Science is an Extraordinary Opportunity for Personal Growth

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Abstract HUMILITY. What' s a DNA molecule? How is it capable of dictating a definite response? How many consequential and regulatory steps are actually needed to achieve the result? How, when and where are they controlled? When we inject in a human body an mRNA molecule how do we control its journey, the site and the level of its expression? When we inject an antigen into a human body are we able to predict the strength, the duration, the type of the immunological response? Are we able to control the changes occurring at the level of the different B- and T-lymphocytes populations? How can we explain the side effects of almost any drug? Do we know the pathways by which they work? These are just a few examples restricted to my own areas of interest that cannot find a conclusive answer and indeed may never find it if we consider that any effect, occurring in a different point of time and space, is likely exerted by multiple molecular and cellular events triggered either inside or outside of a given organism. And this has just to do with humans, a numerical fraction of the whole universe. Not to mention the quantum dimension. Now, do we have to stop doing research, because of these arguments? Stop looking for new answers to our ever-growing questions? NO and NEVER, because this is just the magnificence of our work: accept the challenge trying to uncover new fragments of an unlimited truth. This is also the reason why I have always treasured the most famous teaching of master Socrates when he said that he knew of not knowing. I believe that staying humble is the most straightforward way to grasp a further piece of knowledge.

1 Motivations: How I Developed an Interest in Science

CURIOSITY. My approach to science came relatively late and originated from a humanistic interest. At the time of my youth, the sixties, there was a great deal of interest in sociology and psychology. Urged by an intense political period, spread almost all over the western world, youngsters were idealistically searching new models of life while breaking obsolete social rules and enjoying a fantastic music,

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still unparalleled even nowadays. Grown up in a modest family and forced to attend a high school focused on chemistry, I ended up developing an instinctive interest in the organic base of the living matter wondering if anything, even human attitudes could eventually be traced back to it. I was someone like an anachronistic follower of Descartes' mechanistic view of human life or a pioneer of modern transhumanism theories, depending on the temporal side you want to take. I didn't care so much about intangible, vague matter of discussion or, better, I wanted to know how much of it could find a rational, organic, science-based explanation. Genetics was the field in which I choose to carry on this personal exploration with the idea that if everything was encoded into the DNA then it was just a matter of time to find the answer. This is a fideistic trait not too far away from some current thinking of the genome BIG DATA collectors. At that time, the influence of the environment on the way a genome could be differentially expressed was substantially neglected. Actually, Lamarck and any renovated version of his theory was ignored if not scorned. Cold war ideology, politically influencing science, was also a factor because the Russia of Stalin had promoted the idea of Lysenko by which the environment is stronger than genetic inheritance while the USA of the Statue of Liberty were discriminating amongst immigrants on the basis of their performance on IQ testing thus concluding that some populations were inferior to others, with the Anglo-Saxons as the champions of intelligence. Where was the truth? Speaking about intelligence can we even today explain what it is? How many forms of intelligence exist? Now that we have explored so many human genomes, do we have any genetic clue as to which nucleotides encode for it ? If we could insert the nucleus of Einstein into a human egg cell, mimicking the Dolly approach, would we generate a progeny of genius? If genetics cannot find the basis of intelligence, or any one of its multiple forms, how can bioinformatics work out their algorithms to develop robots that act like humans? Transported to the actual time, these were the kind of questions that were stirring my mind. **So, more of Mendel and less of Freud**. It was with this inclination that, while searching for a tutor of my thesis work, I asked my genetics teacher Prof. Giovanni Magni to help me in joining the lab of Prof. Luca Cavalli Sforza, a world-wide recognized expert/master in quantitative genetics, who was working in Pavia. I was unlucky since right at that time (1980), Cavalli Sforza moved to Stanford University in the USA. **Luck is a factor** as we will see. Ironically, and this gives the reader an idea of the weirdness of bureaucracy when science is ill-administrated, I have just recently closed (2022) an evaluation of a genetic test for lactose intolerance put in place in 2002 under the advice and consultation of Prof. Cavalli Sforza. So quantitative and population genetics were gone, and it would have been forever. I was left with, at that time for me, less intriguing, lab-based qualitative genetics, that by the way was almost in its infancy. Restriction enzymes were crude extracts from microorganisms prepared in the lab, separating columns were made by pouring gels into chemistry pipettes and I was happily transporting radioactive vials on a tray placed on my old bike to reach the closest institute equipped with a beta-counter. A kind of romantic, and dangerous, way of living science. What was supposed to be the occurrence of the moment, an unexpected change of plans, turned out to become a sort of philosophy of my way of

doing science, looking for different subjects of investigation in different organisms with different approaches at different periods of time.

