

SCIENCE NEVER ENDS: A NEW PARADIGM IS BEING BORN IN BIOLOGY

RAFAEL VICUÑA

Does science have an end?

The spiraling advances in our knowledge of the natural world appear to drive the paradox that sooner or later science will no longer have questions left to answer. Distinguished thinkers thought that such a transcendental moment had already arrived. Of note is the case of physicist Albert Michelson, who in 1894, upon delivering the main address during the dedication of the Ryerson Physical Laboratory at the University of Chicago, declared that the more important fundamental laws and facts of physical science had all been discovered. According to Michelson, future research would be oriented towards the application of these principles and to perfect the precision of measurements. The same kind of assertion had been foretold by the eminent Lord Kelvin. A few years following the predictions of Michelson and Kelvin, the revolutionary theories of relativity and quantum mechanics emerged and completely changed the outlook on how the universe is viewed. Ironically, the experiments of Michelson relating to the speed of light helped to inspire Einstein's special theory of relativity.

In a book published in 1996, entitled *The end of science*,¹ the author John Horgan discusses the limits of knowledge with scholars from a broad range of disciplines. Among the interviewees is Gunther Stent, who has been one of the foremost proponents of 'the end of science'. Born in 1924 in Germany, Gunther Stent settled fourteen years later in Chicago, where he would later receive a Ph.D. in Chemistry from the University of Illinois. He was one of several physicists attracted to the biological sciences after

¹ Horgan, J., *The end of science*. Broadway Books, New York, 1996.

reading the now classic work *What is life?*² written by Erwin Schrödinger. Gunther Stent, together with Max Delbrück, Leo Szilard, Francis Crick, Rosalind Franklin and Maurice Wilkins, among others, left the scientific discipline in which they had been trained to tackle the mysteries of living organisms. Stent was soon working along with Delbrück at the California Institute of Technology. Both were members of the famous *Phage Group*, which also included Salvador Luria, Alfred Hershey and James Watson. Later, in 1952, he would establish himself at the University of California at Berkeley, where he works until this day. There he founded the Department of Molecular Biology, and later he entered the fields of neurobiology and philosophy of science.

In 1969, Stent published *The coming of the golden age: a view of the end of progress*,³ in which he develops the hypothesis that reality possesses limits and therefore soon nothing important will remain to be discovered. He utilized the fields of anatomy and geography as examples of scientific endpoints. According to Stent, chemistry had already reached its heights in the 30s when Linus Pauling demonstrated that every molecular interaction could be understood in terms of quantum mechanics. For their part, physicists had already described the physical universe, from the microcosmos of quarks and electrons to the macrocosmos of planets, stars and galaxies. Furthermore, a consensus had been reached in which the universe exploded about 15 billion years ago and that all matter is governed by four forces: gravity, electromagnetism and the weak and strong nuclear forces. The field of biology would be left with only three fundamental problems to explore: the origin of life on Earth, embryonic development and the processing of information by the brain. According to Gunther Stent, students of the nervous system would form the avant-garde of biological research, with the challenging perspective that the inability to even imagine any reasonable molecular explanation for consciousness offers some hope that new laws of physics might be revealed.

The remainder of the larger picture in the biological sciences had been clarified with the publication of the *Origin of the species by means of natural selection* by Darwin, the resolution of the DNA structure by Watson and Crick and the deciphering of the genetic code. These latter two discoveries

² Schrödinger, E., *What is life? The physical aspect of the living cell*. Cambridge, Cambridge University Press, 1944.

³ Stent, G.S., *The coming of the golden age: a view of the end of progress*. The Natural History Press, Garden City, New York, 1969.

seemed not to have left room for new advances in the field of molecular biology, a premise which would lead Stent to publish in the journal *Science* in the year 1968 a provocative article entitled: 'That was the molecular biology that was'.⁴ In the first paragraph of this article, Stent declared '... the approaching decline of molecular biology, only yesterday an avant-garde but today definitely a workaday field'.

