Complexity and Analogy in Science
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Life's Biochemical Complexity

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"In vain we force the living into this or that one of our moulds... Our reasoning, so sure of itself among things inert, feels ill at ease on this new ground".
Henri Bergson, Creative Evolution.

Introduction

Since our childhood we have been able to sense that the world that surrounds us comprises living things and inert objects, which usually differ from each other by qualities that seem easily recognisable. Thus, we can distinguish that a plant, a fish or an insect belong to the first group of entities, while any liquid substance, air, stone or machine belong to the second.

Biology, with its numerous subdivisions (ecology, physiology, genetics, biochemistry, etc.) is the science that studies living beings. Despite the extraordinary advances of this science in both basic and applied (biotechnology), it is paradoxical that the efforts of biologists to reach a formal definition of life have proved unsuccessful.[1]

This issue does not only have intrinsic intellectual or academic interest. Astrobiology, which is the science that studies the existence and evolution of life in the universe, requires certain criteria, if the opportunity ever arose, to be able to conclude that life has been found on a celestial body other than earth. Furthermore, there have been attempts by synthetic biology to obtain life *de novo* in the laboratory. What qualities should an entity fabricated by scientists possess so that we would consider it to be alive? Finally, if we concurred that the origin of life on earth did not come about suddenly by a fortuitous coincidence of numerous environmental conditions, but that it happened gradually, would we all unequivocally agree to distinguish at what point during these successive stages life began?

Efforts made from other natural sciences to define life, such as in physics or chemistry, have also proven unsuccessful. The difficulty undoubtedly stems from the fact that life is a complex phenomenon that transcends the

[1] There are at least five books with the title "What is life?" and none of them seem to have given a satisfactory answer to this question: Erwin Schrödinger, Cambridge University Press, 1967; Ed Regis. Farrar, Straus and Giroux, New York, 2008; Lynn Margulis and Dorian Sagan. University of California Press, Berkeley and Los Angeles, 1995; Joseph Seifert. Rodopi, Amsterdam and Atlanta, 1997; P.H. Pinter. Arima Publishing, ASK House, UK, 2006.

scope of individual disciplines and cannot be explained on the basis of its component material parts.

This complexity of life is manifested in various ways. In terms of the molecular components of each cell, the minimal structural and functional unit of all living things, a hierarchical arrangement is observed: monomers, polymers, supramolecular structures, organelles and the external membrane. The resulting cellular architecture shows a remarkable stability despite the constant turnover of the molecular constituents. At the same time, there is an intricate network of interactions among these components, with multiple connections and feedback controls. These include those that occur in the domains of the metabolic pathways and of the genetic material, as well as those that are established between both domains. Just as the structure is hierarchical, the level of organisation is also hierarchical.[2] Thus, within the cell, there are simple networks that are the basis of other more elaborate ones.[3] There are also interactive networks among cells in a colony of microorganisms or those in a multicellular organism and ultimately, among all organisms that make up an ecosystem. Finally, life is also complex in its expansion, a characteristic that is reflected in its dynamic evolution, in its niche construction ability and in processes such as cell differentiation and morphological development.

In search of a definition

Faced with this difficult scenario, researchers have adopted different strategies to agree on a definition of life. One of these has been to distinguish its most distinctive characteristics, such as nutrition, homeostasis, irritability, etc. To Claude Bernard, for example, these qualities are organisation, generation, nutrition, embryonic development and disease or death.[4] The problem is that there are always situations that do not seem to satisfy one or more of these requirements. Could we say that a mule is not a living being because it is incapable of reproducing? There are also the typical borderline cases, such as viruses, frozen bacteria, spores and dormant seeds. More recently, Daniel E. Koshland described the seven essential pillars

[2] Bairns, W. The parts of life. *Nature Biotechnol.* 19, 401-402, 2001.

[3] There are authors, however, that argue that the idea of organisational hierarchy should not be applied to living beings given that the components are self-produced by organisms themselves in a closed system of metabolic reactions. See for example Letelier, J.C., Cárdenas, M.L., Cornish-Bowden, A. From l'homme machine to metabolic closure: Steps towards understanding life. *J. Theor. Biol.* 286, 100-113, 2011.

[4] Bernard, C. *Phenomena of life common to animals and plants*. Trans. R.P. Cook and M.A. Cook. Dundee, Cook & Cook, 1974.

of life, which are worth mentioning given the great prestige attained by this scientist: these are programme, seclusion, energy, improvisation, regeneration, compartmentalisation and adaptability.[5] However, Koshland's proposition did not receive much consideration. According to Franklin Harold, on the other hand, there are four criteria that would allow life to be distinguished anywhere in the universe: flow of matter and energy, reproduction, organisation and adaptation by random variation and natural selection.[6] In turn, for Michael Morange there are only three pillars: a high molecular complexity, a system of very specific chemical reactions in which an exchange of matter and energy is produced with the environment, and reproduction.[7] But as Morange himself has pointed out, the strategy to describe the distinctive qualities of life is only partially correct, since instead, the real issue is how they emerged and became integrated within organisms.[8] Another drawback of this approach is that some statements are not simple to prove, or are in fact unprovable. For example, the most widespread definition of life is the one proposed by Gerald Joyce now almost two decades ago[9] and which was later adopted by NASA for its astrobiology programme: "Life is a self-sustaining chemical system capable of undergoing Darwinian evolution". How long would it take a probe sent to another planet to confirm these qualities?

