Chapter 9
On the Evolutionary Development of Biological Organization from Complex Prebiotic Chemistry

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Abstract In this chapter we offer a critical analysis of organizational models about the process of origins of life and, thereby, a reflection about life itself (understood in a general, minimal sense). We begin by demarcating the idea of organization as an explanatory construct, linking it to the complex relationships and transformations that the material parts of (proto-)biological systems establish to maintain themselves under non-equilibrium dynamic conditions. The diverse ways in which this basic idea has been applied within the prebiotic field are then reviewed in relative detail. We distinguish between "network" and "protocell" approaches, discussing their specific implications and explaining the greater relevance of the latter in the current state of affairs. Despite the key role that such organizational approaches play (and should keep playing) to advance on the problem of primordial biogenesis, the second half of our contribution is devoted to argue that they must be combined with other explanatory accounts, which go beyond the physiology of any single (proto-)organism. With that aim, we underline the fundamental differences between the autonomous, metabolic dynamics that individual (proto-)cells perform and the evolutionary and ecological dynamics that take place in a collective and transgenerational dimension. Apart from obvious gaps in the characteristic temporal and spatial scales involved, the corresponding causal and interactive regimes also reveal themselves as neatly distinct, what is reflected in the unpaired functional integration and the agent behavior displayed by biological individuals. Nevertheless, any living organism (and life in a wider, general sense) derives from the deep interweaving of those two phenomenological domains: namely, the "individual-metabolic" and the "collective-evolutionary" domains. At the end of the chapter, we propose the principle of dynamical decoupling as the core idea to develop a more comprehensive

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M. Mossio (ed.), *Organization in Biology*, History, Philosophy and Theory of the Life Sciences 33, https://doi.org/10.1007/978-3-031-38968-9_9

theoretical framework to understand how this intricate, causally asymmetric connection must be articulated during the actual process of biogenesis (as it happened here on Earth or anywhere else in the universe), so that life's minimal complexity threshold is reached.

Keywords Primordial biogenesis · Prebiotic transitions · Molecular reaction networks · Protocell models · Organizational integration · Minimal metabolism · Regulation · Origins of agency · Functional domain · Reproduction · Material/trans-generational constraints · Pre-Darwinian evolution · Sedimentation · Proto-phylogenies · Dynamical decoupling · Informational records · Genetic code · Biological organization

9.1 Introduction: Organization as an Explanatory Construct in Origins-of-Life Research

Most of the research work in the field of prebiotic chemistry has been focused, so far, on discovering reaction mechanisms and transformation pathways for the abiotic synthesis of biopolymers, their monomers, or some other biologically relevant molecules. In addition, the replicative and catalytic properties of those molecules have been explored in considerable detail (for an extensive review, see Ruiz-Mirazo et al., 2014). However, all that body of empirical and theoretical knowledge tells us very little, unfortunately, about the main transitions during the process of primordial biogenesis. Somehow, Miller's (1953) famous experiment was a turning point in the field that has been transformed, over the last decades, into a wider and more solid platform to approach the problem of origins of life, but further progress has been quite modest, really: like Sutherland (2017) says, all what we have achieved so far (including his own investigations) is just «the end of the beginning» in terms of solving the question.

Many authors consider that *natural selection*, combined with long enough time periods, up to the geological scale, could lead all the way, from populations of biomolecules (in particular, if the latter developed the capacity for multiplication, variation, and heredity (Maynard Smith, 1986; Szathmáry & Maynard Smith, 1997)) to living cells. Yet, this involves a huge assumption, based on the premise that the principles of Darwinian evolution can be readily applied to bare sets of molecular replicators. There is ample evidence, indeed, to support that molecular structures, like RNA strands, undergo artificial evolution in vitro, being able to reach pre-established target motifs (e.g., ribozymes with specific features (Bartel & Szostak, 1993; Johnston et al., 2001; Tjhung et al., 2020)) or even follow a potentially endless process (Lincoln & Joyce, 2009). Nevertheless, there are no obvious results showing evolutionary dynamics that bring about a relevant increase in the complexity of the individuals that constitute those populations, even when replicators are used in combination with protocellular structures (e.g., Chen et al., 2005; Mansy et al., 2008 – see also the more recent review: (Joyce & Szostak, 2018)). Therefore,

although hopes still remain for an "RNA world" (the hypothesis that all started from RNA molecules (Crick, 1968; Orgel, 1968; Gilbert, 1986)) which could turn into a full-fledged biological world (Higgs & Lehman, 2015; Joyce & Szostak, 2018; Krishnamurthy, 2020), more and more skeptical voices are rising, advocating the need to conceive alternative scenarios (Ruiz-Mirazo et al., 2017; Le Vay & Mutschler, 2019; Kroiss et al., 2019; Preiner et al., 2020). Among other reasons, it could well be the case that a molecularly richer, more varied, and heterogeneous prebiotic milieu is required, right from the beginning, to trigger off those evolutionary processes that may lead to an open-ended increase in functional/phenotypic diversity, as it was argued more extensively in (Wicken, 1987; Moreno & Ruiz-Mirazo, 2009; Ruiz-Mirazo et al., 2008, 2020).

The main alternative to the "RNA world" has traditionally been the "metabolism-first" hypothesis (De Duve, 1991; Dyson, 1999; Morowitz, 1999; Shapiro, 2000), which defends a completely different plan of attack and explanatory framework for life's origin. The key question, according to this approach, would be discovering the combination of energy inputs, material components, and chemical transformation processes that put together a self-maintaining system in non-equilibrium, precarious conditions, in transition toward minimal (unicellular) organisms. It is in this context, precisely, where the idea of *organization* comes to the center of stage, as a fundamental mereological construct through which complex systems, like living cells (or their precursors, "protocells" or "proto-metabolisms"), demand a proper characterization. The work of a number of classical authors in theoretical biology (Rosen, 1971, 1991; Varela et al., 1974; Maturana & Varela, 1980; Ganti, 1975, 2003; Eigen & Schuster, 1979; Kauffman, 1986, 1993; Fontana & Buss, 1994, 1996), trying to determine the idiosyncratic nature of biological organization, in its most elementary and general sense, in contrast to other types of organization one may find in the natural world, also helped to elaborate and support this view. A view that has been reinforced in recent times, as well, with the advent of new research programs like "systems biology" (Kitano, 2002; Westerhoff & Palsson, 2004) and "systems chemistry" (von Kiedrowski, 2005; Ludlow & Otto, 2008) that insist on the irreducible complexity of biological and proto-biological entities (de la Escosura et al., 2015; Kroiss et al., 2019). This does not always mean defending metabolism as the first or most important landmark in the prebiotic process, but it stands much closer to that way of framing the question, as an investigation into the intricate material and energetic couplings that enable non-equilibrium, dynamic systems whose emergence and maintenance rely on the strong functional integration of a variety of molecular components in continuous transformation (Ruiz-Mirazo et al., 2017; Lauber et al., 2021).

In this chapter we will briefly review the different organizational approaches that have been pursued within the field of origins of life and their relative success, classifying them in two main groups: *network* models and *protocell* models. These two types of model can be both experimental (in vitro) and theoretical/computational (in silico): the fundamental feature that distinguishes them relates to the degree of diversity and interdependence required among the various processes, material components, and constraints that constitute the system. Although protocellular models

tend to be more demanding and encompassing in that sense (and, therefore, more interesting in order to develop a complete theory of biogenesis), they are also more complicated to handle and analyze. In any case, we will try to show how the concept of organization, in its various meanings, can be used both as *explanans* (i.e., to describe intermediate hypothetical model-systems, taken as necessary conjectures or stages to make sense of such a long and complex transition) and *explanandum* (i.e., to account for the end result, prokaryotic cell organization, taken as factual "minimal life").