In fact, ***the philosophy of change*** has sustained my freedom of learning, thinking and acting. Only in this way, by this attitude, I could preserve my enthusiasm, my wonder, the very base of knowledge. You are happily assisted in this by the ever-changing wonderful complexity of life, of the world. ***Panta rei***.

As a matter of fact, my thesis work on the time of synthesis, during DNA replication, of different families of human repetitive sequences was appreciable and not only assisted my knowledge on molecular biology techniques but, more importantly, drove my interest toward the less popular fraction of DNA, yet the most abundant, that doesn't encode for protein (ncDNA). Covering more than 97/98% of the Eukaryotic genomes was imprudently termed as selfish DNA or junk DNA even by very important scientists. At that time, I could not know that I would have spent several years in trying to understand the role of introns, elements belonging to that fraction of ncDNA. A lesson was about to be learned that is that the less obvious, the less evident, the less established is also the most attractive, surprising and exciting of the arguments. ***The dark side of the moon***. A course and a book contributed to further shape my personal inclination for science. In 1980 the University of Pavia held an EMBO course on DNA replication, recombination and repair with the participation, as teachers, of a group of the most well-known and respected scientists of the field led by the Nobel Prize winner, Arthur Konberg. I was strongly impressed by their conduct, by their openness, their helpfulness. They cared to spend time with us, answering our questions, transmitting in the simplest yet rigorous way their knowledge and teaching the art of reasoning. **Kings were dedicating time to peasants**. Incredible. Thus, science was a land of democracy where everybody is allowed to raise a question or to offer a line of thinking, a land where the strength and correctness of reasoning was the only thing that mattered. **Brain, not muscle**. In the meantime, I was reading the book entitled "Advice to a young scientist" written by another Nobel Prize winner, Peter Medawar. Another clue that prestigious, successful scientists cared about the rookies, the new entries, their younger colleagues to favor the flow of knowledge***, to raise children on the giant shoulders.*** The book of Medawar is full of advice and descriptions of the scientific environment with important and erudite references on ethics and philosophy. It starts with three important questions. How does one know if he/she is fit for science? What will be one's subject of research? How does one select a good place for his/her scientific training? Over there, in those pages, I found again my philosophy of change. In fact, the young scientist is warmly recommended to move out from his/her original lab, leaving his/her tutors and approaching new lab and subjects of research which I did, once graduated, leaving my Italian group to land in the USA where I was first involved in an immunology-oriented project and then in the discovery of the cellular counterparts of the viral oncogenes, that I searched for in yeast! This was the uttermost and most daring of the changes. But be aware as you'll pay a price for that: productivity hence career. **Freedom is not for free**.

2 Work Done: My Personal Scientific Approach

CHANGE. The major achievement of a 40 year-long career has been the development and the setting up of a method. Working out a method capable of providing reliable and consistent data has been the most intimately rewarding act of my scientific experience. **It is yours**! It is a product of your genius no matter how small it is. Experimental science depends on methods and if one thinks about it, the history of science is paved by methods starting from the *piano inclinato* of Galileo (1604) to genome editing (2012). Of course the one I have developed which is capable of providing, in an easy and reliable way, the genomic fingerprinting of any higher Eukaryote, doesn't aspire to that level of greatness but has provided me with many rewards. The first of them has been my freedom to do research. Let's describe the method in a few words and then discuss the associated aspects (see the right part of the Fig. 2).