A second strategy bypasses the problem by offering definitions from different perspectives: physiological, metabolic, biochemical, thermodynamical, etc. Initially proposed by Carl Sagan, this is what has been adopted by, among others, the prestigious *Encyclopaedia Britannica*. It also seems to be a valid option, although as long as these different approaches are not integrated in one overall vision, it will remain incomplete.

A third strategy has favoured a systemic approach that sees life as a process. According to the concept of autopoiesis, originally enunciated by Humberto Maturana and Francisco Varela, living things consist of a network of processes of production, in which the function of each component is to participate in the production or transformation of the other components in the network, including the production of an external limit that specifies

[5] Koshland, D.E. The seven pillars of life. *Science* 295, 2215-2216, 2002.

[6] Harold, F. *The way of the cell. Molecules, organisms and the order of life*. Oxford University Press, New York, 2001.

[7] Morange, M. *Life explained*. Yale University Press 2008.

[8] Tirard, S., Morange, M., Lazcano, A. The definition of life: A brief history of an elusive endeavor. *Astrobiology* 10, 1003-1009, 2010.

[9] Joyce, G.F. Foreword in *The origin of life: The central concepts*, eds. D.W. Deamer & G. Fleischaker. Jones and Bartlett, Boston, 1994.

the domain of the autopoietic organisation.[10] This definition, probably inspired by another previous proposal by J. Perret and J.D. Bernal,[11] points to a very particular aspect of life: its immanent action. In the words of Robert Rosen, organisms themselves are their own efficient cause.[12] However, for many, the autopoietic approach is not sufficient since it leaves out an aspect essential to life such as its ability to undergo Darwinian evolution.[13] That is why Franklin Harold proposes to combine both qualities in one single statement: "living organisms are autopoietic systems capable of evolution by natural selection".[14] Being very similar to the definition adopted by NASA, it has not prevailed among the scientific community.

Interestingly, the previous difficulties have not been an obstacle for the vertiginous progress of the diverse branches of biology, as has been the case for molecular genetics, bioinformatics and neurobiology, to name but a few. We have come to know in great detail intricate processes such as the replication and expression of genetic material, protein synthesis, signal transduction, cellular transport, etc., but reaching a distinctive understanding of what life exactly is remains elusive. By the middle of the last century, Max Delbrück had stated that "biology is a very interesting field to enter for anyone, by the vastness of its structure and the extraordinary variety of strange facts it has collected. But to the physicist, biology is also a depressing subject, because, insofar as physical explanations of seemingly physical phenomena go, like excitation, or chromosome movements, or replication, the analysis seems to have stalled around in a semi-descriptive manner without noticeably progressing towards a radical physical explanation".[15] Subsequent testimony such as that of Franklin Harold ("life is fundamentally a mystery"),[14] Wendel Berry ("life is a miracle")[16] and Erwin Chargaff ("life is the con-

[10] Maturana, H. and Varela, F.J. *De Machines and living things – A theory of biological organisation*. Editorial Universitaria, Santiago, Chile, 1973.

[11] J. Perret had defined life as a system of organic reactions that was self-perpetuating, sequentially catalyzed, almost isothermically, by complex and specific organic catalysts that are themselves produced by the same system (*New Biol.* 12, 68 1952), assertion reiterated by J.D. Bernal in 1965.

[12] Cfr ref. 3.

[13] See ref. 3 for a recent and very complete discussion of the diverse approaches that life has received as to what constitutes a closed-circuit reaction.

[14] Harold, F.M. Postcript to Schrödinger: So what is life? *ASM News* 67, 611-616, 2001.

[15] Delbrück, M. A physicist looks at biology. *Trans. Connect. Acad. Arts Sci.* 38, 173-190, 1949.

[16] Berry, W. *Life is a miracle*. Counterpoint, Washington, 2001.

tinuing intervention of the inexplicable")[17] appear to give an eloquent testimony of this situation. The laws of natural science, which are applicable to life, seem insufficient to explain it fully.

Different approaches to the understanding of life

There are two main perspectives to address the phenomenon of life. One of these, with a mechanistic or reductionist orientation, is well illustrated by the words of Francis Crick: "The ultimate aim of the modern movement in biology is to explain all biology in terms of physics and chemistry".[18] In his classic work, *What is life*, Schrödinger stated that new laws could possibly be needed to explain life, but always in the field of physics. Consequently, representatives of this view see the functioning of an organism as a machine. The mechanisms that operate both are equivalent.