However, the most important message of our chapter is to remark that the *organizational* framework, being of primary and central importance, is not sufficient to elucidate the nature of the living phenomenon nor the way it came to be, from physics and chemistry. Although the inert and the living worlds are of course linked, in various ways and diverse planes, the jump between their corresponding phenomenologies is too big, or too high, to be taken in just a few steps. Even if the first stages of primordial biogenesis should already involve organized individuals (for the reasons suggested above, see again, in particular Moreno & Ruiz-Mirazo, 2009), additional explanatory principles, beyond the sphere of individual organizations, must be at work for prebiotic systems to overcome the bottlenecks that were surely present throughout such an intricate process. More precisely, increases in complexity (including the internal complexity of the individual organizations leading the process) require dynamics and interactions that take place at the level of population dynamics, as we will see, to ensure minimal robustness at the intermediate phases, paying back for the energetic and material costs involved.[1]

Regulation, for instance, understood in a biologically relevant sense (i.e., not simply as chemical feedback but as a hierarchy of controls operating in minimally adaptive systems, so they can select among a diversity of metabolic/behavioral regimes in response to changes in internal/external variables (Bich et al., 2016)), appears as a key property to be developed by protocells, on their way toward more autonomous, efficient, and sophisticated cells. Although the presence of regulatory mechanisms has also deep implications in terms of how each individual is organized, the appearance and stabilization of such mechanisms cannot be conceived but as the result of an *evolutionary* process. Namely, a process in which a large population of similar, precarious but proliferating systems, in a variable and challenging environment, try various (constitutive or behavioral) options, and come out with "a solution" that spreads and eventually becomes built-in, hard-wired in each system

[1] This makes our position quite different from other authors', like Varela's (1979) or Rosen's (1991) who defended that the living phenomenon could be fully captured in terms of the organization that each organism continuously realizes. In more pragmatic terms, the conception that we will defend here, based on our previous work on the nature of life (Ruiz-Mirazo et al., 2004, 2010), highlights that there are yet many hard issues to address in the field of origins (as well as in theoretical biology) related to the intricate link between the physiological, ecological, and evolutionary spheres of the living. That move also makes more understandable the huge gap in complexity that still lies between our current prebiotic (or "bottom-up") models/systems and any real cell.

(i.e., it is adopted as a reliable mechanism by all subsequent individuals in the population). Similarly, genetic mechanisms are incardinated within individual organizations, where they play their fundamental physiological roles (as a guide for protein synthesis, more prominently), but their raison d'être and functional contribution do not make full sense unless it is considered in the context of a wider and longer evolutionary pathway, an open pathway that transcends any of those particular individuals (Ruiz-Mirazo et al., 2020).

Therefore, we will put forward the thesis that complex chemical systems (self-producing and self-reproducing protocells) progressively transform into hypercomplex biological organisms (living cells) thanks to a combination of factors that operate not only at different spatial/temporal scales and with different weights but also following intrinsically different dynamic principles. Some of these principles have to do with the composition, architecture, and necessarily interactive self-maintaining dynamics of the individuals involved, whereas some others have to do with their reproduction, inheritance, diversification, and open, collective dynamics. Accordingly, *organizational* aspects will be primarily associated to the development – and adequate coupling – of basic control mechanisms at the molecular and physiological description levels (Ruiz-Mirazo et al., 2017), while *evolutionary* and *ecological* aspects (Moreno, 2016) will rather cover the "propagation" (Kauffman, 2000) and "sedimentation" (Walsh, 2018) processes working at the level of the population, or the whole ecosystem/biosphere. We will argue that, even if they seem quite orthogonal to each other, these two phenomenological domains must actually get tied up during the process of origins of life, establishing a mutual – though causally asymmetric – connection that is further reinforced once biological evolution takes off. Our discussion will reveal, in any case, how much ground science must still cover in order to solve the problem of primordial biogenesis.

9.2 Organizational Accounts at the Onset of Prebiotic Evolution: Network Versus Protocell Models

As we just advanced in the introduction, tackling the problem of origins of life from an organizational perspective implies a theoretical scheme according to which different molecular components and transformation processes come together to constitute prebiotic systems that maintain themselves and proliferate in non-equilibrium conditions, on their way toward living organisms. Depending on the diversity and complexity of the material components/transformations involved, as well as on their relationships and interactive properties, one can propose a variety of architectures to characterize such systems. A complete organizational theory of biogenesis should provide a plausible sequence of transitions, starting from relatively simple systems toward increasingly complex ones (both in terms of molecular ingredients and architectural/interactive features), with the aim to bridge the gap currently observed in nature between physical-chemical and biological phenomena.

Living beings, as pointed out so wisely by Kant, long before the development of modern biology, have a very special kind of organization, in which even the mere existence of many fundamental system parts cannot be taken for granted, since they result from the collective transformation dynamics of the whole. This idea is, in our view, of central importance to understand biological phenomena, and, thus, it must also play a key role in any explanation of the process of biogenesis. Yet, what Kant did not anticipate is that the roots of this complex dynamic behavior could actually be found in the domain of physics and chemistry: in other words, that matter is inherently active, given the adequate conditions, as it became apparent in the study of non-equilibrium self-organization and self-assembly processes last century (Nicolis & Prigogine, 1977; Lehn, 1995) and has been, thereafter, reinforced (Showalter & Epstein, 2015; Semenov et al., 2016). These processes, being necessary to understand the workings of any cell (Karsenti, 2008), are nevertheless not sufficient. The capacity for self-maintenance characteristic of biological organisms, their surprising endurance as non-equilibrium, dissipative systems, involves not only the organization of already existing parts into a whole but a proper *metabolism*: continuous constructive and reconstructive transformations, which actually synthesize the key ingredients that rule its complex behavior (i.e., a diversity of material constraints that operate on those same transformations (Ruiz-Mirazo & Moreno, 2004; Lauber et al., 2021)).

This circularity or cyclic/self-referential, collective dynamics was the theoretical target of a number of classical models of minimal biological organization (Rosen, 1971; Varela et al., 1974; Ganti, 1975; Pattee, 1977; Kauffman, 1986). Although the direct impact of such abstract models on the research field of prebiotic chemistry has been relatively modest, we consider that they can still be helpful to draw a conceptual distinction between two general approaches to the problem of origins of life, when this is envisaged in terms of the emergence of metabolic organizations. On the one hand, one can identify the *network* approach, in which the dynamics of a population of reacting molecules in homogenous – typically aqueous solution – conditions is explored, assuming a more or less concentrated "organic chemistry soup" where the potential couplings/interactions among those molecular components can be captured through mathematical mappings or graphs.[2] On the other hand, we find the *protocell* approach, in which both physical and chemical transformations take place in heterogeneous conditions – typically a mixture of aqueous and organic domains, like a lipid vesicle suspension – where the couplings/interactions among the system components must be analyzed making use of additional tools, since they are also influenced by spatial constraints on their free movement/diffusion. With variations (which we will not go into here – see Moreno & Ruiz-Mirazo, 1999;

[2] Some "network" models/theories would not work, strictly speaking, in homogeneous 3D conditions, but on surfaces. For instance, the classical proposal of Wächtershäuser (1988, 1990) would constitute a two-dimensional metabolism working on the surface of pyrite. However, we are not going to pay special attention to this type of scenario here. It could be interesting in terms of finding synthetic pathways to generate some organic compounds, but they are severely limited for any further organizational developments (Ruiz-Mirazo et al., 2020; Lauber et al., 2021).

Hofmeyr, 2007; Cornish-Bowden & Cárdenas, 2020 for more detailed reviews), Rosen's *M-R systems* or Kauffman's *autocatalytic sets* would represent the former (i.e., network approaches) and Maturana and Varela's *autopoiesis* or Ganti's *chemoton* the latter (protocell approaches). Let us briefly review, with a critical eye, the effective progress made in the prebiotic research camp, over the years, by following these two general organizational schemes.

9.2.1 Network Models

Most empirical approximations to the problem of origins of life have been championed by chemists (organic synthetic chemists, in particular), whose main interest is deciphering abiotic reaction pathways that could lead to various, specific biomolecules (Ruiz-Mirazo et al., 2014). This, although important to address, does not lead very far in terms of understanding the first biologically relevant *organizations*, as we said above. Nevertheless, in recent years, with the advent of systems biology and systems chemistry, an increased awareness in the community about the importance of dealing with complex mixtures in a prebiotic context has brought about a much more compelling research scene (Ashkenasy et al., 2017; Kroiss et al., 2019; Wolos et al., 2020), in which strongly reductionist approximations to the problem (e.g., working with one type of molecule, or one type of chemistry – even if this is claimed to be fundamental for life) are no longer valid.