Gunther Stent was not alone in the twentieth century with this fatalistic vision of science. Other protagonists included the physicist Leo Kodanoff and the former president of the American Association for the Advancement of Science, Bentley Glass, who observed that 'experiments of increasing costs are designed to solve more and more irrelevant details'.

A journey through the central dogma of molecular biology

About 34 years after the publication of *The coming of the golden age*, we could ask ourselves how accurate was Stent's prediction related to the end of molecular biology. The so-called central dogma of this discipline, enunciated by Francis Crick in the 60s, seems a viable reference point for a quick analysis on this matter. As it was written in its initial version, the dogma maintained that the flow of genetic information always goes from DNA to RNA and then to proteins. It also established that both DNA and RNA have capacity to replicate themselves.

Subsequent studies on the replication of the DNA confirmed what Watson and Crick predicted in their classic publication in the journal *Nature* in 1953:⁵ 'It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material'. Although there has since been no discovery that could be classified as revolutionary in the field of DNA replication, the synthesis of this fundamental polymer has demonstrated to be extraordinarily more complex than initially imagined. In the bacterium *Escherichia coli*, for example, more than 50 proteins contribute to this process, including five enzymes (DNA polymerases) with the capacity to catalyze the synthesis of DNA. The most prominent of these, DNA polymerase III holoenzyme, is in charge of copying the bacterial chromosome in anticipation of cellular division, a task performed at the astounding speed of 700 nucleotides per second. The discovery of topoisomerases, enzymes that solve the problem of

⁴ Stent, G.S., *Science* 160, 390-395, 1968.

⁵ Watson, J. and Crick, F., *Nature* 171, 737-738, 1953.

the advancing DNA replication fork through two strands that are coiled around each other, also constitutes a conceptual novelty difficult to predict in the early 50s. In this respect, there are still important aspects to solve, particularly the mechanisms that regulate the process in higher cells.

In 1970, Howard Temin and David Baltimore demonstrated independently that the flow of information from DNA to RNA was not strictly unidirectional, as some viruses have an enzyme called reverse transcriptase that is able to copy DNA using RNA as template. These viruses, known as retroviruses, are of great importance to human health as they are responsible for AIDS and certain cancers. Both investigators received the Nobel prize in Medicine in 1975 for this discovery. Another enzyme possessing this reverse action is telomerase, which is of major importance in the synthesis of chromosomal ends and whose action is altered in cancer cells.

The central dogma also failed to predict two unexpected transformations which messenger RNA (mRNA) undergoes before the encoded information is translated into proteins. These alterations consist of the removal of multiple sections of internal sequences or introns, a phenomenon known as *splicing*, and in the chemical modification of the mRNA in a process called *editing*, which alters the information originally encoded by the DNA template. Both modifications to the mRNA, while not contradicting the dogma, certainly shake it in its foundations, to say the least. Today we are still baffled by the existence of splicing and editing, as it would seem a more efficient use of cellular energy if evolution had chosen to directly alter the chromosomal DNA instead of the mRNA. More recently, the phenomenon of trans-splicing has been uncovered. It consists of a covalent union of mRNA fragments originating from both DNA strands, extending the initial concept still further that a gene is a continuous segment of genetic information.⁶

But still, this is not the complete story. Studies on RNA splicing mechanisms lead in 1982 to the surprising discovery that some introns have the capacity to excise themselves without the participation of enzymes. This catalytic activity of introns was later found in several RNAs that participate in diverse pathways of cellular metabolism. Typical examples of these now called ribozymes are the RNAs catalyzing peptide linking during protein synthesis and those which are responsible for the processing of transfer RNA (tRNA) precursors. It was for their work in this field that Thomas

⁶ Labrador, M., Mongelard, F., Plata-Rengifo, P., Baxter, E.M., Corces, V.G. and Gerasimova, T.I. *Nature* 409, 1000, 2001.