The reductionist approach has been extraordinarily successful regarding the generation of new knowledge, though always relative to localised or partial phenomena. The situation becomes complicated when attempting to establish a linear causality between these and the behaviour of the cell or the entire organism. Molecular genetics itself, which originally appeared so promising to this deterministic vision, has been the source of findings that, instead of facilitating this task, are making it more arduous. By way of example, one can mention (among others) RNA splicing, RNA editing, prions, genetic redundancy, epigenetics, pleiotropy and the fascinating new scenario displayed by the various types of non-protein coding RNAs. Today we know the complete genome sequences of many organisms but we don't know how genomes sharing a high degree of homology can give rise to very different organisms. On the other hand, establishing the relationship between genotype and phenotype is also proving very difficult. To the well-known c-value paradox, which shows a profound lack of correlation between the amount of genetic material and the complexity of organisms, the g-value paradox has been added, which surprisingly shows no correlation either with the number of genes that their genomes possess. A recent study of the systemic inactivation of genes in two virtually identical yeast strains, showed that out of the 5,100 genes analysed, just over 900 are essential. But apart from the latter, the same work revealed that a few dozen genes were only essential for one strain or the other, an unexpected result

[17] Chargaff, E., in *Heraclitean fire: Sketches from a life before Nature*. Seabury, New York, 1977.

[18] Crick, F.H. *Of molecules and man*. University of Washington Press, Seattle, USA, 1966.

given their high sequence identity.[19] Results like these help to explain why finding the genetic causes of diseases that affect humans is so problematical. However, it gives the impression that these difficulties are not so relevant for the materialistic view, since after all, its supporters do not see a radical difference between living things and inert bodies. Life only represents an intricate shape of the motion of matter and therefore there is no reason to treat it like a phenomenon that transcends the laws of physical chemistry. Recently, an editorial in the journal *Nature* stated "there is a popular belief that life is something that appears when one crosses a threshold. It would be desirable if the perception of a qualitative difference between living and inert matter had disappeared just as the concept of spontaneous generation emerging from non-living matter did".[20]

On the other hand, there is also a more holistic approach to life, which envisions it as a complex phenomenon that cannot be deduced from its material components. According to this, organisms and machines differ in fundamental attributes. Among these, the most obvious is that organisms can form themselves, while an external agent manufactures machines. Another one is that the network of interactions between the constituents of a living being is synchronously established with the production of said constituents, whereas for machines the interactions between the different parts are established once they have been assembled after being separately manufactured. A third attribute is that the properties of a machine can be deduced from their material components, something that is not possible in the case of organisms. Additionally, the rigidity of the parts of a machine sharply contrasts the structural dynamics that cell components exhibit (membranes, proteins, nucleic acids, etc.), a quality that contributes to its resilience during changes in the environment. Finally, for machines, it is possible to replace one part or another and assembled it with the old parts, whereas in living organisms the components are continuously replaced.

The previously cited physicist Max Delbrück, a disciple of Niels Bohr and one of the fathers of molecular genetics, boldly upheld the argument that biological analysis must be done in terms of the living cell and theories must be formulated without fear of contradicting molecular physics. In a somewhat more cautious, but innovative way, the biologist Sidney Brenner made the following prediction years later: "I think in the next twenty-five years we are going to have to teach biologists another language ... I don't

[19] Dowell, R.D., Ryan, O., Jansen, A. *et al*. Genotype to phenotype: a complex problem. *Science* 328, 469, 2010.

[20] Meanings of "life". *Nature* 447, 1031-1032, 2007.

know what it's called yet, nobody knows ... It may be wrong to believe that all the logic is at the molecular level. We may need to get beyond the clock mechanisms".[21] More recently, the microbiologist Carl Woese, recognised worldwide for his proposal of a tree of life composed of three domains (Bacteria, Archaea and Eukarya), maintained that the great advances in molecular genetics have produced mixed results. On the one hand, the aspects that could be addressed with an objective scientific method based on hypothesis testing were greatly benefited. But this reductionist approach, which came to permeate all of biology, was also pernicious, as it produced a distortion in twentieth-century biology. Not only did it leave the most holistic problems unresolved, but it also succeeded in producing a change to the concept of life itself. According to Woese, this approach stripped the organism of its surroundings, it separated it from its history and evolutionary flow, and it reduced it to unrecognisable parts, this being the cell, a multicellular organism or the entire biosphere. Therefore, Woese makes a fervent appeal for a new biology, with less attention on the deterministic mechanisms and a greater emphasis on the holistic aspects. That is to say, to see living beings for everything that they are, not to break them into parts as required by the reductionist scientific method.[22] Hans Westerhoff and collaborators have contributed to this line of thought with new arguments. According to these scientists, organisms do not fit into two basic attributes that are typical of physical systems, namely, maximum simplicity and minimum potential energy state. The high complexity of living organisms and their functioning as open thermodynamic systems that store energy, distinguishes them from inert matter. Therefore, they propose to give impetus to systems biology with its own principles equipped with quantitative laws that differ from traditional biology.[23]

A sample of the valuable contribution of the reductionist approach

Criticism of the application of the reductionist scientific method that has prevailed in biology is justified if it is thought to constitute the only way to explore life. Nevertheless, one must not lose sight that its contributions to biology have been very beneficial and will remain so as a comple-

[21] Cited in Judson, H.F. *The eighth day of creation*. Simon & Schuster, New York, 1979.