There were some remarkable achievements associated with the idea of autocatalytic networks in the past (e.g., von Kiedrowski's (1986) self-replicating oligonucleotides, or the analogous oligopeptide systems developed by Ghadiri's group (Lee et al., 1996)), but these were just networks of oligomer-pairs with a template that coupled through a single, potentially autocatalytic recognition mechanism, rather than a collectively or reflexively autocatalytic network. Subsequent expansions toward more complex systems (employing combinations of more diverse components and higher-order catalytic and cross-catalytic mechanisms operating in parallel, within the same pot) led to interesting, emergent properties at the collective level (for a review, see Dadon et al., 2008). Similar investigations have also been carried out with populations of different RNA molecules designed to build cooperative relationships among them in order to achieve some collective autocatalytic behavior (see, e.g., Vaidya et al. (2012) or, more recently, Ameta et al. (2021)). Nevertheless, despite their obvious interest, we consider that these emergent phenomena are not so relevant, prebiotically speaking. Although they do reflect a complex global dynamic behavior that could not be predicted from the pieces of the puzzle (like in self-organizing phenomena, in which the more pieces you mix, the more difficult it becomes inferring, from individual molecular/mechanistic properties, what will happen at the overall, network level), their potential to build minimally robust,

integrated material organizations is still unclear.[3] Many similarities can be drawn with the general case of dynamic combinatorial libraries (DCLs), and more so if they are under non-equilibrium conditions: the higher compositional diversity one introduces, the more interesting (and difficult-to-predict/analyze) phenomena one obtains (Corbett et al., 2006; Reek & Otto, 2010) but, without more demanding *systems* requirements (in particular, without the development of spatial and energetic control mechanisms) where do those phenomena lead to?

Many of these works, in addition, are not so concerned about the prebiotic plausibility of the components used. They are just demonstrating that chemistry is much wider than biology, in terms of molecular structures and nontrivial combinations thereby. Nevertheless, complex dynamic behavior does not immediately lead to molecules and transformation processes that establish and develop *functional* relationships, like those so characteristic of living organisms (see Sect. 9.3.1). The key question does not seem to be molecular and interactive diversity per se but playing with the biologically relevant type of diversity, with the aim to open a new window of dynamic behavior, performed by more *complexly organized* systems. Yet, this combination of material ingredients and conditions for viability does not come for free: they need to be physically constructed and maintained. Unlike self-organizing phenomena, which often run spontaneously (given some initial/boundary conditions), biological organization involves a thermodynamic effort right from the beginning (that is probably the reason why proto-metabolisms are not so easy to implement).

A good number of labs and researchers are actually focusing on how fundamental metabolic cycles and synthetic pathways (as they are realized in biochemistry, or in similar versions) could run under prebiotic conditions (i.e., in the absence of enzymes, making use of alternative catalysts) (Keller et al., 2014, 2016; Coggins & Powner, 2017; Muchowska et al., 2017, 2019; Springsteen et al., 2018; Stubbs et al., 2020). These groups are opening the origins-of-life field in a really interesting direction, demonstrating that there could be a natural bridge (or several bridges, right now under exploration) between organic chemistry and biochemistry, to be then reinforced through the development of proteins and enzymes, but not necessarily dependent on the latter at the very beginning. The importance of considering metabolism as the central problem in biogenesis is that the material and thermodynamic hurdles involved become apparent: they actually turn to be the main focus of research. However, the results obtained on these lines, though highly promising, are still far from "minimal metabolisms" because they have not managed to couple the

[3] By an "integrated material organization," we mean a molecular system where all components are strongly interdependent and constitute a coherent, operational unit that self-maintains. Minimal robustness, in this context, requires the combination of different physical and chemical factors. More precisely, compositional and interactive diversity, along with phase heterogeneity (the coupling of chemistries taking place in various reaction domains) seems critical to achieve this kind of collective and operational molecular interdependences (Ruiz-Mirazo et al., 2017; Lauber et al., 2021).

reactions with adequate, endogenously synthesized material constraints that should act as first-order control mechanisms on those same reactions. This is crucial, as we will expand below, for any material organization to be able to *construct* itself autonomously (see Ruiz-Mirazo & Moreno, 2004, 2012 and also Lauber et al., 2021).

9.2.2 Protocell Models

Network approaches are mostly concerned with chemical reactions (in particular, their stoichiometry and kinetics). Yet, the problem of origins of life is not only chemical: physics also plays a fundamental, complementary role in it. In support of that claim, one can always bring to the fore the fact that all biological systems heavily rely upon boundaries and compartments, as their universal cellular character indicates, which has deep energetic and thermodynamic implications (Harold, 1986, 2001). Following this premise (more explicitly stated in footnote 3), the protocell research camp has flourished in the last couple of decades. There were, of course, remarkable pioneers earlier on, starting from one of the founders of the origins-of-life field, Oparin, but also including other key figures, like Deamer or Luisi, who defended the prebiotic importance of lipid compartments in times when it was still a rather marginal line of work (for a nice review on the history of the field, see: Hanczyc, 2009). The situation changed with the turn of the century, when the "lipid world" hypothesis was introduced (Segré et al., 2001) and highly influential researchers, like Jack Szostak, coming from the RNA-camp, started investigating protocellular systems in depth (Szostak et al., 2001).

This contributed to widen the field of origins of life, embracing in the same move some of the non-reductionist postulates coming from the field of systems biology (Ruiz-Mirazo et al., 2014, 2017). Indeed, the assumption that protocellularity is central in the early stages of biogenesis brings forward a concept of prebiotic individual that goes definitely beyond the molecular level: rather than populations of molecules as such, what one should consider is populations of molecular *organizations* constructed within compartments. Or, more accurately expressed, one should consider molecular organizations that also build their own boundaries and constantly traffic with matter and energy through them to achieve a precarious self-maintenance, with potential to propagate through reproduction and evolve as a protocell population. Taking seriously into account a global constraint, like a vesicle membrane, that derives *from* and exerts spatial control *on* a set of encapsulated chemical species/transformations (introducing new rules for dynamic behavior that need not be strictly stoichiometric – e.g., osmotic and volume effects, generation/management of electrochemical gradients) has far-reaching implications, both in a proto-metabolic and in proto-evolutionary sense. Unfortunately, many chemists feel out of their "comfort zone" working with colloidal systems (like lipid vesicle suspensions), hence their traditional reluctance to investigate this domain. But postponing the problem of compartmentalization to later stages in biogenesis only makes it

worse (Piedrafita et al., 2012; Szostak, 2012), and the community is beginning to realize this.

Thus, during the last two decades, there has been a remarkable increase in the scientific exploration of protocellular systems, in diverse directions, and taking up both bottom-up and top-down approaches. The development of synthetic biology, in particular the "synthetic cell" research program, has also contributed to this expansion (de la Escosura et al., 2015), even if most of that work is far from being prebiotic, and often just recreates biochemical processes under well-controlled, artificial conditions (e.g., through the use of synthetic liposomes). Nevertheless, understanding the principles of organization underlying real, prokaryotic cells (the *end result*, from an origins perspective) or simpler, hypothetical versions of them (the *intermediate steps*) also requires making use of material components and conditions that are alternative to the standard, biological ones. In this vein, we will briefly review here experimental work that is especially interesting from a particular theoretical perspective on biology and primordial biogenesis, the "autonomy perspective" (that we embrace and have contributed to develop (Ruiz-Mirazo & Moreno, 2004, 2012; Moreno & Mossio, 2015)),[4] but without paying so much attention on whether the material aspects involved in those (proto-)cellular systems exactly match the actual biochemistry and biophysics that we know on planet Earth.[5] The autonomy view, when focused on the process of biogenesis, is particularly interested in finding paths toward prebiotic systems whose internal complexity (i.e., the diversity of components and interrelations among them) is organized in such a way as to achieve their own sustainability. Namely, systems that can build – at least, part of – the boundary conditions that allow for their existence as precarious organizations in far from equilibrium conditions.

Maturana and Varela (1980) and their theory of *autopoiesis*, forerunners in this way of thinking, were more concerned about capturing the "organizational core" of the living phenomenon than to understand its origins. There were others, like Luisi, who took up the job of trying to implement those ideas in an empirical research program that could illuminate biogenesis (Luisi & Varela, 1989; Walde et al., 1994; Luisi, 2006; Bich & Green, 2018). That research program, established 30 years ago, is still active and giving interesting results, e.g., Hardy et al. (2015); Post and Fletcher (2020). The main motivation that articulates this type of investigation (which blends very nicely with our conception of the origins of life as the evolutionary development of autonomous, protocellular systems (Ruiz-Mirazo & Moreno, 2004; Shirt-Ediss et al., 2017; Ruiz-Mirazo et al., 2020)) is the exploration of how

[4] The idea that biological organisms are autonomous systems has deep historical roots, although the modern explicit use of it can be attributed to the Chilean biologist Francisco Varela (Varela, 1979). The general claim is that the property of autonomy can be naturalized and applied to molecular systems with an organization that produces and maintains itself.

