have mutual causal relations that fit the idea of FD. For instance, the clouds generate the rain, which (contribute to) generates a spring, which generates a river, which (contribute to) generates a lake, which generates different clouds, and so on. However, this is not the case. Not only the water cycle does not exhibit any CoC (see Mossio and Moreno, 2010 for an analytic explanation), but also, the generation of any structure (e.g. a cloud) that may contribute differently (e.g,. heavy or light rain) to the cycle is the result of externally imposed boundary conditions (e.g., sun, air, various types of ground), which since there is no CoC they are not redefined by the operation of the constraints in the system. Therefore, the water cycle does not exhibit any form of functionality.

2 It is noted that even at this level of organizational complexity the system interacts with the environment, although its interaction is limited in the exchange of raw chemical materials.

3 By 'recursively' we mean that the fulfilment of Cond_1 will ensure the maintenance of Cond_2, so that under certain environmental conditions their operational combination (Cond_1 & Cond_2) will ensure Cond_3, whose fulfilment will result in the continuous persistence of the operational combination of Cond_1 & Cond_2 & Cond_3) and so on. At this level of organizational complexity of a biological system exhibiting minimal FD, the mutual dependence of those conditions and of the respective constrained processes is of a recursive nature.

4 At this level of organizational complexity there is no need for the system to be able to alternate between two significantly different modes of operation thus presenting different self-maintaining characteristics and properties, but there is a need for the association of the effects of a set of constrained processes of the system with at least two significantly different contributions to the overall SM organization.

5 In this section, we will discuss the prerequisites for significantly higher levels of FD but we will not necessarily consider open-endedness in an unlimited way.

6 At this level of organizational complexity, it is postulated that a minimum degree of functional differentiation (FD) is already exhibited by the system and hence, what we consider is a possible way for the system to achieve a significant openness of that differentiation.

7 There is also the formation of supramolecular structures, like processes of crystallization, but these bigger structures have poor internal diversity unless they are based on weaker bonds, as it is the case for lipid membranes, micelles, polymer aggregation, etc.

8 It is not easy to tell what degree of molecular complexity is required to satisfy these conditions. But it seems that relatively small ones could act as modules. For example, as recently shown by Manrubia and Briones (2007), certain small molecules of RNA can play the role of modules in a stepwise model of ligation-based modular evolution: RNA hairpin modules could have displayed ligase activity, catalyzing the assembly of larger, eventually functional RNA molecules. These ligation processes allow a fraction of the population to retain their previous modular structure, and thus, structural and functional complexity can progressively increase, even in the absence of template replication.

9 It is very important to keep in mind that the output of these arrangements is a function, and that a structure of parts is only a machine if it serves a function in a given system.

10 "Open" here refers to the idea that functional devices could be used to generate new ones, and that this process can precede indefinitely. Thus, functional 'recursion' (i.e. functional recombination in the context of a SM organization) is a more powerful concept than that of the aforementioned assembling, based solely on structural re-combination processes.

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