[22] Woese, C.R. A new biology for a new century. *Microbiol. Molec. Biol. Rev*. 68, 173-186, 2004.

[23] Westerhoff, H.V., Winder, C., Messiha, H., Simeonidis, E., Adamczyk, M., Verma, M., Bruggeman, F.J., Dunn, W. Systems biology: The elements of life. *FEBS Lett*. 583, 3882-3890, 2009.

ment to new studies of a more systemic orientation. In this sense, one of the more fruitful branches of biology has been biochemistry, a discipline that studies the structure, function, organisation and transformation of the cellular constituents. Biochemistry also serves to support all other branches of biology, because in the search for understanding biological processes, it is common that the inductive method ultimately leads to the molecular level. That is why it may be illustrative to mention some fundamental aspects that biochemistry has demonstrated to us, as a sample of how relevant the use of the experimental method has been for biology.

Firstly, we know that the **materials of life** are developed in an aqueous medium. Its bipolar structure, its smooth tendency to ionise, its ability to form hydrogen bonds, its high specific heat and the lower density of ice, makes water the optimum solvent for life on earth. But water also plays other important roles: it is a source of electrons in photosynthesis, it is the final product of cellular respiration (oxidative phosphorylation), it is also a product of condensation reactions and a substrate of hydrolysis, etc. Biochemistry has also taught us that the molecules of life are the same in all organisms of the biosphere, although some of them may also be formed in environments devoid of life. For example, meteorites that come from space contain amino acids, sugars, lipids, nucleotide bases, etc. These same compounds can be synthesised abiotically in the laboratory simulating environmental conditions of early earth. Among those, and of particular interest, are lipids, compounds that compose the membrane providing confinement and identity to the cell. Far from playing a passive role, membranes participate in the flow of compounds towards the interior and exterior of the cell and in the electron transport systems related to cellular energetics.

One can wonder whether life could arise with a solvent other than water or with other types of molecules. The scientist Michael Denton has devoted an entire book to the argument that the cosmos is particularly conditioned to one type of biology – the one that exists on earth – and the phenomenon of life cannot be sustained in any other exotic chemistry.[24] In agreement with him is the microbiologist Norman Pace, for whom the biomolecules of life that we know of are unsurpassable because of their individual characteristics and their ability to form polymers. Wherever life develops, according to Pace, will be composed of macromolecules, for only they can contain and transmit information.[25] Furthermore, the cellular scaffold re-

[24] Denton, M.J. *Nature's destiny*. The Free Press, NY, 1998.

[25] Pace, N.R. The universal nature of biochemistry. *Proc. Natl. Acad. Sci.* USA 98, 808-808, 2001.

quires a versatile element that is capable of forming different links with itself and with other atoms of life, such as hydrogen, nitrogen, oxygen, sulphur and phosphorus. Carbon, which is abundant in the universe, is superior to silicon, the only other element that could compete with this function, because the linkages that silicon forms to itself are weaker and thus less stable than carbon-carbon bonds. In addition, several metals that are essential in cellular biochemistry and that probably played a leading role in the origin of life on earth, act harmoniously in this carbon-based biochemistry. The universal phylogenetic tree based on ribosomal RNA sequences is the best proof of the interrelationship between living things on earth and a unique molecular logic for all of them. Pace adds that the extreme conditions of temperature, pressure, pH and aridity, that are capable of bearing life on earth makes one presume that be it anywhere, the fundamental biochemistry should be the same.

But there are also other views regarding the material components of life. In a provocative article, Benner *et al.* argue that a self-sustaining chemical system capable of Darwinian evolution could allow for a great diversity of chemical compounds, including a non-aqueous solvent that could well be ammonium. A similar viewpoint is stated in an extensive report elaborated by a committee convened by the U.S. National Research Council to analyse the boundaries of life on earth and to help NASA to adopt criteria to recognise, conserve and study life that could exist in other parts of the universe. According to this committee, it is easily conceivable that the fundamental requirements of life, that is, a thermodynamic disequilibrium, stable bonds between atoms, a liquid environment and an ability to undergo Darwinian evolution, could satisfy a chemistry that is not based on carbon, that does not use water as a solvent and where oxygen does not participate in electron transfer reactions. The report calls to set aside the prevailing "terracentristic" view and explore the possibility of new scenarios. Besides proposing various options of small biomolecules, macromolecules and solvents, it extends to analyse the possibility of life in solid and gaseous states.