It is the story of how elegant biology has been displaced by powerful technology. In fact, it has been through thousands of years that evolution has worked out a fine apparatus, the mitotic spindle, to ensure that, in Eukaryotes, the genetic heritage could coherently pass from mother to daughter cells. The spindle is principally made up by microtubules that bind, pair and move the chromosomes. Microtubules are made by filaments of alpha—and beta-tubulin, monomers added in a head to tail fashion. Hence, tubulins are key components for the maintenance and function of the spindle. As such, their primary amino acid composition is highly conserved. At a DNA level, this reflects in equally highly conserved nucleotide sequences that are only interrupted by two introns (variable, non-coding parts of the genes) at conserved positions in vertebrates and plants. So, if one placed a couple of primers at the two boundaries of the exons that flank the two introns, amplification done by PCR would produce fragments of different length, sequence and numbers in any genome, since that of tubulin is a gene family. **You generate a species-specific DNA code**, which is very simple and handy. I named it: TBP for Tubulin-Based-Polymorphism. This was worked out, and published in a patent, five years before the birth of the COBL (Consortium of Barcoding of Life) where DNA barcoding is instead more conveniently obtained by plain DNA sequencing of targeted mitochondrial or plastidial genes. I was creamed. The whole scientific community gathered under the flag of the COBL sponsored DNA barcode and our invention went neglected. Yet, my lab survived and our work was recognized at experimental, applicative and dissemination levels. These are the principal reasons. Experimentally, even the classical DNA barcode has its own limits, especially, but not only, in plants where the species boundaries are not well definable and there is the need to recognize varieties, landraces, wild species, hybrids. A method that doesn't require an a priori knowledge of the target DNA sequence may turn out to be very convenient. Another advantage is the application to mixtures like feed or food products where the TBP method can easily recognize the different ingredients down to a very respectable and useful quantitative limit. Here is where TBP turned out to be appreciated by farmers and industries allowing them to check their raw material against contaminations and frauds as well

as helping in assisting the release to the market of authenticated products. The money we received from these contracts was used to support our research on more basic scientific issues. Last but not least, the conceptual simplicity of the method favored the dissemination of key genetics concepts to students, farmers, and the general public, which was done by making videos, organizing events or delivering on-site lectures. Here to follow is just one of the stanzas of a small poem entitled TBP-DNA barcoding:

> *But the Microtubules stock/has* ***Tubulin*** *as the building block/This protein piles up in stalks/thank to conserved sequence docks/These* ***conserve aminoacid domains/****in the sequence of DNA are also well retain/so that is almost a game/to selectively amplify them and thus gain/an exclusive profile that renamed/as a* ***DNA barcode*** *acclaims/the diversity of life deserved fame.*

The story becomes even more interesting and instructive if one wonders how I ended up working on tubulin after my first experiences with human DNA repetitive sequences, immunology, under the tutelage of Prof Nicoletta Sacchi who became one of the most famous women in science, and virology. As already mentioned, looking for a biological role of the cellular counterparts of viral oncogenes I decided to address the question by working on the yeast *Saccharomyces cerevisiae*, a simple unicellular model organism that allowed sophisticated genetic approaches. This is a splendid organism with a refined genetics and a superior and precise DNA recombination system, natural precursor of genome editing. I attended a course at Cold Spring Harbor, at that time directed by the Nobel Prize winner James Watson, of the DNA double helix fame, under the training of Gerry Fink and Fred Sherman, two fathers of yeast genetics, and going back to my lab in NIH I was assisted by two other great scientists: Alan Hinnebusch working on campus and Kelly Tatchell who was at Penn State University. Under such an aura of greatness, I was able to give my small contribution to the role of the RAS cellular oncogene, primarily working on its pattern of expression in cell division and in response to external signals, facilitated by the availability of suppressors of its function. I was adopted by the yeast community, a very open circle of scientists continuously exchanging information and strains. I went back to Italy with this background and a lot of hope to further continue my work now that I had accomplished Peter Medawar's advice. But **life is never easy and one must be prepared for unexpected upheavals**. I was to join an Italian Institute that under the ghost direction of the President of the National Research Council (CNR) was at that time sponsoring its participation in the emerging HUGO (Human Genome) project thanks to the involvement of the Noble Prize winner, Renato Dulbecco. At first, my yeast expertise was appreciated since I set up, thanks to a collaboration established with Prof. Maynard Olson and Prof. David Schlesinger (University of Saint Louis), the megacloning of fragments of human chromosomes in yeast and the PFGE techniques for separating large size DNA molecules. Unfortunately, it soon turned out that participation of the Institute in the HUGO project was much more a matter of money and politics and much less of science. I spoke out about it loudly and had to quit. I joined then a Plant Biology Institute trying to rescue the authenticity of my yeast period, ignoring the