[5] This is often taken as a criterion for prebiotic plausibility, but we consider it is somewhat narrow-minded (too Earth-chauvinist, as it is commonly expressed), especially from the wider perspective that fields like astrobiology, artificial life, and synthetic biology have given to the problem of origins.

the generative power of chemistry (typically, autocatalysis) can be coupled to the (self-assembly) dynamics of the compartment, so that relatively simple protocells stay in non-equilibrium conditions, through that mutual reinforcement, becoming *active* and, potentially, *reproductive* systems.[6] This (the capacity to make thermodynamically viable the synthesis, growth, and reproduction of a system) is of fundamental importance not only to understand how chemistry may get organized *biologically* but, furthermore, to realize how such an achievement actually requires the unfolding an evolutionary dimension – an aspect that was utterly disregarded by the autopoietic school.

Other "bottom-up" approaches, like the one pursued by the Szostak's lab, have provided key insights into protocell growth and division processes, usually in the context of a population of vesicles competing for the available lipid monomer, either through osmotic effects (Chen et al., 2004), differences in the membrane lipid composition (Budin & Szostak, 2011), or internal synthesis of a hydrophobic compound (e.g., a dipeptide) that could spontaneously join the membrane (Adamala & Szostak, 2013). However, despite some interesting excursions into aspects like vesicle homeostasis (Engelhart et al., 2016) or membrane functionalization, combining lipids with peptides and RNA (Izgu et al., 2016), this group has not focused on the development of autonomous protocell behavior, as such, but on finding an adequate companion for RNA evolution, so that natural selection starts operating at a supramolecular level. Yet, as we already argued above, when an evolutionary scenario is advocated as necessary to tackle the origins-of-life problem, this should be done taking into account the organizational complexity of the primitive individuals involved (like it is shown, for instance, in Piedrafita et al., 2017). On those lines, a former researcher of Szostak's lab, Sheref Mansy, has recently established an independent line of research that is more directly tackling the issue of how complex should the "original protocells" be. In other words, is there a minimal threshold of complexity, like we suggest in Ruiz-Mirazo et al. (2017) and Lauber et al. (2021) for prebiotic evolution to get started? How many different constraints (i.e., material controls) must be put together to reach the platform for taking off? The Mansy group are pushing quite promisingly in this direction, in an effort to combine compartments, catalysts, energy currencies within the same experimental system (Bonfio et al., 2017, 2018), keeping also an eye on how chemical diversity can contribute to protocell growth and division processes (Toparlak et al., 2021).

In addition to these "bottom-up" strategies that start from scratch, so to speak (i.e., from physics and chemistry), other researchers take minimal-life exemplars (microorganisms or parasites) and try to simplify or deconstruct them. From such a "top-down" perspective (which should show us the finish line for the process of primordial biogenesis), there have been very interesting results in the last years, as well. The new Craig Venter *Mycoplasma* construct (Hutchison et al., 2016) was of course a landmark, in that regard: it has provided plenty of opportunities for further

[6] That coupling between chemistry and compartment may actually be considered as the key feature to define what a "protocell" is (Ruiz-Mirazo, 2011).

exploration, not only about its physiology and metabolism (a highly complex, genetically instructed metabolism, as one could expect (Breuer et al., 2019)), but also about its reproductive potential or reliability (Pelletier et al., 2021). Although these minimalist approaches push in a direction in which both the autonomy of the cells (i.e., their actual capacity to survive in "free-living," changeful environmental conditions) and their reliable reproduction (i.e., their ability to generate "normal offspring") are taken to the limit, their study is critical to discern, precisely, the boundaries of biology. In a similar vein, "semisynthetic" constructs, like the bioreactors developed by Noireaux et al. (2011) or, more recently, Blanken et al. (2020), are also very informative. These involve biomolecules and other parts/subsystems of biological organisms under compartmentalized (*in vesiculo*) artificial conditions, with the aim to investigate the complementary relationship between membrane and endogenous reaction pathways, specifically focusing on the implications for autonomous behavior – an illuminating and very interesting line of work for the future.

Nevertheless, in order to conclude this section, we must acknowledge that the empirical evidence available to date is still clearly insufficient to elaborate a minimally consistent and complete *organizational* account for the origins of life, in the sense of establishing a plausible sequence of transitions that cover all the ground from complex, non-equilibrium chemical systems to the simplest biological ones. There are theoretical models (in particular, protocell models – from the classical (Varela et al., 1974; Ganti, 1975; Dyson, 1982) to much more recent and refined ones (Ono & Ikegami, 1999; Castellanos et al., 2004; Macía & Solé, 2007; Mavelli & Ruiz-Mirazo, 2007; Ruiz-Mirazo & Mavelli, 2008; Van Segbroek et al., 2009; Mavelli, 2012; Shirt-Ediss et al., 2015; Piedrafita et al., 2017; Pechuan et al., 2018; Attal & Schwartz, 2021) that try to fill in the current holes and open new avenues of research. The advantage of the latter (as compared to strict molecular simulations of prebiotic chemistry, usually linked to the network models reviewed above – or to other protocell models that tackle evolutionary dynamics but simplify so much organizational aspects that cannot be called properly "protocellular," e.g., Kamimura and Kaneko (2010, 2019) is that they offer a richer picture in terms of "constraint-based" or "rule-based" modeling techniques (see Lauber et al., 2021 and references therein). Thus, they are probably much closer to the complex reality of the first protocells that were involved in the process of biogenesis. Yet, without more solid, ample, and informative experimental results, it is very difficult to move forward through theoretical-computational approaches.

Furthermore, by focusing on the organization of individual protocells, like most of the previous works do, we may be limiting ourselves, "hitting a wall" that is there, but not so easy to see. In other words, we may be overlooking a fundamental bottleneck that needs to be addressed at the population level, as will be discussed in the remaining of the chapter. The crux of the matter will be finding the adequate balance between the two perspectives, organizational and evolutionary, and how they actually get intermingled. The discussion that we open here, in any case, points at one of the most difficult issues in the problem of origins of life, for which scientific insights and methods are still to be developed, so our aim is just to pose it in conceptual terms and draw some implications for future research.

9.3 The Interweaving of Organizational and Evolutionary Processes in Biogenesis: A Complementary but Causally Asymmetric Relationship

The historical dimension of life is a commonplace, a recurrent theme. While life is manifested in the form of organisms (and associations of organisms, whose spatial borders are often not so trivial to determine), the complexity of their material components and organization seems inexplicable unless appealing to a long process of evolution, beyond the time span of each of those individuals. «Nothing in biology makes sense, except in the light of evolution», as Dobzhansky famously remarked. This means that we must consider systems that, before disintegration, reproduce the essential features of their organization (what is usually understood, in general terms, as "heredity") and generate, in this way, a set of causal entailments that propagates in time and space, transcending the limits of such an organization (i.e., of the actual organization that constitutes each individual). The collection of temporally similar systems brought about through reproduction across "successive generations" constitutes a "lineage" (or a "phylogeny" – when genetic mechanisms are under focus). In that context, where the analysis must obviously scale up to a "population" level, variability also tends to be assumed (linked to some inevitable, random modifications) in the reproductive success of the individuals (i.e., their "fitness"), which leads (through a combination of selective pressures and cooperative dynamics) to a highly complex phenomenon that shows both long-term maintenance, in a basic sense, but also continuous change and diversification along the way.