An intermediary position between a universal biochemistry and the existence of several biochemistries is that of Paul Davies, who has promoted the concept of the "shadow biosphere" originally coined by Carol Cleland and Shelley Copley. According to Davies, given that it is possible that life arose more than once on earth, there could be organisms that exist (meaning microorganisms) whose biochemistry partially differs to that of what we know. For this reason, we have not known how to detect it. Davies, who recently co-authored a controversial article describing a bacterium that replaces phosphorus with arsenic, invites us to search for this shadow life in isolated environments such as underwater hydrothermal vents or the Dry Valleys of Antarctica.

An additional distinguishing characteristic of biomolecules is their chirality (derived from the Greek *kheir*, hand). This concept applies when an object and its mirror image are not super imposable. A textbook example to illustrate this situation is that of hands. Something similar happens with several molecules of life, including amino acids and sugars. In these cases, the chirality arises when a carbon atom binds to four different chemical groups. There can be two types of configuration or spatial distribution of these groups, one being the mirror image of the other. These are known as optical isomers. In life, as we know it, there is only one type of configuration in both amino acids and sugars. That is, in each case life chose only one of the optical isomers. By contrast, the analysis of the organic molecules found on meteorites or those obtained in the laboratory always show the presence of both optical isomers. This selection that resulted in life on earth is explained by reasons of stabilisation of the three dimensional structure of the macromolecules and the specificity in enzymatic reactions. The reasons why life chose a particular isomer in each case is unclear and the mechanisms involved in this selection are the subject of intense studies. It could well be that elsewhere in the universe there is a life based on the same biomolecules as our biosphere but of opposite chirality.

One aspect of the molecular logic of life that does not cease to attract attention is its **metabolism-genetic duality**. The integrated and harmonic coexistence of a component of chemical reactions with an informational one evokes the before mentioned autopoietic and evolutionary processes of Harold's definition, respectively. It is possible that self-replicating informational macromolecules such as RNA, or maybe another initially simpler molecule, may have appeared at the origin of life on earth. There are also supporters of the theory that metabolism was born first and that genetics appeared later. It may be that both began at the same time. Whatever the case, it is difficult to imagine how this association and interdependence between metabolism and genetics was produced.

Would we consider to be alive an RNA molecule that makes copies of itself? Or, could we say that a system of chemical reactions sustained by an external power source and which lacks informational polymers is alive? Neither of these situations exists in natural environments nor have they been obtained experimentally in controlled conditions in the laboratory.[26]

[26] With the exception of the autocatalytic system of reactions known as the formose cycle, which gives rise to sugars based on formaldehyde.

The self-replication of an informational polymer such as RNA proves particularly problematic, since it implies a series of difficult obstacles to overcome: i.e. the abiotic synthesis of precursors, the initial copy of a complementary RNA that will later serve as a template for the synthesis of the original RNA, accuracy of the copy so as not to lose the self-replicative capacity. Moreover, one can speculate that if at the origins of life certain conditions were present, such as an energy source, availability of precursors, an external boundary and the presence of catalysts, reaction systems could have appeared with the capacity to grow and "evolve" by incorporating new ingredients.[27] This hypothesis has both supporters[28] and detractors.[29] If this had been the case, there is no doubt that the genetic-metabolism duality of life that we know of would have constituted a substantial improvement, since the presence of a genome provides many advantages. Among them, a mechanism of replication, variation, and evolution of organisms; a mechanism of internalisation of its phylogenetic history; an expression of functions program (i.e. metazoan development, tissue-specific expression, energy saving in the adaptation mechanisms, etc.) and the encoding of catalysts that are much more efficient and specific than minerals. But above all, the genome provides stability and gives identity to all organisms.

An essential requirement of the chemical reactions of life is **catalysis**. Without catalysis, these reactions would be extraordinarily slow and, we presume, life would not be possible. At the origin of life, it is presumed that metals fulfilled this role, a hypothesis that is reinforced by the participation of these in numerous cellular reactions. However, enzymes, proteins that greatly exceed metals because of both their efficiency and specificity, catalyse the vast majority of the reactions. The information for the synthesis of all enzymes is encoded in the DNA, reinforcing the functioning of the metabolic and genetic domains as a whole. There is another layer of complexity to catalysis, though, as for some years now we know that some reactions in the cell are catalysed by RNA (ribozymes), which could be a remnant of a catalysis that preceded that of proteins.

Finally, a few words about the **energy** that sustains life. The laws of thermodynamics tell us that the high organisation and complexity of life cannot occur spontaneously. The increase of the internal order, which is compen-

[27] Shapiro, R. A simpler origin of life. *Sci. Am.* 46-53, February 2007.

[28] Segré, D., Ben-Eli, D, Lancet, D. Compositional genomes: Prebiotic information transfer in mutually catalytic noncovalent assemblies. *Proc. Natl. Acad. Sci.* USA 97, 4112-4117, 2000.

