# Science in search for visible in the realm of indiscernible*

## Dedicated to Gerald Stranzinger

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Close to the end of the second millennium, several journalists started a kind of a public-opinion poll by virtually addressing two questions to several prominent persons: which invention or discovery had the largest impact on humanity in the past millennium, and in the past century? Among many smart and ingenious answers – short to very long – I was impressed by two shortest ones, both by my friend Rudolf Zahradnik, the former president of the Czech Academy of Sciences: book-printing and chip. Of course! Where would we stay today if Johannes Gensfleisch called Gutenberg had not discovered his printing with movable types? And how would our life be today without the integrated circuit, the chip? It would be, by all means, very different and less comfortable (Still, how many of us ever met the names Walter Brattain and John Bardeen? The two received the Nobel Prize – the latter one even twice – for discoveries which led to this genuine breakthrough in the twenties century). The chip is omnipresent and in its substance invisible for most of us.

"Invisible" is one of the keywords which accompanied science since the great change of its strategy at the beginning of Enlightenment. Besides typography and the chip, it was this new science which formed and stills forms our world and lives by seeking for explanation of natural phenomena around us and using them for very pragmatic purposes. Therefore, I would suggest addressing an additional question, namely, as to the most important discovery that enabled science to search in the realm of invisible at the time of its origin. The answer will probably be: the telescope and the microscope. There is not only a physical similarity between the two. Both rose from the idea to bring closer things which cannot be properly seen by an unarmed eye. Both were first constructed at the time in which scientists recognized that a bare perception of an object cannot in many instances offer an explanation of its substance. And the birthday of the two dates back to roughly the same time, the 17th century. As to the epistemological goals, however, there is a slight difference between the two inventions. The objects of interest in astronomy, phenomena of the Universe, were empirically known: stars on the sky and other celestial bodies were frequently "visible" but not "discernible". There was a call for a closer look at them that might bring explanations of their substance and mechanics. This was indeed a purely scientific interest. Telescope was the desired instrument to give such a closer look at the sky, and the expectations placed in it were not dashed. In addition, however, telescopes contributed to very pragmatic aims like measuring of time or determination of geographic position of merchant ships on high seas. The focus on pragmatic aims, on the other hand, motivated the construction of the first microscope: merchants and craftsmen sought for magnified pictures of objects to facilitate their daily tasks. Its inventor, Anton van Leeuwenhoek, experienced it during his apprenticeship in a linen-draper's shop when he had to count yarns in textile stuffs. Astonishingly to many, the microscope enabled to discover a fully unexpected, new field: the living microscopic world. Thus, these old optical instruments represent two different trails of experimental science: testing of hypotheses by designing experiments, and a random observation.

This article is dedicated to Gerald Stranzinger, a man whose scientific life and career are closely associated with microscopes. His area of research is cytogenetics – the *"science which links the study of the visible appearance of chromosomes with genetics",* as the Penguin's "Dictionary of Biology" says. The definition is interesting: it connects a visible structure (chromosome) with a property – the heredity. Historically, the heredity was discovered first as an abstract phenomenon, as an observation without any material – a contemporary biologist would say "cellular" or "molecular" – basis. Johann Gregor Mendel described its basic laws by a both simple and astonishingly accurate mathematical model between 1864 and 1866. It is very likely that no one ever thought at that time about a material basis of this abstract feature. The cell as the elementary structure of the living matter was naturally known – Mathias Schleiden discovered it in plants as soon as in 1838, Theodor Schwann in animal organisms one year later. Did Gregor Mendel think about a possible "cellular basis" of his discovery, did he even know the works of Schleiden and Schwann? Let's not forget that Mendel had no formal training in life sciences: he

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studied mathematics, physics, philology, philosophy and ethics in Olomouc (Moravia) and later took courses in agriculture (fruit-growing and viniculture) in Brno. Be as it may, the basis of heredity remained invisible in the time of its discovery. It is indeed well known that Mendel's conclusions were forgotten for long thirty-five years. First Carl Correns in Germany, Hugo de Vries in Holland and Erich von Tschermak-Seysenegg in Austria rediscovered this fundamental work in independently published articles, all of them in spring 1900.

Unnoticed for quite many years remained also cytological discoveries which later turned out to be in close connection with it. Formation of nuclear filaments – a "visible" phase of mitosis, was first seen in 1880 by Walter Flemming; and in the same year, Eduard Strassburger and Otto Bütschli described the whole process of mitosis, the former in plant, the latter one in animal cells. Also Paul Ehrlich, the *spiritus rector* of modern biomedicine, takes credit for a part of this development: he invented tissue staining and in 1881 reported the use of methylene blue as a suitable tool for vital staining of cells. This all facilitated the discovery of chromosomes by Wilhelm Waldeyer in 1888. However, it took other 25 years until Walter S. Sutton from the Zoological Laboratory at the Columbia University in New York made the following statement in his paper about chromosomes of the lubber grasshopper Brachyostola magna:
*"I may finally call attention to the probability that the association of paternal and maternal chromosomes in pairs and their subsequent separation during the reducing vision … may constitute the physical basis of the Mendelian law of heredity".*[1]

Probably also Theodor Boveri from the University of Würzburg independently assumed between 1902 and 1904 chromosomes as carriers of hereditary features in animals. This historical period of "making cellular basis of heredity visible" was then completed by Thomas Hunt Morgan from the California Institute of Technology in Pasadena, who made crucial *"discoveries concerning the role played by the chromosome in heredity",* as the laudatio of the Nobel Committee runs. He was awarded the Nobel Prize for Medicine in 1933.