self-interest of humans. I could not go on working on yeast though and so I decided to translate my yeast interests into the two different issues of plant signal transduction and plant cell growth and division. Calcium protein-dependent kinases (CDPKs) and tubulins were selected as the two respective champions in the hope that one day I would have been able to uncover some functional link between the two. There was an even subtler reason. While CDPK was a somewhat confined issue, tubulin would have been, and indeed it was, and it is, a field of investigation that would have allowed me to move in many different directions satisfying my thirst for change and protecting me from any change in the leadership of the scientific direction of my institute, at the time under dispute by three major scientists who were working on gene expression, protein synthesis and accumulation and stress response, respectively. If you work on tubulin you can address all these issues, uncovering inherent unique aspects, and many more such as: DNA methylation and parental imprinting, pseudogenes, promoters, naturally occurring anti-sense RNAs, co-translational control and post-translational modifications, polyploidy, anti-mitotic drugs, embryo plane of division, motor protein interactions, viral propagation, pathogen attacks, plant morphogenesis, weed control, pollen development, endosperm ontogeny, cellulose biosynthesis. Actually, my group has been recognized, by the cytoskeletal community, for the work done in the characterization of plant tubulin gene families, their regulatory elements and pattern of expression (Breviario et al. 2013).

However, **it is the funding availability, or lack thereof, that will orient your research**. In fact, CDPK were the first to be abandoned despite the fact that for a while my group was a point of reference for many labs to ask for specific antibodies and cDNA clones. Studies on introns and their effect on gene expressions lasted longer but when it came the time of funding obtainable just with applied science projects I had to turn to ILP (Intron Length Polymorphism) that is TBP and thus we are now closing the circle of this short story which, I hope, should tell the reader about the vast possibilities of study and change that Science can offer and the resilience ability a scientist must have.

3 Science Today and Tomorrow

COMPLEXITY. Figure 1. What comes next depends on the idea you have of science, knowledge and progress. If you stand with Thomas Kuhn, who is considered the father of extant philosophy of Science, the progress in knowledge is neither granted nor linear. Each axiom must be experimentally verified to proceed with the acceptance of the current theory until the emergence of one or more anomalies, that cannot be explained, requires new thinking and propositions. This eventually leads to the definition of a new paradigm. In other words, knowledge in Science, that is different from progress in technology (see below), proceeds by discrete steps, and it is hard, if not impossible, to predict the direction of it while it is illusory to think of reaching the ultimate, revealing TRUTH. At the very least, this inference can be brought back to the birth of quantum physics, to the Heisenberg principle of uncertainty,

Fig. 1 My office, where chaos hides an order

and to Godel's incompleteness theorems about the intrinsic limitation of any logical system. Well known and often cited examples of paradigms are those that marked the change between the Ptolemaic and Copernican astronomy systems, the Newtonian revolution in physics, Einstein's theory of relativity, quantum mechanics and now is time for genetics to enter into this perspective (Fig. 2).

In fact, a century and a half of progress in genetics, starting from Mendel's laws of inheritance, has brought knowledge to a transition phase between an old over-simplified paradigm, where the central dogma has been the cornerstone, to a new paradigm yet to be defined. Premonitions such as the C-value (total DNA content doesn't correlate with complexity) and the G-value (estimated gene number does not correlate with complexity) paradoxes had been known for a long while but a decisive, yet problematic, contribution has been given by the massive sequencing of genomes that has revealed an extraordinarily large amount of dark matter, meaning the presence of non-protein coding DNA and RNA, the former accounting for more than 95% of the higher Eukaryote genomes and the later surpassing by far the sizes of the genome of reference. Neither can be easily traced back to the descending flow of information of the central dogma, from DNA to protein, since they are often attributed, but rarely demonstrated, to multiple regulatory functions and interactions. A dark matter that is still waiting to be deciphered and, when done, it could possibly lead to changes in the way we have been referring to genetics so far. For instance, what do we actually know about the molecular mechanisms defining speciation in plants