[29] Orgel, L.E. Self-organizing biochemical cycles. *Proc. Natl. Acad. Sci.* USA 97, 12503-12507, 2000.

sated with an increase in disorder (entropy) of the environment, is made possible thanks to a constant flow of energy. Because of this, it is said that life is in thermodynamic disequilibrium with its environment. When this flow of energy is detained, death occurs and the molecular components of the organism are dispersed and eventually degraded, thus terminating the state of imbalance. We know that there are two primary energy sources to sustain life: sunlight and inorganic redox reactions. Organisms that use them (plants and bacteria) are also capable of manufacturing their biomolecules from atmospheric CO_2, although there are some bacteria that use sunlight for energy purposes only and are dependent on the supply of organic compounds as a carbon source. There is also an important group of organisms, including humans and all animals, which use organic compounds synthesised by plants as their source of energy and carbon.

Whatever the source of energy, all living beings follow the same strategy to capture and store it for later use. This consists of a flow of electrons from levels of high energy to lower energy, which produces a proton gradient whose natural tendency balances and culminates in the synthesis of ATP. This molecule, which constitutes the universal energy reserve of the cell, is principally used for the synthesis of biomolecules and polymers, and for mechanical work. The formation of a proton gradient due to the flow of electrons activated by solar photons or redox chemistry is undoubtedly one of the most unique and ingenious signatures of life on earth. It is the fundamental support of the distinctive autonomy of living things to be permanently sustained in disequilibrium with the environment. This could be the reason that inspired Hungarian Nobel Prize winner Albert Szent-Györgyi to metaphorically say that life is nothing but an electron looking for a place to rest. Therefore, it should not surprise us that upon finding extraterrestrial life, the same strategy to provide energy could be used.

A renewed impetus in the studies of the complexity of life

Leaving aside that some might consider that an isolated self-replicating polymer possesses life, the simplest autopoietic system seems to be a polymer that self-replicates and that replicates another polymer involved in the synthesis of membrane lipids that confines both.[30] Indeed, this would be hypothetically possible if the precursors of both polymers are synthesised abiotically using any available energy source. However, life as we know it has a threshold of complexity far higher than the previously mentioned minimum. *Mycoplasma genitalium*, one of the simplest bacteria known to

[30] Szostak, J.W., Bartel, D.P., Luisi, P.L. Synthesizing life. *Nature* 409, 387-390, 2001.

exist, has a total of 580 genes, of which 482 code for proteins. Several years ago, while trying to answer the question of what could be the smallest genome of a microorganism that is not exposed to any form of stress and that has all the nutrients it needs, Craig Venter and collaborators methodically inactivated each of the genes of *M. genitalium* and discovered that 382 protein-coding genes are absolutely essential for the viability of the bacterium.[31] To these we must add 43 genes that code for RNA. This seems to be the minimum size that a system requires to be autopoietic, that is to say, to function autonomously, far from equilibrium, and exchanging matter and energy with the environment.

Autopoiesis is a process that involves several interconnected systems of metabolic reactions. Some may be intended for energy production, others for the supply of macromolecule precursors, others for biosynthetic purposes. Indeed, also included in these reaction systems are compounds destined for replication and expression of genetic material. One approach to studying the complexity of life is precisely the comprehensive analysis of macromolecular complexes and of the networks within the cell, which seem to represent a growing trend in contemporary biochemistry.[32] This view of systems biology is in line with the more holistic approach proposed by Woese and provides a complimentary perspective to that of the traditional work at the molecular level. Always within the scope of the scientific method, systems biology operates by gathering, processing and integrating information to later develop mathematical models that describe the structure and organisation of a particular system. These models are also useful for analysing the robustness of the systems, allowing the simulation of different disruptions to the structure. This networks approach is even being used as a tool in evolutionary studies, analysing the effect that a mutation produces in the functioning of a network and how its alteration is translated into a phenotypic change.[33]

There are different types of interaction networks, such as: protein-protein, signal transduction, metabolic networks and regulation circuits of gene expression. While each network has its own identity, all together they form an interactive system. This systemic approach is not simple. It firstly requires the identification of the components of the network, taking into account

[31] Glass, J.I., Assad-García, N., Alperovich, N., Yooseph, S., Lewis, M.R., Maruf, M., Hutchinson, C.A., Smith, H.O. and Venter, J.C., Essential genes of a minimal bacterium, *Proc. Natl. Acad. Sci.* USA 103, 425-430, 2006.

[32] Arkin, A.P., Schaffer, D.V. Network news: Innovations in 21st century systems biology. *Cell* 144, 844-848, 2011.

