Chemistry or Biology: That Is the Question

Enzo Martegani

Abstract My first interest was for chemistry. I was fascinated by the possibility to make simple experiments with reagents commonly used in any house or readily available in a drugstore or in groceries such as baking soda, vinegar, bleach, ammonia, slaked lime, iodine, quicksilver. I also tried some electrochemical experiments with low voltage batteries and salt solutions obtaining the electrodeposition of copper, zinc or water electrolysis. Then I "discovered" organic chemistry with its almost infinite possibilities to generate new compounds with a marked interest for naturally occurring molecules like alkaloids, plant pigments and dyes, and natural flavors like terpenes, organic esters etc. Subsequently I realized that the most complex and sophisticated chemistry was invented by nature in the generation of living organisms. Living organisms in fact use organic chemistry to make an incredible variety of compounds and to construct the macromolecules (DNA, RNA, protein) and lipids that working together generate the complex phenomenon that we called "life". At that point my choice was made and I decided to study biology with a focus for biochemistry and molecular biology and this opens the way to a long career in research laboratories and universities.

1 Motivations: How I Developed an Interest in Science

The beginning: I don't remember when and why I got interested in Science, but surely it was a very early predisposition related to a curiosity and a thirst for knowledge that occurred very early, probably during the period of primary school (6–10 years old….). I was fascinated by nature, by flowers and plants and I remember our old teacher (a nice woman of about 55–60 years old) took us out of the classroom in spring time and brought us to green fields showing the blossoming of flowers and the buds on tree branches ready to open for a new beautiful season. My interest in biology increased when my parents gave me as gift a small microscope (really

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it was a toy, not a serious one…) but at that time (I was about 10 years old) was a continuous discovery of new wonderful things, like the different type of pollen spores, the delicate structure of flowers, small insects etc. These early observations were also documented by hand-made drawings to record my observations. However at the end of the primary school my interest in science widens to the other scientific matter like physics, chemistry and astronomy with the help of books provided by older relatives. During this period (between 10 and 14 years old) I became deeply interested (may be even fixated) in chemistry and not only I tried to do small experiments in my kitchen, but I studied also basic stoichiometry laws and calculations. My obsession for chemistry took me to choose as a secondary school a technical one (Chemistry, really I graduated in Nuclear Chemistry in 1969) (Fig. 1). During this period I continued to do experiments in the kitchen sometimes with hazardous results, like the synthesis of iodo-acetone (a strong tear gas), or experiments with explosives that once gave fire to the kitchen table! But the real turning point occurred when at the age of 17 I read a book of Biochemistry (The Biochemical Approach to Life, by Frederic Jevons, Edizioni Scientifiche e Tecniche Mondadori, 1965). I was fascinated by the complexity of life and realized that chemistry was relevant for living things so that it would be better to study Biology and Biochemistry instead of Chemistry alone. And this actually happened: I got a Master degree in Biology at the University of Milano, with an experimental thesis in Biochemistry followed by a specialization in Biological Research.

Biology became my principal interest and was relevant for all my working life, but I had also interest for other more technical things, like the understanding and the realization of electronic devices (audio amplifiers, radio receivers and transmitters, oscillators, etc…) and I remember that during one summer I read and studied books on the theoretical basis for working with Radio and Television equipment and performed several radio transmission experiments with home-made radio-frequency generators. Later I was also interested in computers and in coding, initially in the lab where we had the fortune to have a Digital PDP-1 computer (just at the beginning of ‘80s) and then also in my home with the Commodore VIC-20 and several personal computers. In the free time I was (and I am still now) also interested in astronomy (I have an 8-inches telescope) and in playing bass guitar and/or keyboards in a local band.