Cech and Sidney Altman were awarded with the Nobel prize in Chemistry in 1989. In recent years investigators have selected synthetic RNAs of such catalytic versatility, that the hypothesis that ribozymes must have played a fundamental role in the first evolutionary stages of the life on Earth has been given a strong fortification. Examples of ribozyme activities generated in the laboratory by random sequence selection include phosphodiester cleavage, RNA ligation, RNA phosphorylation, RNA aminoacylation, peptide bond formation, glycosidic bond formation, RNA alkylation and cyclic phosphate hydrolysis, among others.⁷ It has further been demonstrated that under specific conditions, RNA has the ability to catalyse the synthesis of its own nucleotides and moreover to replicate itself.⁸ This *in vitro* selection of specific ribozyme activities is of such effectiveness that it has been used in the selection of deoxyribozymes. That is to say, the traditionally inert DNA molecule can also be compelled to perform a surprising variety of chemical reactions, such as RNA transesterification, DNA cleavage, DNA ligation, DNA phosphorylation and porphyrin methylation.⁹

The flow of information from RNA to proteins has also been a source of interesting surprises with respect to the central dogma. When the genetic code was solved in 60s, the attention was immediately drawn to the observation that this code was universal. All organisms in nature seemed to use the same language to store and transmit genetic information. In the course of the following years, it was discovered that several organisms fell outside this norm, particularly in their expression of the message contained in minute cytoplasmic organelles called mitochondria.

Additional findings substantially extending our perspective on the central dogma, relate to unexpected properties of some proteins. For example, certain proteins from bacteria and yeast have the capacity to remove internal fragments from themselves in an autocatalytic manner. The intervening polypeptide (intein) is precisely excised from the precursor protein and the flanking polypeptides (exteins) are ligated to form the mature protein.¹⁰ The biological meaning of this splicing of proteins is still unknown, although most inteins harbor homing endonucleases which turn inteins into infectious elements by mediating horizontal transfer of the intein coding

⁷ For a review, see Bartel, D.P. and Unrau, P.J., *Trends Biochem. Sci.* 9, M9-M13, 1999.

⁸ Johnston, W.K. *et al*, *Science* 292, 1319-1325, 2001.

⁹ Li, Y. and Breaker, R.R., *Curr. Op. Struct. Biol.* 9, 315-323, 1999; Breaker, R.R., *Science* 290, 2095-2096, 2000.

¹⁰ Paulus, H., *Ann. Rev. Biochem.* 69, 447-496, 2000.

sequence. Prions are also a good example of a novel concept within the dogma. These protein agents, which affect the mammalian nervous system leading to diseases such as Creutzfeldt-Jakob, kuru and scrapie, can cause a non-physiological modification of other proteins seemingly without the need for genetic material.¹¹

The Functional Genome

It is quite possible that if Gunther Stent had known that after the publication of his work there would appear exceptions to the universality of the genetic code, splicing and editing of the RNA, the reverse transcription of RNA, the splicing of proteins, the presence of catalytic DNA and RNA, etc., he may have abstained in 1968 of his prediction about 'the approaching decline of molecular biology'. And yet, it is highly likely that molecular biology has yet to reveal many of its greatest and surprising secrets, upon the unfolding of functional genomics. This novel field studies the organization of the genes, the mechanisms that control their expression and the interactions that are established among them to make up the physiology of an organism.

The fundamental discovery of Watson and Crick took center stage only one year after Martha Chase and Alfred Hershey, based on the observations of Oswald Avery, confirmed that DNA was the genetic material. Doubt no longer existed that this polymer was the structural key to the development and organization of living organisms. Then, it was assumed that a simple relationship between phenotype and genotype would allow an interpretation at the genetic level for every characteristic exhibited by living organisms. Possibly, this somewhat straightforward and ingenuous vision of the problem was influenced by the extreme reductionism championed by Francis Crick.