[33] Moore, A. Towards the new evolutionary synthesis: Gene regulatory networks as information integrators. *Bioessays* 34, 87, 2012.

that some of them may have a transient presence. It is then necessary to establish interactions with the corresponding cooperative and feedback elements. It requires also an idea of its spatial topology: participation of elements in solution, associated with membranes or within an intracellular compartment. Next, the robustness of the network must be analysed: how it responds to external stimuli, to states of stress or disease, how it varies during the cell cycle or in cells of different tissues. Finally, it is necessary to integrate all the networks of the cell, establishing the connectivity map and the spatiotemporal relationship that exists between them.

Computer modelling and the availability of software that allows the integration of data, together with new high-performance technologies, are greatly facilitating network analysis. For example, a recent study detected the presence of 178 protein complexes with some multifunctional proteins participating in several of these complexes in a different species of *Mycoplasma* to the aforementioned one.[34] The case of *Saccharomyces cerevisiae* is paradigmatic, because of its unicellular eukaryote nature. Studies from two groups with this microorganism have identified 547 protein complexes, although it is estimated that these could be about 900. The average amount of polypeptides per complex is close to four and they have found 429 additional interactions between pairs of complexes.[35,36] Another study that analysed more than 5.4 million possible interactions between gene products, led to a functional map of the yeast cell, in which genes that participate in the same processes show connections between them.[37]

Apart from yeast, another organism used extensively in diverse types of molecular studies is the worm *Caenorhabditis elegans.* Its close to a thousand cells and the sequencing of its genome make it a very good study model. Several groups have contributed to building networks of protein-protein interactions, networks of transcription factors with promoters and with mi-

[34] Kühner, S., van Noort, V., Betts, M.J., Leo-Macias, A., Batisse, C., Rode, M., Yamada, T., Maier, T., Bader, S., Beltran-Alvarez, P., Castaño-Diez, D., Chen, W-H., Devos, D., Güell, M., Norambuena, T., Racke, I., Rybin, V., Schmidt, A., Yus, E., Aebersold, R., Herrmann, R., Böttcher, B., Frangakis, A.S., Russell, R.B., Serrano, L., Bork, P., Gavin, A-C. Proteome organization in a genome-reduced bacterium. *Science* 326, 1235-1240, 2009.

[35] Gavin AC, Aloy P, Grandi P, Krause R, Boesche M, Marzioch M, Rau C, Jensen LJ, Bastuck S, Dümpelfeld B, *et al.*: Proteome survey reveals modularity of the yeast cell machinery. *Nature* 2006, 440:631-636.

[36] Krogan NJ, Cagney G, Yu H, Zhong G, Guo X, Ignatchenko A, Li J, Pu S, Datta N, Tikuisis AP, *et al.*: Global landscape of protein complexes in the yeast *Saccharomyces cerevisiae*. *Nature* 2006,440:637-643.

[37] Costanzo, M. *et al.*, The genetic landscape of a cell. *Science* 327, 425-431, 2010.

croRNA, networks of interactions between gene products, and signal transduction networks.[38] Moreover, the phenotypic effects that have disruptions in some of these networks have been studied, a strategy already being applied to study the genetic causes of human diseases.[39]

Another approach to the three-dimensional architecture of the cell is the study of the spatial arrangement of the different regions of the genome. One of them made with the fly *Drosophila*, reveals that the active genes are grouped in different domains to those that are not expressing, producing physical interactions between them despite the fact that they can be found on different chromosomes.[40] Since it would seem that the gene sequence is not sufficient to express a phenotype alone but that the structural organisation is also critical, this methodology is being applied to study human diseases, particularly cancer.

Is science sufficient to explain life?

Lord Rutherford argued that physics is the only science. Anything else was the equivalent to collecting stamps.[41] Though entitled to his opinion, his judgement seems like an insult to biologists. It has to be considered that it was a type of "philatelic" activity that led Darwin and Wallace to the theory of evolution. It is possible that descriptive biology at that time motivated Lord Rutherford to deliver such an arrogant judgement. Possibly, had he witnessed the progress of contemporary biology, his opinion would have been different.

The current outlook, with its impressive achievements, is mainly the result of a successful application of the experimental method. First, enzymology, then molecular genetics, followed by structural studies of macromolecules, and more recently by massive genome sequencing, has led to the accumulation of information that is only possible to analyse with computational tools. It has been a successful journey and it is compulsory to continue along this path, because it is the only way to know how the molecular components of life function. But this approach has its limitations, because it is methodologically reductionist. A subsystem is isolated and is

[38] See Gunsalus, K.C., Rhrissorrakrai, K. Networks in Caenorhabditis elegans. *Curr. Op. Genet. & Develop.* 21, 1-12, 2011 and the references included.

[39] Cordell, H.J. Detecting gene-gene interactions that underlie human diseases. *Nature Rev. Genet*. 10, 392-404, 2000.

[40] Sexton, T., Yaffe, E., Kenigsberg, E., Bantignies, F., Leblanc, B., Hoichman, M., Parrinello, H., Tanay, A., Cavalli, G. Three-dimensional folding and functional organization principles of the Drosophila genome. *Cell* 148, 1-15, 2012.

