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The challenging biology of transients

A view from the perspective of autonomy

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Contemporary biology struggles to advance a systems' perspective to explain global emergent phenomena and, ultimately, how certain systems become alive. The production and study of transients that are somewhere between the inert and the living has become an important scientific goal, which is sometimes associated with the aim of designing living systems for practical application. This sets an additional difficulty to the already challenging task of defining what life is, as the research fields that are focused on such transitions need to consider that there are intermediary steps between non-life and life, which are either gradual or punctuated. However, if the intention of building artificial living beings prevents us from thinking that there is a difference between life and non-life, the scientific question of what life is could become meaningless.

A recent Editorial in *Nature* commented as follows: "There is a popular notion that life is something that appears when a clear threshold is crossed. One might have hoped that such perceptions of a need for a qualitative difference between inert and living matter—such vitalism—would have been interred alongside the pre-Darwinian belief that organisms are generated spontaneously from decaying matter" (Anon, 2007). Similar views claim that our definitions of life are historical, and are moulded by convictions or scientific practices that do not necessarily reflect natural categories.

Conceptual changes in science affect the kinds of phenomenon that are brought to view. The notion of life emerged historically as a shift of perspective in the classification of nature and initiated the field of biology—as Michel Foucault showed, life does not establish an obvious threshold in nature but rather is a category adopted only at the end of the eighteenth century (Foucault, 1966). Owing to this fact, some authors conclude that life is not a natural kind to be unambiguously characterized by science (Keller, 2002), but rather that different scientific fields might define life and identify living beings in diverse ways (Dupré, 1993; Dupré & O'Malley, 2009). Although we understand and even share some of these arguments, we believe that the investigation of transient systems needs to be guided by at least an intuitive idea of the difference between the inert and the living.

Clearly, the difference between what is life and what is not life has also changed with the advance of science. For a long time, especially in antiquity, when life had total primacy, the opposite of the living was 'dead'. Later, it changed to simply 'inorganic', reflecting the fact that during the twentieth century the question of what is life mostly concerned physicists, who sought to understand the peculiarities of living matter, as opposed to the inorganic (Keller, 2009). Today, the opposite of life is generally 'inert', which is a category that includes organic materials. The difference between the inorganic and the inert reflects an increasing awareness that life is so complex that the scientific study of transients cannot attempt to start from raw inorganic

materials, but rather needs to begin with organic compounds and processes to study how these, or similar alternatives, are produced in living systems and the laboratory. This topic has now become interdisciplinary, involving teams of biologists, engineers and computer engineers.

However, the distinction between the physical and the biological perspective of life raises the problem of how to bridge the gap between the physics of self-organizing patterns and the biology of organisms. To understand systems as alive—rather than as mere collections of components or processes—biologists seek a functional understanding, according to which the global properties of the system depend on an organization of parts causing one another to function. Yet, models of self-organizing networks lead to emerging global patterns without apparent function (Keller, 2007), whereas designing models with externally assigned functions is too arbitrary to be able to explain the systemic properties of biological phenomena (Krohs & Callebaut, 2007).

The usual analytical approach does not provide an adequate perspective from which to study life as an organization of material processes. The French philosopher Georges Canguilhem (1904–1995) commented as follows: "We suspect that to do mathematics it is enough to be angels, but to do biology, even with the help of intelligence, we sometimes need to feel like beasts" (Canguilhem, 1969). For Canguilhem, biology as a form of knowledge requires analysis; however, once isolated, parts cannot provide the kind of intuition or empathy that one living being experiences for another. This difficulty may be assumed and tackled with the development of synthetic approaches in biology that aim to understand life as autonomy, which is to say, as the complex material organization that is responsible for the phenomenology of the living.

The study of transitions from inert matter to life establishes a new challenge for biology. It has produced a range of synthetic material configurations that are intermediates between the physical and the biological, which we have termed transients. In fact, any scientific approach to life and its origins requires accepting the 'hypothesis of continuity' (de Duve, 1991; Morowitz, 1992; Fry, 1995) and, therefore, that certain boundaries will become fuzzy or vague. But a focus on autonomy allows one to keep an eye on the organization and determine the sense in which transitions actually occur.