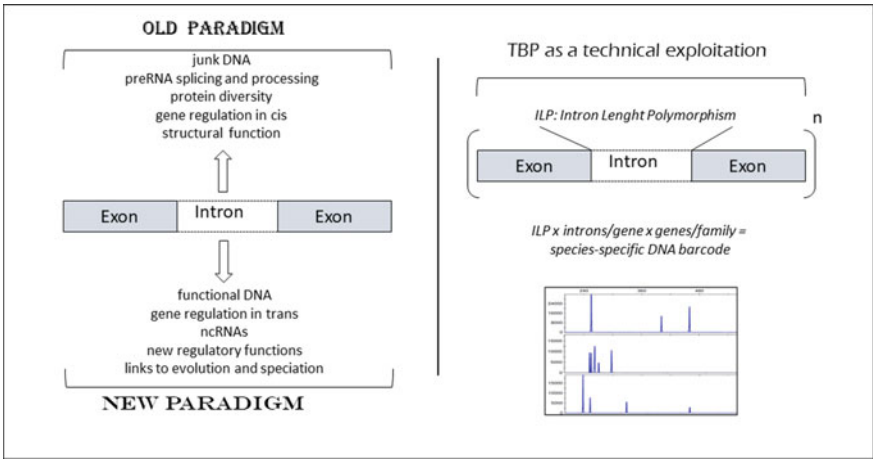

Fig. 2 The old and the new paradigm in genetics

where liberal outcrossing is defying sex barriers? Referring to my limited experience with the method described above, how comes that length and sequence variations in tubulin introns strictly correlate with speciation? It may be an effect or a cause but still remains an open and challenging question and thousands more can be similarly posed when investigating the complexity of genomes and transcriptomes. In order not to disregard proteins, always referring to the central dogma as the stronghold of the old paradigm, we should not forget the puzzling issues raised by prions. So, there is still a lot of work to do even if one restricts oneself to classical biology and genetics but of course we have to consider the contribution that may come from quantum physics to the possible unravelling of a new biological paradigm. After all, molecular genetics deals with the molecules world, not atoms, neither electrons, nor other subatomic elements, which together represent a higher and deeper level of resolution of biological matter. How are they going to impact on the current status of genetics knowledge? DNA mutations have been already explained through a quantum model. What will be coming next? And in which context? Reading the capital and visionary book of Erwin Schrodinger entitled What's life, which by the way I would make mandatory in any course of science, it is understood that the macro-genomic order that supports, preserves and inherently propagates biological life is dominant on the chaotic single- and sub-atomic events that would eventually lead an organism to its maximum of entropy that is death. By eating, drinking, breathing, assimilating, and very likely thinking, biological organisms replenish themselves of that amount of orderliness required to remain alive. Quantum biology is definitely an issue for next generation science.

Let's now go back to the concept that massive genome sequences have shaken the central dogma but still they have not paved the way to a new paradigm. In my opinion this is due, to a certain extent, to what I would call the survival instinct of scientists who find it more convenient and immediately rewarding to stick to the

coding part of genomes rather than bravely heading toward the dark matter. Scientists must publish to boost their reputation and to progress in their career and it is much more convenient and a lot easier collecting data and information on genes and their patterns of expression than muddling with the unknown. This would be part of a more sophisticated reaction that, as argued by Lakatos, brings scientists to shield the nucleus of the existing theory by a so called "protective belt". In turn, this is instrumental to **the easy construction of models to be applied under the most different physiological and environmental conditions**. Models are quite distant from data corroboration and methods realization. They can easily vary depending on the variables that are incorporated. Models built up on data collection are even less consistent, semantically incorrect, since are often presented regardless of their proper justification into the currently accepted axioms of the theory of reference. Because of their intrinsic inconsistency, models cannot even be falsified, in the most classical Popper view. **Models represent examples of inductive, sometime adductive knowledge which can yield different conclusions depending from different possible starting premises.** On the contrary, deductive reasoning is based on widely accepted facts or premises. Only when models are not opportunistically proposed, they can offer some idea on where to move for new investigations. In a way Plato had already warned us about this pernicious attitude when he made a clear distinction between **knowledge and numbers**.

Let's take a typical whole genome sequencing project that provides numbers, by search and definition. If the investigated organism is an Eukaryote it will be very easy to find out the number of the alpha and beta tubulin genes but the question is: how this numerical information is going to provide knowledge on even just one of the following issues? **Multi-tubulin hypothesis.** Since its first proposition in 1976 (Fulton and Simpson 1976) the question of why a highly conserved structural protein such as tubulin is actually encoded by a discrete and variable number of genes has not found an answer yet. Is it a regulatory or a functional issue or a mix of the two? Sporadic and not fully convincing evidence has been obtained so far and nucleotide sequencing is not going to help. **Intron length polymorphism**. The first question here is why tubulin genes conserve introns since, at present, no form of tubulin has ever been observed that could result from alternative splicing. Is thus a purely regulatory matter and, if so, why does intron length vary within the members of the same plant species and among the members of different plant species? Since speciation is the final result of the accumulation of DNA new arrangements and mutations, inevitably reflected in the length and sequence of the tubulin introns, it may be that the introns also influence the efficiency of chromosome pairing by some yet unknown ribonucleoprotein complex. After all, colchicine, a well-known anti-microtubular drug, it is used to overcome the sterility of hybrid species, by producing chromosome doubling. **Pseudogenes**. When looking for tubulin-like sequences within a genome you can also find a certain number of tubulin pseudogenes, forms of the genes that cannot encode a functional product because of the presence of several mutations that affect the correct frameshift. What actually are pseudogenes? The useless remnants of previously functional genes or an intermediate form that will eventually evolve into a new, more adapted tubulin isoform? That would be to say that the most evolved