2 Work Done: My Personal Scientific Approach

Don’t care about money. Although my family was typical working-class people, I have never been interested in accumulating wealth as such but instead always tried to do a job that would stimulate my thirst for knowledge and give me intellectual satisfaction. This was the basis of all my work focusing on experimental research activities without disdaining theoretical-speculative activities associated with a university teaching function. As a result I have been teaching biochemistry and molecular biology for more than 40 years through the whole career development: fellow, assistant professor, associate professor, full professor. Taking an average of

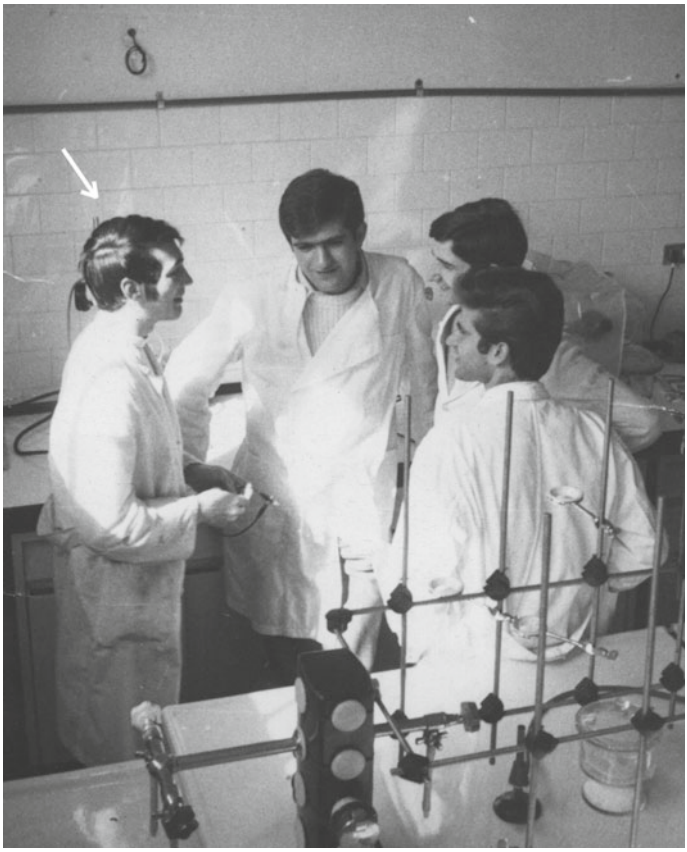

Fig. 1 ITIS-Stanislao Cannizzaro, Rho (MI), Italy, 1969. A photo taken in a laboratory of Chemistry during a discussion with classmates. The author is marked by an arrow

200 students/year, I have taught to more than 8000 students over my career. Of course teaching occupied only a fraction of my working time and most of the time was occupied by research activities done in the laboratory and also at home. The work of a scientist (or of a researcher) is not really a job but a way of life. Your work does not end when you leave the laboratory but continues outside since it is often not possible to stop to think about your experiments and your positive or negative results. It is a sort of continuous occupation and some of the more interesting and positive products from this thought process were originated by ideas generated in this way. It could be after dinner or before going to sleep, or even during summer holidays!

In the course of my experimental work I followed many fields of research that however could be grouped in almost three different topics: (1) Molecular mechanisms that regulate growth and cell cycle in eukaryotes; (2) yeast biotechnology; (3) systems biology and modeling.

The first topic is one that gives me major interesting results with also an unusual jump from budding yeast to mammalian brain. This story started with the biochemical characterization of a temperature-sensitive (ts) cell cycle mutant of budding yeast called *cdc25*. This mutant showed and interesting phenotype: when transferred to restrictive temperature (37 °C) it stops growth in a way resembling the lack of nutrients, suggesting that the principal defect was in a mechanism that links nutrients sensing to growth regulation (Martegani et al. 1984).