In contrast to the intricate molecular and energetic couplings that constitute the organizational core (i.e., the metabolism/physiology) of organismic processes, as we discussed in the first part of this chapter, evolutionary processes cover a completely different dimension of the phenomenon of life, where causal connections extend across much larger temporal and spatial scales. In fact, the interesting point is not just that evolutionary processes are spatially and temporally wider than organismic processes: they are also ontologically different. They concern population dynamics, in which remarkably looser "organism-environment" and "organism-organism" causal interactions (i.e., less demanding or stringent than the molecular interactions *within* each organism) are the key. An additional peculiarity is that the relevant effects of these interactions can only be adequately analyzed statistically and, even more importantly, through a very long time window: a time window during which most of the causally responsible entities or agents (the actual organisms, the "tokens") have already disappeared, after participating in a *sedimentation* process of the most successful lineages (i.e., the "types," which are conserved).[7] Thus,

[7] We will use here the term "sedimentation" (Walsh, 2018, personal communication) as a generalization of the idea of "selection." Evolution does not only result from competition dynamics but also from cooperative relationships among the individuals/agents of a population, which play an active role in the process (Walsh, 2015). The idea of sedimentation conveys long temporal scales, in which different types of hereditary mechanisms, with different degrees of reliability (i.e., different "trans-generational depth," genetic and nongenetic) could be operating in parallel (Danchin et al., 2019).

the organismic and the evolutionary dimensions of life, despite being deeply entangled (and necessarily so, as we will expand below), hold an essential asymmetry: the former relies on molecular components, processes, and interactions that continuously sustain each other in a tightly cyclic, self-constructing, and self-referential manner, whereas the latter is the result of an open, long-term, and much wider process of sorting out that takes place in populations of reproducing agents, across many successive generations (Ruiz-Mirazo et al., 2020).

How can all this get started? And in what sense does the origin of such a complex, asymmetric entanglement help us understand the unfolding of a biological domain? Well, a central issue that must be highlighted straightaway (in line with what we just described in the previous section) is that the first chemical systems with potential to start turning biological were relatively complex but still precarious, given their far-from-equilibrium nature. How were these systems, then, capable of increasing their stability and robustness? We should realize that this is not a trivial task, especially if it requires an effort of synthesis of progressively more complex molecular ancillary. Fortunately, steady self-maintenance in this context would be the exception, rather than the rule: vesicles in heterogeneous, changing conditions naturally tend to undergo fission and fusion processes and more so if they are coupled with physical gradients and chemical reactions (see, e.g., Carrara et al., 2012; Oglêcka et al., 2014; Toparlak et al., 2021) for an experimental survey of this type of scenario). In other words, it is more realistic to consider that the large majority of such primitive protocells were very dynamic (favoring either growth or shrinkage, potential division, intermingling, decay, etc.) not organized for the stabilization of a steady state – like it is often assumed in theoretical models about minimally autonomous (autopoietic) protocells. Therefore, one should imagine this setting as a mess of diverse "populations" of organizationally similar systems, i.e., groups of growing and dividing protocells with their own suite of dynamic and plastic behaviors, which also brought about many processes of merging and content reshuffling (e.g., through vesicle fusion).

The advantage of such a scenario is twofold: (i) on the one hand, protocells would have an intrinsic tendency to grow and divide, to reproduce and propagate;[8] (ii) this intense activity would be an obvious source of novelties, which could eventually be kept in the system if they contributed to the far-from-equilibrium maintenance of a given type of protocell (including here the first point, too – i.e., *maintenance through reproduction*). Nevertheless, these mechanisms for preservation through statistical reproduction and generation of molecular novelties (that could be recruited for the protocellular organization) were probably quite poor during the initial stages. Under such conditions, the main "driving forces" for prebiotic complexification would depend on some specific boundary conditions (a range of temperatures, osmolarity, pH values, gradients, etc.) that could sustain protocell

[8] By "reproduction" (or "propagation" (Kauffman, 2000)) of an organization, we mean here the process through which a complex system (in this case, a protocell) generates physically detached similar systems (i.e., other protocells with a similar material composition and organization).

synthesis and dynamics, rather than on the robustness of their internal self-constructing organization or on their agency.

Anyhow, that incipient capacity for propagation of an organization could explain that, at a given stage, growth and fission led to the generation of protocells capable to reproduce through some primitive (still statistical, stochastic) mechanisms of transmission of their compositional and organizational identity (as suggested, for instance, by Segre and Lancet (2000) through their "composome" idea).[9] The iteration of self-reproducing cycles would generate a somewhat longer-term continuity of a specific *type* – the incipient lineage – constituted by populations of similar self-reproducing protocells. At the level of each protocell – as a particular *token* – the mechanisms involved in its reliable reproduction would trigger a diachronic succession of similar self-reproducing organizations, and in this way the innovations may have been retained beyond the particular fate of each individual protocell (Ruiz-Mirazo et al., 2020).

Hence reproduction (viz., growth and fission ending up in at least one new, physically separated, similar entity) would become the way in which the system displays its own far-from-equilibrium self-maintaining dynamics, and, at the same time, the consequence of these dynamics is the maintenance of a similar type of protocells (a particular "protocell lineage") through the continuity of generations. Interestingly, some proto-organismic innovations could be thus stabilized, and, even more importantly, this trans-generational continuity (type preservation) would also allow organizational changes, because it may have involved the accumulation of variations across long time periods. All this, of course, would be enhanced if molecular mechanisms to *record* (at least, to some extent) the increasing complexity of the protocell were developed, in parallel, at the molecular level (e.g., template mechanisms) giving way to progressively more reliable "hereditary" transmission of various features – even if the evolutionary (trans-generational) depth of these mechanisms would be rather small at those early stages (nothing comparable to later, genetic mechanisms).

The central issue here, in a situation in which nature must have faced a huge bottleneck (perhaps the biggest bottleneck it has ever faced), would be to develop material constraints that would enable these systems to solve two fundamental problems at once: (i) increase the robustness of the precarious individuals/agents and (ii) preserve the level of complexity they reach, in a way that is both operational for each individual, during its existence as a protocell (its "proto-ontogeny"), and for the collection of individuals it may bring about (its "proto-phylogeny" or "proto-lineage"). Solving these two problems requires obviously higher metabolic efficiency of the protocells, which is necessary both to ensure maintenance against perturbations and reliable reproduction. But the key is to realize that the solution is not at reach for any kind of metabolism: a remarkable threshold of molecular and organizational complexity must be reached, and systems below that threshold will

[9] This proposal was made in the wider context of a "lipid world" (Segré et al., 2001), some of whose assumptions we share, but some others we don't (in particular, the open-ended character of the evolution that such systems could implement – see: (Ruiz-Mirazo et al., 2008)).

naturally tend to decay. Von Neumann's idea of the "universal constructor," of course, resonates with force in this context (i.e., the problem of determining the logic of a system, the architecture of relationships among its operational modules, so that it builds itself, avoiding disintegration, across generations – see McMullin (2000) and Ruiz-Mirazo et al. (2008) for a more extended discussion). Yet the way this issue was originally posed avoided many aspects that had to do with the physical/material implementation of the systems that could become universal constructors, which might be crucial, as von Neumann (1966 [1948]) himself acknowledged. By focusing on the question of primordial biogenesis, we are precisely trying to naturalize the problem, going all the way back to its primary roots, and taking up a conceptual but unmistakably nonabstract standpoint.

Thus, as we were saying, evolutionary changes are the consequence of a long, historical series of causal actions performed by particular protocells belonging to a "proto-population" (or a family of protocells). Most of these changes (generated through a large number of reproductive cycles in a pool of similar systems) will be lost; but some variations in certain protocells will contribute to an increase in organizational integration and adaptive potential, generating more stable and somewhat deeper lineages (proto-phylogenies). In this way, trans-generational continuity may afford the maintenance and slow transformation of protocell lineages, facilitating the appearance of new protocellular types, whose organization is metabolically more efficient and has more and more control over external conditions. This is why reproduction with heredity is so important for the progressive complexification of such proto-organisms, allowing for their transition toward full-fledged biological organisms. Therefore, the evolutionary dimension indirectly (but with an increasing weight) affects the composition and organization of new generations of protocells, and more so as their reproductive capacities become more reliable, enabling higher and higher levels of sustainable organizational complexity.