[41] Birks, J.B. *Rutherford at Manchester*, Ed. Heywood. London, 1962.

studied outside the multiple connections that it normally has with other systems within a cell or the whole organism. For this reason, the application of systems biology and the development of complexity science are giving a new impetus to biology, always within the scope of the scientific method of exposition and refutation of hypotheses. Most likely, problems such as the lack of correlation between gene number and the complexity of organisms, the significance of RNA editing or the consequences of pleiotropy, will be solved this way.

However, the underlying question is whether the exclusive use of this method and the mere knowledge of the molecular mechanisms will lead us to an exact understanding of not only life, but also living beings. Nowadays, no one disputes that molecular mechanisms do not violate the laws of physical chemistry. Is this, however, sufficient to explain life? The analysis of such a distinctive aspect of life, such as its autonomy to operate far from the thermodynamic equilibrium while exchanging matter and energy with the environment, seems to require more than experimental data. The same could be said about self-organisation and emergent properties, or about the effect of the relationship between parts of a system in the behaviour of said parts (*downward causation*).[42] Supposedly, a science of complex systems should be able to explain these aspects, but it is not at all clear that that is how it will be.

In his *Critique of Judgement*, Kant maintained that mechanical causes are not sufficient to understand the phenomenon of life and that teleological arguments are indispensable for its systematic and complete comprehension.[43] Later on, Niels Bohr would reaffirm this line of reasoning.[44] By this time, Bohr had already proposed the principle of complementarity in physics, ac-

[42] Mazzocchi, F. Complementarity in biology. *EMBO reports* 11, 339-344, 2010.

[43] "For it is quite certain that in terms of merely mechanical principles of nature we cannot even adequately become familiar with, much less explain, organized beings and how they are internally possible. So certain is this that we may boldly state that it is absurd for human beings even to attempt it, or to hope that perhaps some day another Newton might arise who would explain to us, in terms of natural laws unordered by any intention, how even a mere blade of grass is produced. Rather, we must absolutely deny that human beings have such insight. On the other hand, it would also be too presumptuous for us to judge that, supposing we could penetrate to the principle in terms of which nature made the familiar universal laws of nature specific, there simply could not be in nature a hidden basis adequate to make organized beings possible without an underlying intention (but through the mere mechanism of nature)". Immanuel Kant, *Critique of Judgment*, 1790.

[44] "The asserted impossibility of a physical or chemical explanation of the function peculiar to life would in this sense be analogous to the insufficiency of the mechanical

cording to which, matter at the atomic level should be studied as a particle and as a wave. Similarly, he thought, the reductionist and holistic approaches, mutually exclusive epistemological concepts, could be used in a complementary form for a complete understanding of life. The first one is called to account for molecular mechanisms, while the second is directed towards the functioning of living organisms as such, even taking teleological arguments into consideration. This is not to later make an amalgam of both, but to accept the idea that the understanding of life may require approaches from different points of view.

In more recent years, Vicuña and Serani-Merlo[45] have made an epistemological proposal comprising three types of approaches to the study of life, as follows: a) an experimental one, appropriate to answering questions that can be resolved with the scientific method in the areas of biochemistry, physiology, cytology, genetics, etc; b) a historical one, to address scientific aspects that cannot be answered experimentally, such as evolutionary speciation, the effects of mass extinctions, etc.; palaeontology and dating methods are valuable tools of the latter approach; and c) a third one, from the standpoint of philosophy of nature, for matters that cannot be studied with the experimental method either, such as the existence or nonexistence of purpose or design of living things. In a similar vein, Powell and Dupré have recently indicated the importance of looking at both sides when studying the transition from molecules to systems, also suggesting that philosophical ideas can be valuable in this exercise.[46]

It is difficult to estimate the degree of acceptance the invitation that Kant and Bohr gave us would have today, namely, to resort to arguments from natural philosophy together with those from the natural sciences to attain a better understanding of the phenomenon of life. Given the so far unsuccessful efforts of biologists to attain this goal, this seems to be a path worth exploring.

analysis for the understanding of the stability of atoms….It is due to this situation, in fact, that the concept of purpose, which is foreign to mechanical analysis, finds a certain field of application in problems where regard must be taken of the nature of life". Bohr, N. Light and life. *Nature* 133, 421-423, 457-459, 1933.

[45] Vicuña, R., A. Serani-Merlo, A. Chance or design in the origin of living beings: an epistemological point of view, in *Life in the Universe: from the Miller experiment to the search for life on other worlds*. Series: Cellular Origin, Life in Extreme Habitats and Astrobiology, Vol. 7. Seckbach, J (Ed). Kluwer Academic Publishers, pp 341-344, 2004.

[46] Powell, A., Dupré, J. From molecules to systems: the importance of looking both ways. *Stud. Hist. Philos. Biol. Biomed. Sci.* 40, 54-64, 2009.