Parallel to this development run another one, in some of its features even more interesting for biochemists: it aimed at the molecular basis of heredity. Its history started as early as in 1871, when the Swiss physician and chemist Johann Friedrich Miescher discovered in various biological materials a substance which was later, after its isolation from salmon's sperm cells, named "nucleic acid" by one of his students and co-workers, the German pathologist Richard Altmann (1889). In a pure and minimally degraded form, the deoxyribonucleic acid was obtained by Rudolf Signer from the University of Bern in 1938, and his pure DNA then greatly facilitated the famous conformational analysis resting on crystallographic studies by Rosalind Franklin and Maurice H. F. Wilkins. As notoriously known, the double-helix model based on that studies was then formulated by James D. Watson and Francis H. C. Crick at the end of nineteen fifties.

However, important knowledge concerning the chemical basis of heredity was attained already before. The great fellow countryman of Gerald Stranzinger, Erwin Schrödinger, was talking about a kind of "code-script" in each chromosome and about the importance of "complicated organic molecules in which every atom, and every group of atoms, plays an individual role" already in his famous lecture *"What is Life?"* at Dublin Institute of Advanced Studies in 1943[2]. And as an excellent theoretical chemist he ascribed a chromosome to be "an aperiodic solid". The structure of these complex "solids" was identified one year later (1944) by another great biologist, Oswald T. Avery. He and his co-workers, Colin M. McLeod and Maclyn McCarty showed, in one of the first transfection experiments, that DNA from the virulent strain of *Pneumococcus* Type III (S) can transform the avirulent Type II (R) into a virulent form. This "transforming principle", as Avery wrote to his brother, *"may be a gene"*. And, as we now know, it was really a chemical basis of a gene!

In the last two decades, an enormous progress was achieved in elucidation of genetic basis, not only in human but also in many animals, among them also farm animals, and prokaryotes. Complete structures of chromosomal DNA were deciphered, within them coding sequences of many genes delimited, the universality of the code was recognized, and genes were mapped on individual chromosomes. Does this solve all problems of genetics? The answer may be perhaps yes, on condition that not only a gene structure but also all structure-function relationships linked to it are known. This knowledge is still largely missing, even when the structure of the genome may be a key to *all* laws of genetics. First, not all gene products are known – the new area of proteomics aimed at this target with by part newly developed tool. Second, even if all gene sequences were identified, not all genes are active within the same space (cell) and time period; moreover, epigenetic factors, cross-linking rules and many

[1] Biological Bulletin 4 (1902) 24–39
[2] First published 1944 (Cambridge University Press)

other phenomena could probably be explained at others than purely genomic levels. And on top of it, the complexity of experimental tools which the "pure" genomics is presently using would be prohibitive for pragmatic genetic aims.

This was well known to Gerald Stranzinger and his colleagues. Geneticists of this generation were aware of the necessity to possess empirical tolls which would facilitate links between some tangible entities and genetic features, even though the true material nature of heredity was not yet "visible". One of such links were chromosomal maps obtained by specific "band staining"; it is to the merit of Gerald and his co-workers that these maps were completed for the most important farm animals and also some other animal species, sometimes even quite exotic. Another link were marker genes, the use of "quantitative trait loci" (QTL), and later gene mapping by means of *in situ* hybridization, polymerase chain reaction and other tools of the new technology developed by molecular geneticists for this purpose. In all of them, Gerald Stranzinger left his traces.

Let us return to the phenomenon of visibility. Clearly, the same task, namely, to make the invisible visible, is in the aim of any scientific field – depending only what one understands by "visibility". Also an abstract interpretation of visibility is possible. Some years ago, I came across a unique book by the Stanford mathematics professor Keith Devlin; its title reads *"The Language of Mathematics.* ***Making the Invisible Visible****"*[3]. Obviously, also mathematics can discover the "invisible", by using its specific language. Already an exact formulation of a problem and substitution of known elements by abstract, symbolic terms can lead to a detection of invisible elements, however, in the same symbolic form. This is frequently the way which physicists follow: a prediction achieved by theoreticians is given over into the hands of experimental physicists. In the past century, it brought remarkable knowledge about basic laws of Nature.