In Sect. 9.4, below, we will give a rationale about the actual transition from these initial stages toward a situation in which the interweaving between the organismic and evolutionary dynamics becomes really profound, inextricable, as it is necessary for the unfolding of biological phenomena. But, once the complementarity between these two dimensions of life has been brought to the fore, let us say a few more words on the asymmetry involved in their relationship. Metabolic organization (the core of the individual dimension) is run and maintained in each (proto-)organism through a set of "rate-dependent" causal connections (Pattee 1977): namely, causal connections that crucially depend on specific conditions of distance, velocity, and energy requirements. In Ruiz-Mirazo et al. (2017), following Ruiz-Mirazo and Moreno (2004), we propose a minimal set of (first-order) control mechanisms that would be necessary to keep basic autonomous systems, like these protocells, in far-from-equilibrium conditions (kinetic, spatial, and energetic control mechanisms, more specifically). In any case, what is important to highlight for the discussion here is that this kind of organization can only stand robustly and efficiently on its own feet if, and only if, its constituent parts are highly *integrated*. The idea of organizational integration (as it was discussed in the first part of the chapter) expresses the fact that the different parts and processes of a system are highly interdependent:

there is a need to coordinate the distances, times, rates, and energies involved in all of them. And when the system's complexity increases, the need to introduce regulatory mechanisms that reorganize some parts in differentiated levels (constraints on top of constraints) also becomes apparent (again, see Sect. 9.4, below, for further explanations).

Thus, the pressure for integration is inherent in any system whose identity is based on a far-from-equilibrium, cyclic set of synthetic processes (always coupled to matter and energy sources from the environment), namely, on a logic of self-construction that depends on the specific energy requests and the actual rates of their (always precarious) constitutive/interactive dynamics. That is why such systems cannot increase in complexity unless they enlarge the web of endogenous (higher-order) constraints and their assorted integration – including mechanisms to control the relationship with the environment (which will lead to the development of minimal forms of agency). In sharp contrast with this, the maintenance of an evolutionary process, per se, is much less demanding. Or, rather, it is demanding but in a completely different way: what matters there is the reliability in the transmission of constraints across generations, within the dynamics of populations of reproducing systems, all of which is averaged out in a very long and complex sedimentation process. In this context, part of the causal connections operate as if they were "rate-independent" (Pattee, 1977), even if they must be continuously supported by the set of (rate-dependent) cyclic causal connections that constitute, maintain, and reproduce each protocell in far-from-equilibrium conditions. As the mechanisms of heredity (or "control on variability" (Ruiz-Mirazo et al., 2017)) become more and more reliable, the relevant historical series (i.e., the "trans-generational depth" or the average number of generations through which those constraints do no suffer relevant changes (Danchin et al., 2019)) becomes longer, more relevant, and profound in evolutionary terms. This has very important implications for "open-ended evolution" (Ruiz-Mirazo et al., 2008), as we will recall in the next section, but a fundamental related issue must be explicitly addressed first: functional expansion and diversification.

9.3.1 Trans-generational Constraints and the Expansion of Functional Space

The emergence of a functional domain (a world where material systems exist by virtue of what they do – i.e., by virtue of their dynamic causal effects (Mossio et al., 2009; Moreno & Mossio, 2015)) is important in this prebiotic context precisely because it is behind the key fact that during biogenesis chemical diversity gets reduced and narrows down to a relatively small subspace of "the molecularly possible." As the rich mess of prebiotic processes and material transforms into more elaborate chemical organizations, a progressive selection takes place, favoring those molecular components capable of putting together cohesive far-from-equilibrium systems. Regardless of the time this may take, only those components that have

allowed further complexification will be retained, and that has some important implications. In particular, it means that the chemical diversity will suffer a significant decrease, as this is a condition for systemic and highly integrated material organizations. The development of the necessary mechanisms of control (spatial, catalytic, energetic) actually requires fixing some of the molecular rules and components operating in these systems. More specifically, a subset of chemicals and reaction processes must be chosen both to generate components of control (internal constraints) and to be amenable to that autonomous control.

This, in our account, coincides with the emergence of "minimal metabolisms" (Lauber et al., 2021) and is actually the first moment in natural history where one can begin to speak properly in terms of *functions*, the claim being that these don't emerge "one-by-one" (Ruiz-Mirazo et al., 2017): a combination of endogenously produced and tightly coupled constraints (operating as first-order control mechanisms on the underlying, far-from-equilibrium reaction network) must come together, from the very beginning, so as to constitute a minimally robust chemical system, similar to the protocell systems that we described at the end of Sect. 9.2. Therefore, the basic idea of "functional organization" (as an enduring form of self-maintenance) is deeply linked to that of material control and organizational integration. In this context, we should remark that the appearance of self-reproducing protocells already requires, as a precondition, the existence of populations of protocells with – still strongly limited but – nontrivial functional domains. The reason is that the reproduction of a protocellular and minimal metabolic organization involves managing quite a number of processes, like the duplication of certain structures of the system, coordinated with surface increase (and other modifications) in the compartment, as well as with an adequate temporal and spatial allocation of the components during growth (so as to ensure that, when fission actually occurs, the new entity is able to repeat a similar self-productive cycle). In other words, reproduction requires a fair degree of control of the proto-metabolic processes, since growth and fission are the specific expression of the self-production regime of these protocells (Mavelli & Ruiz-Mirazo, 2007). One could say that spatial, kinetic, and energy control mechanisms, including the suitable coordination among them, constitute the necessary functional basis for any reliable trans-generational propagation of protocell organization (Moreno, 2019).

One should also recognize that the very idea of reproduction (see footnote 8) implies, right from the start, a minimal degree of reliability or "inheritance," namely, the new, spatially separated entity has to be molecularly and organizationally similar to the parental entity. The first forms of reproduction would have been statistical, which means that similarity among the different members of the progeny was ensured only partially – at some percentage, so to speak. As it is argued in (Danchin et al., 2019), an increase in the reliability of reproduction was most probably a consequence of a stronger degree of functional integration, and this, in turn, through reproductive steps, resulted in the selection – or sedimentation – of the most efficiently integrated protocells, which nicely illustrates how the aforementioned link between evolutionary and physiological (proto-metabolic/protocellular) processes can be, in practice, coherently articulated.

Conversely, the incipient connection between these two phenomenological dimensions (that will develop and get reinforced throughout primordial biogenesis) has really interesting and far-reaching consequences with regard to the functional domain itself, which can expand through novel ways of contributing to maintenance that become available to those protocellular populations. Indeed, such a connection opens the door to a completely new set of functionalities, which lie beyond the strictly physiological sphere of each protocell. A function in this extended functional domain can acquire "temporal/historical depth," in so far as some feature/property of the system is linked to the new ways of ensuring organizational maintenance across generations. This allows to establish a natural conceptual bridge between the *organizational* and the *evolutionary* interpretations of function (Saborido et al., 2011). Indeed, from this stage onward, it makes sense to say that a trait (a component, a mechanism, a property of the organization) X in a population is there because it has been selected/sedimented through a complex evolutionary pathway. In other words, in our prebiotic context, X would be there because those protocells that bear X – and were capable to transmit it to their offspring – have a (relatively long-term) history of reproductive success through which X remains in the population. In this sense, we must open ourselves to the possibility that there are functions whose contribution to the current individual organization of the system is not so obvious, and they should be analyzed in a wider time frame, i.e., there could be functional traits that contribute to the maintenance of the *type* and, thus, only indirectly to the maintenance of any particular *token*.

Let us explain how, in just a couple of paragraphs, before moving on. As we have discussed, the possibility that some protocells managed to achieve relatively reliable reproduction cycles would depend critically on the synthesis of a number of material constraints controlling the processes of growth and fission. Certainly, there would be an organizational and material continuity between the initial, "mother protocell" and its subsequent offspring and, in this sense, the functional role of the constraints more specifically involved in the reproductive processes would not be distinguishable, in principle, from the nonreproductive functions. However, more and more reliable self-reproductive systems require additional control mechanisms: in particular, hereditary mechanisms that should be focused in managing the variability generated in these protocells, preserving the level of complexity reached and making "statistical numbers," so to speak, "no-longer-statistical." But this, in turn, requires, as we will expand in the next section, a *dynamic decoupling* with regard to the current organization and the specific times, rates, and energies required by each individual metabolism. In a concurrent way, the organizational architecture of the protocells must be profoundly modified, through a *hierarchical coupling* with these new mechanisms that can no longer be considered, simply, as the result of the constructive power of each individual but rather as the result of a much more complex evolutionary process in which whole populations are involved.

Somehow, we are facing a scenario in which the organization of individual entities transforms the way in which evolution occurs and evolution also transforms, more and more profoundly, those individuals. But what should be especially underlined here is that all this takes place in the context of – and thanks to – the capacity

of these protocellular systems to enlarge and diversify, enormously, the space of possible functions though which they are realized (starting from that initial, minimal set that we mentioned above). Although a good part of such a space will be filled by strictly physiological control mechanisms, some other regions will not simply belong anymore to individual "tokens" but to the "types," the lineages, that consolidate through longer-and-longer-term population dynamics. Therefore, the main problem at this stage (and from this stage onward) is not dealing with chemical diversity, heterogeneity, and messiness (what is classically regarded as the combinatorial explosion of molecular interactions and transformations) but dealing with an increasingly rich space of functionalities, expanding in different – though interconnected – directions. A fundamental issue will be addressed next, in Sect. 9.4.