form of a gene is when it loses its coding capacity. **Natural occurring anti-sense mRNAs**. For tubulin, they have been found at least in maize. The question then is: what's the role of these molecules and are they present in other organism besides maize?

All these issues ask for answers which cannot be provided by the simple numbers of a genome sequencing project, neither by their further bioinformatics elaboration but they rather call for a new, wild way of thinking that must be supported by a new scientist attitude for enduring the hardship of a long time spent in experiments that will not lead to easy publication. But, **what does the world really want: papers or knowledge?** As my friend Peter (Nick) says: a lot of data doesn't mean a lot of knowledge.

Once the current inebriation for models built up on any numerical assembly of known functional parts of the genomes will find an end and the scientists will start to look convincingly into the dark matter, withstanding a certain degree of unproductiveness, a fundamental step toward a new genetic paradigm will be taken. This goal could be more easily achieved if, simultaneously to the explosion of the BIG DATA era, some adjustments in the more general terms of doing science will also be introduced. I am here referring to a more careful and moderate rate of papers production (*Einstein: An academic career, in which a person is forced to produce scientific writings in great amounts creates a danger of intellectual superficiality*), on a stronger request for data repeatability (possibly with dedicated Journals), on a better control on raw data acquisition and elaboration, on the definition of new statistical limits of significance, since false positives may be orders of magnitude higher than real data and undesirable behaviors like that of p-hacking must be alienated. On the contrary, the time is due to publish negative results if the construction of the investigation plan is solid and to make reviewing a more responsible and gratifying job. So accepting the Kuhn model of progression of scientific knowledge, that cannot be linear as the whole is unlimited, there are **two quite distinct ways in which future science can be performed which we can call the accumulating and the breaking free way**, each one with its own role and references. A good quality accumulating science is based on experimental evidence, corroborating concurrent data and theories formulated in a new positivism milieu, qualified by high impact factors and citation index of the publications. The breaking free way, evidently moving toward the new paradigm, is based on wild discoveries, original ideas in a yet undefined new theory, supported by a post-modernism thought and qualified by a high disruption index. Technological progress is not necessarily bound to any paradigm. The old paradigm may contribute as well since it is a matter of practical tools and applications. This calls for the last consideration of this section which I leave to Peter Medawar in the following quote: *science must face the problems that trouble the humans struggling to find technical remedies and solutions but the direction to be taken, the priorities to be assigned, the distribution and coordination of the activities into the society go to politics that has to take the responsibility. Science provide new and diverse solutions but doesn't stand for a specific one.*

4 Advice to the New Generation of Scientists

WONDER

Aristotle stated that: "*Learning things and wondering about things, as a rule, is pleasant. For wondering implies the desire to learn and to know"* (Rhetoric 1371). I do strongly believe that science is for those people who are capable of wondering, who have a positive and enthusiastic way of looking at the miracle of life, who are constantly and intimately asking questions and try to find reasonable answers, who are not afraid of cultivating daring ideas and take the challenge to verify them. The one who is sustained by such a sacred fire will be content, no matter how many difficulties she/he will encounter. On the contrary, if one thinks to take science as an ordinary job, she/he will soon feel to be out of place and, by carrying on, she/he will eventually damage science and society. She/he will become an unhappy clerk or an ambitious bureaucrat but not a scientist. So the first basic question one has to answer is: **how I feel about Science?** If the answer is positive then the next question automatically follows: **am I fit for doing science?** Indeed, because the job is not that easy and, as I said, one must feel a strong commitment. Let's start by saying, partially contradicting what I have just written because fanaticism can be a mistake as well, that, once the commitment to science is there, one's important contribution can also be deployed on the path of accumulating additional evidence and data from experimental approaches designed to corroborate the extant theory. After all, there are **four roles** that a dedicated scientists can perform. **The explorer**, who produces new data, unravels new evidence, makes new discoveries. **The inventor**, who develops new methods, new materials, new algorithms sustaining innovation and technology. **The philosopher**, who takes the challenge of more fundamental and radical questions. **The teacher**, who has the responsibility of a correct education and of the dissemination of scientific theories, information and data. All the four types jointly contribute to improve human knowledge and their role in society is and must be fully recognized. This is more appropriately laid down in the European Charter for Researchers where rights and duties are also well defined.