Later investigations, nevertheless, demonstrated that the genome is considerable more complex and that multiple factors influence phenotypes. An initial source of astonishment came from the observation that the amount of DNA contained within a genome and the place of organisms in the evolutionary scale do not follow a linear relationship. Thus, for example, many plants have more DNA than mammals, and still more surprising, the amoeba, a very small unicellular organism, has 200 times more DNA than *Homo sapiens*. This phenomenon is referred to as the *C*

¹¹ Prusiner, S.B., *Proc. Natl. Acad. Sci. USA*. 95, 13363-83, 1998.

value paradox. Although today we correlate this phenomenon with the fact that only a fraction of the genome has a coherent message (around a 1.3% in the man and a still smaller percentage in plants), the function of the non-coding DNA is for the most part unknown. For whatever reason, this portion of the genome must be essential, as its maintenance requires high energy consumption.

The Human Genome Project has brought new surprises that have come to defy the basis of genetic determinism, i.e. the traditionally sustained belief of the existence of simple causality between phenotype and genotype. As it is commonly known, this project anticipates reading (sequencing) of the genome in its totality, the elucidation of the genes encoded and their corresponding chromosomal locations (genetic map). The Human Genome Project also incorporates the study of genomes from other organisms, with the purpose of making comparative analyses among them.

Without a doubt, the most remarkable discovery that has been contributed by the Human Genome Project was not only the confirmation that there is no simple correspondence between the degree of morphologic complexity and DNA content, but neither is there a correlation between this physical property with the number of genes in the different organisms. Thus, one sees that solely within the group of the bacteria, the number of genes ranges from 473 (*Mycoplasma genitalum*) to nearly 8,000 (*Myxococcus xanthus*). Among them, the *Escherichia coli* bacterium, widely used in laboratory experiments, has a genome made up of about 4,500 genes. The yeast *Saccharomyces cerevisiae*, also unicellular, possesses 6,034 genes. Since the latter is larger and possesses a more elaborate structure than bacteria, a greater difference in the number of genes had been expected. Among the metazoans (multicellular beings), the fruitfly *Drosophila melanogaster* appears with 13,061 genes, whereas the roundworm *Caenorabditis elegans*, that measures a millimeter in length and displays a more basic morphology, has 19,099 genes. Furthermore, *Arabidopsis thaliana*, a cress plant whose genetic simplicity makes it a useful model for laboratory studies, has a genome of 25,500 genes.

How many genes should be expected for the human species? Until a few years ago an estimate near 100,000 was postulated, although some experts elevated this number as far as 165,000. Then, in February of 2001, data published in *Nature* by the Human Genome Project consortium,¹² as well

¹² International Human Genome Sequencing Consortium, *Nature* 409, 860-921, 2001.

as that published in Science magazine by the biotech company Celera,¹³ threw out an unexpectedly low number. The genome of the human species seems to have about 30,000 genes, little more than the cress *A. thaliana* and only 50% more than *C. elegans* worm. Other genomes whose study has not yet been concluded, such as those of the mouse and the chimpanzee, are expected to contain a gene number very similar to that of our own.

But it is not merely the low gene number that draws our attention. Something equally unexpected is that the genomes of the yeast, the fruitfly and the worm share 46, 61 and 43 percent similarity with the human genome. These observations raise fundamental questions. How is it that this low number of genes contains all the information required by a complex organism such as man? How do we explain that genomes sharing such a high degree of homology can give rise to such different organisms?

We do not have answers to these questions yet. The observation that more than a third of the human genes can undergo remodeling leading to the production of several functionally distinct proteins from each gene – a phenomenon called alternative splicing – does not appear to be a sufficient explanation. The DSCAM gene of the fruitfly *Drosophila*, which is involved in nervous system development, could theoretically give rise to 38,000 proteins by means of this alternative splicing. Therefore, it is clear that we must change our traditional vision of the genome and analyze its behavior like that of a complex system whose final product is superior to the mere sum of its parts. In other words, it is becoming more and more evident that although the number of genes are a determining factor in the phenotype of an organism, of equal importance are the inter-genetic interactions (epistasis), as well as the influence in gene expression exerted by the environment.