9.4 "Dynamical Decoupling": A Key Principle to Understand the Evolutionary Development of Complex Material Organizations

Taming complexity in systems that develop numerous functionalities and thus, a large space of possible dynamic states/behaviors is not a trivial task. These systems can realize in multiple ways their basic constitutive regime, as self-constructing protocells (minimal metabolisms) in constant interaction with a variable environment, shifting from one stationary state to another, depending on the conditions that they meet at any given time. More precisely, dynamic multistability poses a remarkable organizational challenge in an evolutionary setting like the one we just described above: the challenge of how to navigate efficiently that space without wasting time and resources that could be critical for the persistence of the individuals involved. This is a problem that cannot be taken for granted, nor assumed to be spontaneously solved by nature: it requires work, literally (viz., in a thermodynamic sense), and time, plenty of time, to develop the necessary mechanisms. In line with other authors that have previously addressed it (in particular Christensen, 2007), we consider that simple feedback mechanisms, or even combinations of positive and negative feedbacks, if they work "online" (at the same rates/conditions in which metabolic processes take place), are not sufficient to deal with it. Let us try to explain, briefly, why.

In principle, when facing perturbations, a functional system can restore its constitutive and behavioral coherence through *self-organization*[10], namely, through parallel local interactions that generate emergent outcomes (without making use of specifically devoted mechanisms of regulation or higher-order controls). Yet, this solution only works when the number of different functions to be coordinated is not very high. As the complexity of a system increases, these dynamically coupled

[10] In the context of our discussion here, this would apply to *minimal metabolisms (*i.e.*, basic "self-construction").*

("online") mechanisms become clearly insufficient. As Christensen (2007) rightly points out (reasoning in a cognitive context but using arguments that are perfectly applicable at a much more basic level), self-organization has intrinsic limitations to achieve functional coordination. The reason is that the process of reaching a certain global state, in such a case, depends on the reliable concatenation of state changes through local interactions, and those cascades of events add a delay as the functional diversity of the system increases. Nevertheless, robust global coherence/behavior against variations requires selecting very precisely a given dynamical attractor and maintaining the system there during a given period of time. Therefore, if the organization of the system stays "flat" and "online," it faces an obvious dilemma: either its capacity to generate multiple finely differentiated global states is limited, or, instead, the system will have to sacrifice the reliability of attaining a specific state out of that multiple choice. In Christensen's own words, «slow action and poor targeting capacity severely limit the capacity of self-organization to achieve the kind of coherence that functional complexity requires (…) Consequently, the most effective means for achieving the type of global coherence required for functional complexity is through regulation, including feedback mechanisms and instructive signals operating at both local and larger scales. The key feature that distinguishes regulation from self-organization is the presence of a functionally specialized system that differentially specifies one or a restricted set of states from the range of possible states the regulated system might take, based on the sensing of system conditions and the production of control signals that induce changes in functional state» (Christensen, 2007, pp. 265–266).

In Bich et al. (2016) we argued, precisely, that (biological) regulation involves second-order control hierarchies that necessarily work "offline" in a relevant sense, or to a relevant extent. In other words, achieving effective control when the complexity of a system is very high requires a subsystem that is endogenously synthesized but operationally decoupled from the dynamics of the controlled processes, so that it can be modified without disrupting those underlying synthetic processes (Bechtel, 2007; Bich et al., 2016). Minimal metabolisms, as generally characterized in Lauber et al. (2021), do not constitute completely "flat" organizations, in the sense that they do require first-order controls (i.e., a set of elementary constraints) to operate. But regulation involves constraints on constraints, which make decoupling mechanisms effectively feasible in an autonomous organization. Basic, first-order controls are required to put the system together, but they are too closely engaged in the metabolic dynamics to be able to work "offline." The question that we must address here, in any case, is why minimal forms of regulation, interpreted precisely in this vein (i.e., already implying a *dynamically decoupled* but *hierarchically coupled* individual system organization), were necessary during primordial biogenesis and how they were actually implemented in the (pre-Darwinian) evolutionary context of protocell populations described above.

As for the first point, we concluded the previous section highlighting that self-reproducing protocell systems demand, right from the beginning, a rather elaborate set of basic functions (those first-order, material constraints acting as "process controllers": catalysts, compartments, etc.) just to realize themselves and that the

prebiotic evolutionary dynamics they bring about would contribute to expand their potentially available space for functionalities (including trans-generational constraints – such as hereditary mechanisms of various kinds). We consider that this hypothetical but plausible protocellular scenario is, indeed, complex enough to defend the need for second-order control mechanisms that help those systems navigate an internal dynamic space with multiple stationary states. The reason why such mechanisms should be considered as "second order" is because they must operate *on top of* the basic set of functions that already put together the constitutive regime of the system, with a variety of accessible dynamic attractors. In brief, the new controllers must be constituted by material constraints operating on other material constraints: there is no other way for nature to do it. And the reason why this action is "offline" has to do with the second point, which we must address now: how were such *hierarchical* autonomous organizations (Pattee, 1973) actually implemented for the first time?

In concrete operational terms, regulation is commonly understood as the harnessing of a system according to a set of rules (e.g., «in case of situation X, do Y»). Contrary to what occurs in artificially designed systems, where the rules are a collection of external norms, in natural (biological or infra-biological) systems, the idea of regulation points to an internal set of constraints that *functionally select* some specific dynamical configuration of the system, among several possibilities, as we expressed above (and as some other authors have also argued, to distinguish this estate of affairs from strict or minimal autopoiesis (Di Paolo, 2005)). Yet, what kind of "function" is this? In principle, it looks physiological, difficult to distinguish from the other, elementary ones – since it is exerted in ontogenic time scales, as an adaptive response of the individual, here and now, to a given environmental challenge: e.g., «if this nutrient is detected, swim up its gradient» or «if this toxin is found, do not absorb it». Nevertheless, these behavioral *shortcuts* are quite more complex than direct controls on a process. In fact, when one thinks carefully about their emergence, they cannot be easily understood outside an evolutionary perspective: regulatory mechanisms definitely seem to require a different time scale to appear and get stabilized in the population. They look anticipatory, when they are analyzed at the scale of a single individual – who "seems to know," in advance, the outcome of its actions. Instead, these self-imposed instructions most probably come from a history of interactions that have taken place in the population and are linked to the persistence of those individuals, but throughout many generations.

As we explain in more detail in Bich et al. (2016), the nature of regulatory mechanisms is not straightforward: they involve material gears to shift from one constitutive regime to another depending on circumstances that must be associated to internal/external variables but without responding directly (through online mechanisms) to those actual variables (e.g., the concentrations of metabolites in the system). The system must "detect" internal/external circumstances selectively, which means distinguishing some inputs as "signals" that will trigger a rapid shift (the adaptive shortcut) to a given behavior (an alternative stationary state). Thus, it is not easy to describe in detail how these regulatory meta-constraints (including the molecular machinery that determines accurately when and how their action should

be executed) could have appeared. Further empirical and theoretical research needs to be carried out on this topic, within a pre-Darwinian evolutionary setting where different stages are distinguished and compared to the pre-regulatory (i.e., minimal metabolic protocell) phase. Yet, it seems quite reasonable to conjecture that regulatory mechanisms should be the result of a long series of "trials and errors," in the context of protocell population dynamics in which subsequent generations of prebiotic individuals were developing, competing for resources, probing their local environments, etc. How it actually happened, putting all the pieces of the mechanism together, avoiding potential disintegration pathways, overcoming external perturbations, and keeping internal coherence, will not be obvious, but if it came about, the regulatory device would for sure be retained, because of its immediate contribution to the persistence of those protocellular systems that integrate it in their organization.