With the effort of my young colleagues David Baroni and Gianni Frascotti and under the supervision of Prof. Lilia Alberghina we cloned the *CDC25* gene of *S. cerevisiae* in 1985 (Martegani et al. 1986). This gene was then found to code for a 180 kDa protein that activates the two Ras proteins (Ras1 and Ras2) present in budding yeast. In fact this protein was the first GEF (Guanine Nucleotide Exchange Factor) identified and its activity was found to be essential for the activation of adenylate cyclase and of Protein Kinase A that in turn regulates growth of yeast cells. In the following years we studied the regulation of this pathway, (now called cdc25/Ras/cAMP) and several other GEFs were discovered in lower eukaryotes (*S. pombe*, *K. lactis*, etc…) all characterized by the presence of a well-defined and conserved RasGEF-domain (or CDC25 domain) of about 250 amino acids that is responsible of the interaction with Ras proteins and catalyzes the exchange of GDP with GTP, thus generating the active form of Ras (Ras-GTP bound). At that time however, no Ras activators have been identified in mammalian cells, although it was well known that mammals have different form of Ras proteins (h-Ras, K-Ras and N-Ras) that are involved in the control of cell growth, differentiation and tumorigenesis. A relevant fraction of human tumors showed activating mutations in a Ras protein. Starting from this observation we were convinced that GEFs should be present in mammals and we decided to use yeast as a tool for identification and cloning of mammalian GEFs. Using limited resources and the effort of many students, we made a cDNA library in yeast expression vectors using RNA extracted from mouse brain. We chose the brain since the nervous system is known to be rich in h-Ras. The library was used to transform a *cdc25* ts mutant strain of budding yeast, and we recovered clones able to grow at the restrictive temperature (37 °C).

These clones were characterized and we found that all of them contained a mouse cDNA responsible for complementation of the *cdc25* mutant defect. This cDNA was sequenced and found to code for a protein containing a well conserved CDC25 domain, that we called Cdc25Mm (Mm for *Mus musculus* the scientific name for mouse…) (Martegani et al. 1992), now renamed RasGRF1. This was the first mammalian Ras activator to be found and identified. My interest for regulation of small G-proteins continued in subsequent years with several works done either in budding yeast (Fig. 2) and in mammalian cells and with some interesting efforts to develop small inhibitors for Ras protein activation in collaboration with colleagues organic chemists (Peri et al. 2005).

The interest in yeast biotechnology was a relevant follow up of the necessity to use molecular biology and recombinant DNA technology in yeast as an obvious improvement for studying mechanisms of signal transduction and growth regulation in *S. cerevisiae*. Indeed this aspect was also supported by the necessity to develop

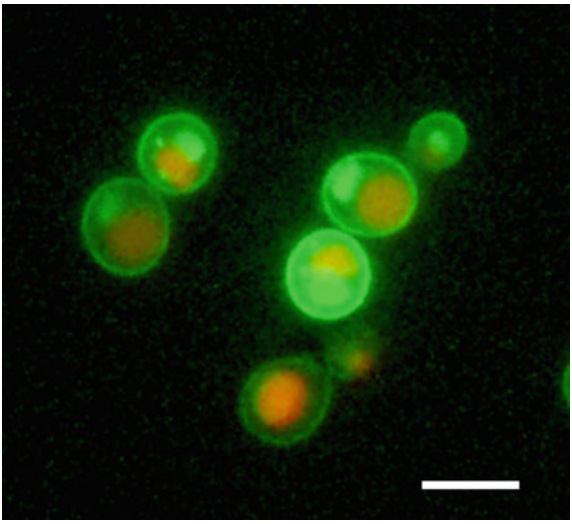

Fig. 2 Localization of active Ras (Ras-GTP) in budding yeast cells. Yeast cell were transformed with a plasmid expressing a fusion protein between eGFP (enhanced Green Fluorescent Protein) and three Ras binding domains (RBD-3) (Broggi et al. 2013). The fluorescence microscope image shows the localization of Ras-GTP in the cell periphery and in the nucleus (green fluorescence), while the vacuoles are evidenced by a red fluorescence. Bar = 5 μm