And wasn't actually the prediction of atoms a similar history? The original notion of the Greeks Leukippos, Demokritos and Epikuros around 400 B. C. was merely a philosophical one, formulated in 17th century by Pierre Gassendi as a physical phenomenon. But first the Italian chemist Stanislao Cannizzaro established it as a base of chemistry by clarifying the terms "atom" and "molecule" and by applying Avogadro's laws on chemical reactions. Manifold evidence for their existence was brought since then, mainly by visualizing traces of their particles; a great step forward in this direction was the recent development of atomic-force microscopy. This is sufficient for having a clear notion of their existence and composition; but, however, until now the atoms were not "seen". Should we consider the collected evidence as circumstantial? I am sure that such a classification wouldn't find much support.

Deep in my memory remain many disputes with Gerald Stranzinger together with other colleagues at the lunch table in the faculty club at the ETH or on numerous other occasions. Similarities and divergences of our own research areas – cytogenetics and endocrinology – were frequently their subject. There are certainly more similarities than divergences between them. Ideas, deductions, analysis of known facts and search for clarity – visibility! – are common goods for any scientific field. Frequently, an idea or an abstract phenomenon, like heredity, stands at the beginning of a new development. Then, the progress in methodology enables to find correlates to "visible elements". By using them, the deductive process results in formation of a theory.

This was the epistemological history of heredity. In my own research area I have encountered a similar history: the transfer of information between cells. Hormones were defined as "chemical messengers which control the homeostasis" by William Maddock Bayliss and Ernest Henry Starling in their famous textbook of 1904. It was recognized later that these messengers conduct signals from endocrine ("sender") to target ("recipient") cells in different body compartments. Single hormones were then continuously discovered in the course of the following years (until, in fact, the present time) but the mechanism of their action on the target cells remained unclear. The answers were searched for at the borderline between pharmacology and biochemistry and came sometimes from far away. Already in 1894, Emil Fischer recognized that the binding of a substrate to its enzyme is the *conditio sine qua non* of any enzymatic reaction: substrate and enzyme have to fit together like a "lock-and-key" (This notion, which in fact is just another dogma of science, has been accepted until now). Paul Ehrlich extended this notion in his address to the Seventeenth International Congress of Medicine in London in 1913 to a biological action of drugs: a substance requires a binding to an attachment site on the cell. *"Corpora non agunt nisi fixata"* was his motto, perhaps as a slight parody on *"Corpora non agunt nisi liquida",* a similar dogma in contemporary chemistry. Ehrlich used the term "receptor" already in his Nobel Prize lecture in 1908. Later on, in 1926, A. J. Clark ventured

[3] First published 1976, last edition 1996 (W. H. Freeman and Comp., New York)

to present a scheme of the interaction between an active substance – a drug, a hormone, or a neurotransmitter – and a receptor in terms of chemical reactions. A receptor, however, was a fully abstract notion, without any recognized material basis.

However, in the first half of nineteen sixties, the first steps to endorse the abstract notion experimentally and to make receptors visible, were undertaken. The support rested on the evidence that substances can really bind to cell or membrane components and that this binding follows the saturation kinetics. Newly introduced methods like radioactive tracers, techniques for cell dissociation from a tissue, cell separation and purification, ultrafiltration procedures and, last but not least, a highly efficient computer technology enabled to investigate the interaction of a – still largely invisible – receptor and its small molecular partner, the hormone. This progress offered a closer look into biophysical mechanisms of these fascinating molecular mechanisms in living cells.

"More visible" were receptors only about twenty years later. Around 1983, the first receptor genes were cloned and their sequence was determined. Since then, several hundreds followed and we are at present witnessing attempts to discover their conformation in their native surrounding, within the cell membrane or in the intracellular space. In this last example, the invisible phenomenon was first formulated operationally, as a part of a physical system. When the generation of Gerald Stranzinger and me started to work in research, some forty to fifty years ago, there was not the slightest evidence that a receptor is a physical entity, or at least, what its nature might be. Here, the brilliant abstract prediction was correct, and after years it lost its abstract character. It became *visible.*

Is there an approved method how to arrive at such brilliant predictions, in the realm of invisible? Such a standard way of predicting would point out to future scientists the right direction, guide them on that way, save resources which become scarce in the modern society, and also protect a scientist against depressive feelings, each time associated with a failure of his or her research. But we all know that such logical or other tools do not exist. Albert Einstein explained it in the following fitting words:
*"Höchste Aufgabe der Physiker ist also das Aufsuchen jener allgemeinsten elementaren Gesetze, aus denen durch reine Deduktion das Weltbild zu gewinnen ist. Zu diesen elementaren Gesetzen führt kein logischer Weg, sondern nur die auf Einfühlung in die Erfahrung sich stützende Intuition."*[4]
We all can only hope that this intuition, probably a rare gift by our Creator, will be granted to many scientists of the coming generations. This, I am sure, will also be the best reward to Gerald Stranzinger for his scientific work and for promotion of so many young scientists.

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[4] Speech to the 60th birthday of Max Planck, on 23rd of April, 1918