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At present, the following four fields of scientific research are exploring biologically relevant transients in one way or another: 'origin of life', astrobiology, 'artificial life' and synthetic biology. Although it is difficult to make generalizations, the first two fields are interested in the physical/chemical processes that generate organic matter and more complex molecules (for example, biopolymers), which are believed to be a part of living systems on the Earth or other planets. Methodologically, they are relatively traditional, with a strong emphasis on 'wet' *in vitro* experiments.

'Artificial life' and synthetic biology have larger and more challenging goals, at least in terms of producing complex transients. These fields seek to expand the domain of biological phenomena in different directions and use novel synthetic methods to create new kinds of entity. Such entities, regardless of the motivation behind their development, constitute tentative intermediate systems between the inert and the living, or are simplified versions of extant living organisms. The different transients produced by these two more provocative research fields can be distinguished as the result of either a construction from scratch or an intervention in already existing life.

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The first approach—construction from scratch—aims to build systems from elementary components that show some of the properties of living cells. To do so, it is crucial to choose—or design—the initial constituents and the experimental conditions to generate self-organizing or self-assembling dynamics. It is also critical to decide how elementary these units are—monomers (such as lipids or amino acids), macromolecules (including proteins and nucleic acids) or more complex parts (such as ribosomes, plasmids, full genomes or cell-free systems), because this choice determines the level at which the self-organizing or self-assembling takes place. Strictly speaking, bottom-up approaches should ideally start with the most basic units, the monomers, and construct the rest from there. However, although living cells are doing it all the time, this has not proved easy to achieve *in vitro*, except in relatively simple cases, such as the formation and reproduction of lipid vesicles (Hanczyc *et al*, 2003).

Many researchers have managed to combine the spontaneous self-assembly of lipidic molecules with other processes in which—previously or independently synthesized—macromolecules are involved. Following such 'semi-synthetic' (Luisi *et al*, 2006) or 'reconstructive' (Solé *et al*, 2007) strategies, liposomes are being extensively used as containers for a wide range of biochemical reactor systems, from gene- or protein-expression kits (Nomura *et al*, 2003) or DNA template-directed processes (Mansy *et al*, 2008) to full bioreactors (Noireaux & Libchaber, 2004). The large number of these mixed experimental approaches shows that there are important hurdles in the construction of whole-protocell systems from scratch, probably because achieving the right combination of self-assembly and self-organization processes, as it is realized in all living systems, is no trivial task.

Different 'shortcuts' are therefore being tried to obtain functionalized protocell systems (Luisi *et al*, 2006; Solé *et al*, 2007; Rasmussen *et al*, 2008), under the assumption that the minimal properties of a living cell can be summarized in a few basic functionalities. In general terms, these protocellular transients or "unfamiliar forms of life"—as Steen Rasmussen and colleagues call them—exhibit one or more of the following capacities: containment, reproduction and/or metabolism (Gánti, 2003). What remains an open issue (Szathmáry *et al*, 2005) is the order in which these should appear and, again, how to build the whole lot from scratch.

The second main research line, which is focused on the creation of alternative cells or subcellular engineered devices, seeks to modify extant forms of life, in particular by acting on their genomes. One of the main scientific tasks in this regard has been to determine the minimal genome that is able to support life, for instance, by

knocking down auxiliary genes in some of the simplest free-living organisms, such as *Mycoplasma* (Hutchinson *et al*, 1999). Changes at the level of whole genomes are also being induced with more practical and control-oriented purposes (Chan *et al*, 2005; O'Malley *et al*, 2008). The idea is to create a simplified and standardized host cell—a so-called 'chassis'—to make the implementation of new devices, so-called genetic circuits, easier and more effective than in a more complex living system.

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In any case, most of the top-down intervention work on cells involves modifications at the level of DNA and other macromolecular structures that affect gene expression. This part of synthetic biology resembles its precursor, genetic engineering; however, both the techniques and the knowledge used have established significant differences between the two. In particular, synthetic biology is not just about changing or introducing a few genes and analysing their effects on the metabolic activity of the cell; it is not even about artificially synthesized bases, orthogonal aminoacyl–tRNA synthetases or some unnatural amino acids, all of which are already being employed. The main goal of synthetic biology is the ability to manipulate the architecture of genetic networks, and to design and fabricate new circuits and interacting modules; this accounts for the importance of standardizing biological parts—BioBricks™ (Endy, 2005)—that can be rationally combined to build a 'bio-machine'.