Thus, rather than saying that we have identified the gene for obesity, the gene responsible for cognitive abilities or the gene responsible for Alzheimer's disease, it would be more accurate to state that these genes are involved in the expression of these characteristics. In reality, the phenotype of each individual is dependent both on the properties of the genome as a whole and upon the interaction with the environment. This explains why the same mutation in a particular gene can give rise to dissimilar effects in different individuals, including failing to be expressed. Although this phenomenon is less frequent in characteristics arising from

¹³ Venter, J.C. *et al*, *Science* 291, 1304-1351, 2001.

a single gene, it is certainly evident in the case of characteristics of multi-genetic origin. A textbook example of this latter point is observed with the gene associated with increased risk of mammary and ovarian cancer, BRCA1. When both alleles of BRCA1, that is to say, when the genes derived from the father and the mother are mutated, the risk of contracting cancer is not greater than if only one allele is present. It is as a result of situations such as this one that geneticists have coined the concepts of penetrance and genetic expressivity, to mean, respectively, the proportion of individuals with a specific genotype that is manifested as a phenotype and the degree to which this expression occurs.

In relation to the previous example, it is possible to deduce that although certain risks can be affected by alterations in a single gene, it does not necessarily imply that altering the dose of this gene by means of genetic manipulation is necessarily going to harness the expression of this characteristic in beneficial and harmonic form. This is perhaps the most important challenge that faces gene therapy, the practice that was initiated over two decades ago as a promising alternative to alleviate the monogenetic diseases. To complicate matters still further, it has been known for some time that mutations of genes whose alteration simultaneously affects multiple functions does not always shed light on relationship among these functions. The existence of these genes, referred to as pleiotropic, constitute further evidence as to why genome related studies must be approached in both a systematic and open-minded manner.

Research in microorganisms has demonstrated this apparent lack of direct correspondence between genotype and phenotype. Comparative analyses of genomes of microorganisms that live at high temperatures, have not explained the genetic bases of thermostability. Equally puzzling is the failure to elucidate the genes responsible for the remarkable resistance exhibited by the bacterium *Deinococcus radiodurans* to radiation. Recent investigations on minimal genomes using the knockout approach have brought to light unanticipated findings in this issue. This technology involves introducing mutations that disable a gene in order to examine the consequences on the viability of the organism. Observations in yeast, for example, show that of the 6,034 genes already mentioned only about 1,000 are essential for survival. It is assumed that functional redundancy occurs, in which similar genes (paralogues) can assume the tasks of the deleted ones. Statistical extrapolations of these works throw out a number of 300 genes which are absolutely essential to sustain life. In metazoans, knockout technology has also demonstrated some highly unexpected results, an

example being that the deletion of both oestrogen receptors still allows the birth of a healthy, although sterile, individual.

The concept of genomic plasticity had already been applied to the discipline of evolutionary genetics, accounting for the observation that certain morphologic characters remain unchanged in spite of a substantial genetic variability. These characteristics have been named canalized characters, since their manifestation stays within narrow limits in spite of stimuli having the potential to disturb them. A classic example is demonstrated by HOX gene clusters, which define the vertebrate body plan. All vertebrates, from sharks to man, have a similar body plan brought about by the presence of four HOX clusters. The bony fish have undergone a genome duplication of these gene clusters and now possess seven HOX clusters, yet still maintaining the same body plan. Further studies in this field have demonstrated that distant organisms in the evolutionary scale have very similar genes (orthologues) which possess completely different functions. One of the notable examples on the matter is the *otx* gene, which in the vertebrate lineage participates in head formation, whereas in the aquatic coelenterate Hydra this gene is associated with movement. In the same vein, genes that code for the eye crystal proteins have orthologues involved in responses to thermal shock and other stimuli that induce cellular stress.