Why the experimental job is so difficult? Hereafter, my multiple answers and humble advice.

The rate of success of even a properly planned new experiment is exceedingly low, This causes frustration. As I have always said to my students and young scientists, the percentage of success in a truly new experiment is less than 10. If you are not motivated you cannot seriously face such a high level of failure, that goes reiterated for any experiment that is not routine and confirmatory, and even there you may find problems.

You feel the pressure of being a productive scientist. Publish or perish, remember? If you do not manage this, and it costs energy, you may end up producing a series of irrelevant papers, or plagiarize the work of others or, even worse, publish biased and wrong data. You need your own money to work and competition is very high. Either you manage, because of your recognized expertise, to enter a consortium of people

who are interested in having you or you must be firmly convinced of your idea, your project, and yet it may not always suffice.

Writing a paper or a proposal is not that trivial and it may cost a lot of time, effort and energy and yet you may be facing failure, and sometimes you feel you have not been judged fairly.

Developing an entirely new method, not an upgrade of an old one, is a difficult task but it is even harder to make it fully reproducible in any lab and context. It requires time and patience and you are not likely to be funded for this.

Science is a very competitive area and yet you must be fair with yourself and colleagues. You should admire and not envy the ones who are better than you. You must acknowledge your limits and always try to improve your standing. **It is always better to be the last of the firsts than the first of the lasts**. At the same time you should not envy the ones who are successful even when you think that they did not deserve it. Once again, remember Aristotle and his saying: *dignity does not consist in possessing honors, but in the consciousness that we deserve them.* **Be content with your dignity**.

Follow your idea. Do not anticipate in your mind the results you will obtain from your experimental plans. Just do them and analyze the data. Do not think a priori of any impediment that will determine the failure of your approach. It is often a useless and ill-based speculation. **Do and then think. Do not think so that you never do**. Be aware that the stronger part of any of your experimental design is provided by the right controls. Controls are more important than results.

Do not follow the stream of the most fashionable science of the moment. Be an expert on something. At the next round science will come to knock at your door.

In presence of a recognizable and documented reputation for both, think and decide which you like better: a renowned large high technology lab with big numbers or a small science team with a more radical thinking?

Do not restrain yourself from conceiving several ground-breaking ideas, just apply for them. It is like in finance: you invest in many products but one will be enough to pay you back. You'll be content and have the lead of that field. You also will be content if and when one of your ideas, untimely and for this not financed, will eventually become a major field of investigation for others. That means you have a good brain, a good perception of science and on that you can count. That has happened even to me many years before the start of metagenomics. I was thinking and proposed to trace the geographic origin of cow milk, at cattle sheds, by making subtraction libraries (at that time there was no massive sequencing) counting on the presence of different bacterial strains, different feed and different bovine race DNAs.

Do not prolong indefinitely your training period, no more than 3–4 years says the European Charter, because that will make you dependent on your senior. You must leave her/his lab and change the subject of research to find your own way. On the other hand do not pretend to be a genius if you are not, and yet if you are you would not read these lines because you would instinctively find your way, as Einstein at the patent office of Zurich.

Do not be afraid of changing the subject of your investigations and be multidisciplinary in your approach establishing good and fair collaborations.

Do not value differently basic and applied science. You will damage both. Just make your choice. Ultimately science is performed to improve human life through newly acquired knowledge and tools. In fact, the old society based on private property has been replaced by the new one based on intellectual effervescence, the so-called Knowledge Based Bio Economy (KBBE). Distinction between basic and applied science, although the latter clearly depends on the first, may become fuzzy and petty and can be easily manipulated. **Just go for good, new and tangible results**.

Your salary, at least at the beginning and even longer in some countries, is going to be low when compared with less prestigious jobs but be content because you will have the freedom of thought and action, which you won't find in many professions.

Be a philosopher not just a scientist. Cultivate your mind with good lectures and classic novels. There are a lot of things that philosophers and writers can teach you. Take Karl Popper for instance and his falsification principle. Karl Popper believed that scientific knowledge is provisional—the best we can do at that moment, and this is in agreement with our post-modernism time.