Again, these are usually mixed 'semi-synthetic' strategies because, even though this new type of research cannot be done without the use of living cells, it still aims to produce *de novo* organized systems through constructive bottom-up steps. Nevertheless, these approaches do not, and might never, go 'all the way to the bottom', as that is not their objective. Even the synthesis of whole genomes from synthetic oligonucleotides (Smith *et al*, 2003) is not the same as a system that self-assembles and self-organizes from different types of monomer.

Consequently, the two main directions in which to explore biological transients remain distant from one another; there is still a big jump from the complexity that results from attempts to start from inert components to that arising from modifying extant living systems. This means that computational models, which are already instrumental to these new approaches, will be of even greater importance in the future. For instance, models might help to investigate the ways in which self-assembled structures can benefit from the formation of far-from-equilibrium self-organizing patterns generated around them, and, vice versa, how self-organized dissipative structures can become robust in the presence of more stable self-assembling units and aggregates. When these two types of process, self-organization and self-assembly, come together under the right conditions, it is highly probable that a completely new type of transient—closer-to-autonomous—will emerge.

The idea of autonomy is relevant in this context because it points to the missing link between physics and biology; it provides a framework to account theoretically for the phenomenon of life as a state or form of organization. As a prominent precursor of this way of thinking, the theory of autopoiesis (Varela *et al*, 1974; Maturana & Varela, 1980), coming from the tradition of cybernetics and systems science, understood life as an arrangement of processes to produce components enclosed within a boundary or membrane, which was also of its own making. This theory aims to bring forth an organization, the operation of which will appear as living to an observer, because it results in the continual regeneration of the system, actively distinguishing an internally constituted 'self' from the environment. However, autopoiesis was conceived as an abstract machine—independent of the nature of the materials constituting the process—and a largely idealized one at that, as it fails to meet the thermodynamic criteria required to maintain an ongoing far-from-equilibrium activity (Ruiz-Mirazo & Moreno, 2004).

The notion of autonomy is appealing for characterizing life owing to the systemic and organizational view that it introduces. The study of transients, however, requires exploring forms in which autonomous organization is produced materially. This obliges us to take into account how the material constituents themselves are informed, as well as the role of historical contingencies and natural selection in shaping the most basic functionalities of life—such as metabolism or heredity (Lazcano, 2008). If material, historical and evolutionary aspects must be included in the study of autonomous organization, it cannot be readily assumed as an *a priori* precursor—because it might well be an *a posteriori* consequence—of material evolution.

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The perspective of autonomy also needs to take into account the role of the environment, not only in the sense that it should warrant viability but also to reflect the fact that an autonomous system reacts to, and within, the environment as an agent (Kauffman, 2000); autonomy should not be misinterpreted as independence. Related to this, autonomy has largely taken an individualistic point of view, which contradicts the complex web of interactions—parasites, symbionts and so on—that constitute the complete metabolism of living systems (Dupré & O'Malley, 2009). Then, a significant issue is whether the first or most basic living beings emerge as autonomous individuals, as we usually think of life, or whether cooperative relations or associations among transient configurations gave rise to the appearance of individuality in evolution.

Despite these remaining difficulties, the concept of autonomy brings forth a pertinent scenario for inquiries about the nature of life, which is lacking in other approaches. For constructive approaches, it sets the goal of achieving a lifelike self-producing complex metabolism; for intervening strategies, it discourages attempts to understand living organisms as straightforward machines. By examining transients from the perspective of autonomy, we can take advantage of a systemic framework to judge attempts to create artificial life.

Contemporary science still aspires to understand the nature of life. Transients are

challenging because, by exploring the intricacies and ambiguities of the distinction of life and non-life, they may lead us to conclude that there is no frontier. If we accept this conclusion, the various successes in creating artificial life, as reported these days, cannot be significant; if we reject this conclusion, transient systems explore intermediary steps starting from two edges that have not yet met. Bottom-up constructs do not reach the living end, whereas interventions in existing cells at most preserve life, rather than create it.

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At least since the 1970s, theoretical biologists have considered that in order to understand complex systems, it is necessary to develop a pluralistic framework of alternative models (Rosen, 1991; Pattee, 2007). As the British biologist Conrad Waddington (1905–1975) once said, "science is not, after all, merely a one-eyed Cyclops […] man is Argus with innumerable eyes, all yielding their overlapping insights to his one being, that struggles to accept them in all their variety and richness" (Waddington, 1969).

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