Science has no end

The biologist Adam Wilkins, after examining the influence of Mendel, Darwin and Watson-Crick, suggested that in biology a Kuhnian style revolution which entails a new paradigm replacing a still effective one, has not occurred.¹⁴ Strictly speaking, contends Wilkins, none of the seminal contributions of these prominent scientists constituted a new theory that substituted an existing one, as what really existed previously in each case was simply ignorance. In accordance with this, Richard Strohman maintains that a true revolution is currently taking place, where the existing traditional genetic determinism is being supplanted by a more systematic approach to genetics. The prevailing paradigm of the last several decades, reinforced by the reductionism of some leading scientists, found support in the statement: DNA to RNA to protein to phenotype. This axiom continues in its validity, declares Strohman, solely for those characteristics that are

¹⁴ Wilkins, A.S., *BioEssays* 18, 695-696, 1996.

encoded by a single gene. But the vast majority of the cellular functions depend on the interaction of several genes, which are also influenced by the environment. It is for this very reason that it is easier to predict the appearance of a monogenetic disease (haemophilia, serious immunodeficiency, hypercholesterolemia) than those of a multifactorial origin (schizophrenia, Alzheimer's disease). According to Strohmman, the new paradigm that is being heralded is that of epigenetics, the discipline that incorporates the study of mechanisms that impart spatial and temporal control of gene expression in the development from the zygote to the adult stage of complex organisms.^{15,16} In this complex epigenetic network it is implied that once synthesized, proteins can establish a series of interactions using guidelines not originally encoded in the DNA. To phrase this another way, the network of interactions between the genes that is established by the proteins they encode, in conjunction with the influences of environmental factors on these interactions, constitute an epigenetic adaptive system that is complex and incompatible with the marked determinism that prevailed in the last century.

It will not be long before the views of Richard Strohmman are verified. Either way, it seems clear that the application of a reductionistic logic in science can lead to false interpretations by limiting the confines of what remains to be explored. We must consider that biological systems are complex and experience demonstrates that as knowledge progresses new scenarios appear that could not have been foreseen with the previously available information. Scientific research always leads to new questions. For this reason, molecular biology, far from having found its limits proposed by Stent, is more vigorous than ever and most likely it is about to give birth to a new paradigm that will revolutionize the biological sciences.

¹⁵ Strohmman, R., *Bio/Technology* 12, 156-164, 1994.

¹⁶ Strohmman, R., *Nature Biotechnology* 15, 194-200, 1997.

DISCUSSION ON THE PAPER BY VICUÑA

CABIBBO: A great question is: will biology continue? Will physics continue? Who knows?

RAO: I think other than biology, there are a lot of other sciences, so let me say something. It's foolish of people to say that chemistry ended with the Dirac equation; Dirac himself said that, and that is unfortunate. And of course people say that Linus Pauling created modern chemistry when he put two dots and said there is a chemical bond.

CABIBBO: Nanotubes, etc.

RAO: The real point in chemistry is not based on this premise. The fundamental premise that explaining a chemical bond is not the end of chemistry. It's a wrong assumption: statements about the end of science, the end of the world, etc., are generally misplaced.

CABIBBO: I tend to agree.

RAO: This seems to be wrong in all these cases.

CABIBBO: I tend to agree. In fact probably even geography still has a lot of interesting aspects to be discovered.

VICUÑA: Well, at the beginning of my talk I mentioned the book by Paul Horgan. He interviewed many scholars in different fields. Supposedly, all of them were more or less in favour of the end of science. But I heard this morning from the previous speaker, Dr. Shea, that many of the interviewees of Horgan in that book are not very pleased with the interpretation of their statements made by this journalist. But there have been prestigious scientists in favour of the end of science. I didn't mention for example Leo

Kodanoff and the former President of the American Association for the Advancement of Science, Bentley Glass, who also said things such as 'experiments of increasing cost are designed to solve more and more irrelevant details'. As I said, he was President of AAAS. So, we have to be careful.

CABIBBO: Individual people may become tired of making experiments, but there'll be new people doing that.