We have now reached the end of this essay and I guess that the final question to ask is Schrodinger's: "*I", what is this "I"? If you analyze it closely you'll end up with the impression that "I" is just the facts, little more than a collection of single data made up by individual experiences and memories. Namely the canvas upon which they are collected.* This brings me back to my beginning and my appeal to humility.

ACTION

If you become a scientist and you really want to make a difference reassigning science to the field of freedom of thought where it belongs (remember the words of the Galileo in the Brecht play: … *What are you working for? I maintain that the only purpose of science is to ease the hardship of human existence. If scientists, **intimidated by self-seeking people in power**, are content to **amass knowledge for the sake of knowledge**, then science can become crippled, and your new machines will represent nothing but new means of oppression. With time you may discover all that is to be discovered, and your progress will only be a progression away from mankind. The gulf between you and them can one day become so great that your cry of jubilation over some new achievement may be answered by a universal cry of horror. I, as a scientist, had a unique opportunity. In my days astronomy reached the market-places. In these quite exceptional circumstances, the steadfastness of one man could have shaken the world. **If only I had resisted, if only the natural scientists had been able to evolve something like the Hippocratic oath of the doctors, the vow to devote their knowledge wholly to the benefit of mankind!***) then you could adhere to the following manifesto, or something alike. Hence, you could sometime place yourself in front of a mirror and read the OATH of the post-Galilean scientist.

I SWEAR TO FULFILL, TO THE BEST OF MY ABILITY AND JUDGMENT, THIS COVENANT

- I humbly recognize that life, in its more comprehensive definition, is marvelously more complex and perfect than I could ever grasp and that universal truth is just

unreachable and yet I will do my best to improve the knowledge of humans and the quality of the terrestrial life.

- I will remember that I remain a member of society, with special obligations to all my fellow human beings. Above all I vow to devote my knowledge wholly to the benefit of mankind and resist the intimidation of the self-seeking power people and their evil distortion of knowledge and applications.
- I will always stand for freedom of thought and free circulation of scientific information and data, fighting against manipulation and anti-Science. In accordance, I will always stand for peace and will be firmly and always against any war. I will not subjugate to any ideology and religion and I will not offer my knowledge to warmongers.
- I will make every effort to ensure that my research will be relevant to society and will not duplicate research previously carried out elsewhere. I will avoid plagiarism of any kind and abide by the principle of intellectual property and joint data ownership in the case of research carried out in collaboration with other colleagues, as also stated in the European Charter for researchers.
- I will remember that there is art to science and that collaboration, information and respect may outweigh the uncontrolled urge for publishing papers or any other kind of personal recognition.
- I will not be ashamed to say "I know not," nor will I fail to call in my colleagues when the skills of another are needed, a contribution I will gladly and duly recognized. On the other hand I will not pretend to be recognized for simple supports such as providing an information, a reagent, a cell line, a strain, a sequence information and stuff of this matter.
- I will respect the hard-won scientific gains of those scientists in whose steps I walk, and will gladly share such knowledge, and the new advancements I will be able to produce, with those who are to follow.
- If I do not violate this oath, may I enjoy life and science, respected while I live and remembered with affection thereafter. May I always act so as to preserve the finest traditions of my calling and may I long experience the joy of uncovering even the most tiny piece of new knowledge.

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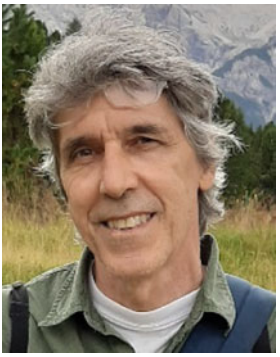

Diego Breviario Graduated in Biology at the University of Milan, Italy in 1980, he moved to USUHS, NIH and NCSU (USA) where he gained experience in molecular biology applied to the fields of human immunology and oncology. Back to Italy he continued his work on signal-mediated growth control characterizing the plant CDPK and tubulin gene families. Working on the control of tubulin synthesis, the main constituent of microtubules, he ended up in studying the role of introns in gene expression. In turn, because of some specific features of tubulin gene organization, he developed a new method for the easy genotyping of plants and vertebrates. He has been working for more than 30 years, at the Institute of Agriculture Biology and Biotechnology of the Italian National Research Council (CNR).