Interestingly, regulatory mechanisms may constitute one of the most prominent pieces of evidence to demonstrate that a sedimentation process is taking place at larger and longer scales, with very important implications at the level of the individual, here and now. The history of interactions of a population of similar protocells with their environment (including the interactions among them) gets eventually distilled or condensed into a relatively complex, built-in mechanism that ensures higher robustness and better adaptivity (quicker responses) by the members of the population to certain variations in the medium. In other words, regulatory metacontrols somehow reflect, also due to the intrinsic dynamical decoupling they involve, the interweaving between the physiological and the evolutionary dimensions of biological (in this case, proto-biological) phenomena. This interweaving is asymmetric, as we discussed in Sect. 9.3, because the physiological sphere always has causal priority (real self-constructing individuals are the material agents performing all relevant interactions, after all) even if the evolutionary sedimentation process, working at larger and longer scales, has a deep impact on the physiological mechanisms and organization of the resulting individuals.

However, regulation by itself is not enough to ensure reliability in the transmission of increasingly complex molecular and organization features to the offspring (including the regulatory apparatus itself, which could also face the risk of getting lost on the way). Hereditary mechanisms must be specifically developed for such a fundamental task. The conservation of system features across generations can be implemented through different means (and then interpreted according to different theoretical frameworks – e.g., Bonduriansky and Day (2018) or Mossio and Pontarotti (2020)), but we are particularly referring here to *molecular records* (Pattee, 1969, 1977) that, through their template properties, are capable of replication with conservation of their monomeric sequences. These hereditary mechanisms would be completely futile if they were not linked to concrete functionalities of the protocells in evolution, in either metabolic or global reproductive terms. Under the hypothesis of an "RNA world," the same kind of molecule could be carrying catalytic power ("proto-phenotype") and replicative potential ("proto-genotype"), but such a reductionist interpretation does not fit in our account. From a more encompassing organizational perspective, like the one we embrace here, the phenotype-genotype mapping would be quite more complex, right from the beginning.

In such a context, as primordial biogenesis proceeded forward, protocells would inherit, among many other components, molecular records whose functionality would be the result of a longer and longer evolutionary process, beyond the "life span" of each of them. At the same time, each hereditary record would play a key causal role in the metabolic organization of the protocell where it exists. In other words, a trans-generational constraint of this kind also embodies two different temporal scales: one that corresponds to its causal activity in the current physiological processes of the protocell and another one that corresponds to the long evolutionary history that has shaped the specificity of its functional sequence. Thus, hereditary mechanisms bring some other kind of dynamical decoupling into these systems. Records are *dynamically decoupled* from (but *functionally connected* to) the metabolic organization because (like regulatory mechanisms) they have been shaped in a different temporal and spatial domain. But in contrast to the rest of the system components, which functionally depend on each other (in the sense that the effects of some components generate and transform the others), hereditary records are not strictly generated within the metabolic organization of each protocell (although they are physically constructed, repaired, and replicated by it). More exactly, they are materially regenerated, preserved, and used within that metabolic organization, but they are not *informationally* generated within each protocell. And yet, it is precisely for this reason that the specific sequence of those hereditary components – what will come to be their "informational content" – allows for a much more robust and efficient mechanism of reproduction, even if the complexity of the metabolism would be much higher at these later stages. Eventually, "genetically instructed metabolisms" would introduce a completely different way of exploring innovations and variation in time: "open-ended evolution" (Ruiz-Mirazo et al., 2008).

As hereditary mechanisms (and phenotype-genotype mappings, in general) develop in protocell populations, regulation can also be applied, in turn, to all the processes in which those material records (which are meta-constraints, too, of course – but with their own specificities (Pattee, 1977, 1982)) are involved. In fact, the constructive and transformative power of combining these two different modes of dynamical decoupling, as it is reflected in the basic organizational architecture shared by all living beings (prokaryotic cell metabolisms, already endowed with a translation apparatus and a common genetic code), was surely crucial to reach the "hypercomplexity" that life required to maintain itself on the surface of the Earth in the long run (a situation that, we guess, should be similar anywhere in the universe, since the problems addressed here would apply to any material organization dwelling close to the von Neumann threshold).[11]

[11] A more extended analysis and conceptual reflection on these issues lie beyond the scope of this contribution but should be an interesting topic for future work.

9.5 Concluding Remarks

The aim of this chapter has been, as indicated in the title, to provide a theoretical framework, a plausible and reasonable account to understand how a biological domain could unfold from complex, nonliving matter. We are convinced that such an intricate transition must be a very long process, involving myriads of molecular systems that generate a great diversity of reaction networks which, over time, lead to increasingly complex material organizations. As this process of biogenesis proceeds, something quite intriguing happens: the phenomena taking place at a given stage, being based on the previous, somehow manage to redefine the conditions, the rules of the game, bringing about systems/organizations that overcome in efficiency and performance the preceding ones and ruthlessly eradicate the latter, leaving no traces behind. However, at a given stage, things radically change: systems sharing an organization with a set of fundamental features similar to what we call nowadays "prokaryotic life" come about and that evolutionary dynamics of "continuous substitution of the old by the new" stops. Not only because there is an unprecedented explosion of diversity and proliferation of these systems (probably all over the surface of the planet) but also because, from that moment onward, subsequent organizational innovations do not (perhaps, cannot) erase this basic type of organization. Instead, all novel biological complexifications become dependent and supported by prokaryotic life – they become, so to speak, curlicues, "convoluted redefinitions" of that same type of phenomenon. Hence, the target of any theory of primordial biogenesis should be to explain how such a fundamental but far-from-trivial material organization (genetically instructed metabolic cells) could naturally emerge.

Within this general context, we have focused the discussion on several key issues. The scientific work reviewed in Sect. 9.2 was mostly related to the early stages of the process: in particular, we collected evidence on how under favorable environmental conditions catalytically driven sets of reactions could turn into self-sustaining protocellular systems, which probably constitute, at those first steps, just a mess of growing and shrinking individuals, only later leading to more sequential fission and fusion events. Then, in the following sections, we explained how, over time, some of such protocells could manage to reproduce their characteristic type of organization, opening in this way a completely new scenario, which has not been explored empirically yet. On the one hand, more and more integrated functional systems (protocellular *individuals* of higher complexity) should start developing. But, on the other hand, lineages of different families of protocells (evolutionary *populations*) would also begin to form. These two apparently orthogonal dimensions of the phenomenon, unfolding in very different scales (both spatially and temporally), get nevertheless deeply entangled. And, through that entanglement, a really powerful driving force is generated that overcomes the apparent physical and material bottlenecks present at those stages, bringing about much more integrated protocells. In turn, these new protocells would not only be more robust but also capable of more reliable reproduction, which would then increase the weight of evolutionary aspects in the process.

Finally, we also discussed the importance of having protocells that develop hierarchical relationships within their organization, namely, complex functional mechanisms that operate on top of the (first-order) controllers of metabolic processes (i.e., only indirectly on metabolism), at rates significantly different from the ones involved in those basic transformation processes, and thus, look as if they were working "offline." Embodied in two very different modes, regulation and heredity, this *dynamic decoupling principle* also seems to play two complementary roles in prebiotic evolution: in the first case, enhancing individual (i.e., ontogenetic) adaptiveness, and in the second, increasing lineage (i.e., phylogenetic) fidelity. Nevertheless, both modes (an effective combination of the two, more precisely speaking) are apparently crucial to complete the process of primordial biogenesis, leading eventually to complex material organizations similar to prokaryotic cells. The physiological plasticity of these cells, together with their capacity for open-ended evolution, lies at the heart of the impressive robustness and long-term sustainability of the phenomenon of life. Modeling all these prebiotic transitions, from initial families of minimal metabolic protocells, to full-fledged living organisms (individuals with a translation apparatus, a complex, code-mediated, phenotype-genotype mapping, etc.) is still a great challenge for science. But making the challenge conceivable, under realistic assumptions, is a first, necessary step to tackle it.

Acknowledgments This research work has been supported with grants from the Basque Government (ref.: IT 1228-19, recently extended to: IT1668-22) and the Spanish Ministry of Science and Innovation (PID2019-104576GB-I00), as well as from the John Templeton Foundation (Project Title: "Integration and Individuation in the Origin of Agency and Cognition" – Grant 62220). KR-M also takes part in a Horizon 2020 Marie Curie ITN ("ProtoMet" – Grant Agreement no. 813873) from the European Commission. We would like to thank Cliff Hooker and Juli Peretó, as well as the editor of the book, Matteo Mossio, for carefully reading a first manuscript of this contribution and providing critical feedback that certainly helped us improve it.

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