Biotechnology in Italy and we had several specific grants by the CNR (National Council of Research) on this topic. To learn the basic techniques for recombinant DNA I spent a few months in the laboratory of Prof. Vittorio Sgaramella at the University of Pavia (a pioneer of recombinant DNA research in Italy) and then a short period in the autumn of 1981 at the University of Sussex in Brighton (UK) in the laboratory of Dr. Paul Nurse to learn the basic methods for yeast transformation (https://www.nobelprize.org/prizes/medicine/2001/nurse/biographical/). The more interesting results of this research line were the expression in yeast of several heterologous proteins, like maize zein seed protein, maize B32 endosperm albumin, beta-galactosidase, human tissue-plasminogen activator (tPA), the development of computer controlled fed-batch fermentations and the generation of engineered strains able to grow and produce ethanol on cheese whey or to produce lactic acid by fermentation.

The development of mathematical models for cell growth and cell cycle was due to a singular coincidence of interests and events that occurred after my graduating in Biological Sciences, in July 1973. A few months after my Thesis defense I started for a obligatory military service, and I used part of the free time available to study System Theory and Dynamics using an universitary handout with a friend of Napoli. At the end of military service I had a fellowship of CNR for working in the lab of my thesis supervisor, Prof. Lilia Alberghina who was also very interested in System Theory and mathematical modelling of cell growth and I was immediately involved in developing this topic. We used a computer simulation approach (with a mainframe computer facility at the University of Milano) to model the dynamics of growth and cell division in eukaryotes and in bacteria, indeed this was a side-work since my primary activity was to stay in the laboratory to study growth, RNA synthesis and protein turnover in the filamentous mold *Neurosposa crassa.* In the following years Lilia Alberghina started a collaboration with Prof. Luigi Mariani, director of

the Laboratory of System Dynamics and Bioengineering at the CNR (LADSEB) of Padua that give us a strong mathematical support for the development of cell cycle models and modeling of yeast populations in the period 1980–1989 (Alberghina et al. 1986). After that my interest in modeling and computer simulations weakened but it was still present for several years to further re-emerge when a new collaboration with colleagues of the Department of Computer Science of my University started in the period 2005–2015 with the development of a comprehensive model for the Ras/cAMP/PKA signal transduction pathway in yeast.

3 Science Today and Tomorrow

Has science moved from an artisanal to an industrial dimension? That's not true at least for biology. The industrial dimension is especially relevant for technology which is an application of known knowledge but not for the true discovery which is "curiosity driven" and subject to serendipity, therefore in itself not predictable or industrializable, but reserved for the thought and genius of the individual researcher and obviously to chance. The discovery of new phenomena in biology, their understanding and use has also recently occurred thanks to the intuition of individual researchers and without the need for stratospheric economic resources. Just think as an example to the discovery of the CRISPR-Cas bacterial systems that have revolutionized our ability to modify eukaryotic genomes (including higher plants and animals) in a simple and inexpensive way, within the reach of any small laboratory (Lander 2016). Therefore, I believe that in biology the craftsmanship dimension is necessary to truly arrive at new developments that increase our real understanding of the "life" phenomenon. Does this mean that biological research is cheap? Not always there are some aspects that require substantial investment of materials and time and that allow the generation of large amounts of data (like large genome sequencing, metagenomics, transcriptomics, and all omics "sciences" in general). This type of research can be industrialized but it is a routine research that generates data and resources (a lot of data) but not a true knowledge of the mechanisms and causes! It is certainly useful for biomedical and/or biotechnological applications but in my opinion, it leaves little room for revolutionary ideas that change the way we understand living systems. Obviously in other scientific fields the new discoveries, and therefore the expansion of knowledge, are often linked to expensive technological developments that can only be addressed at a national or transnational level, see for example nuclear and subnuclear physics or discoveries related to space and to the exploration of solar system and extrasolar planets.

In the course of the last 50 years a real revolution occurred in Biology and I had the choice and also the opportunity to live and work in this straightforward period. The breakthrough happens with the "discovery" of the recombinant DNA technology that open the way not only to a better understand of the basic mechanism that generate "life" but also to a possibility to directly modify the code and to enter in the "button room" of a living organism as stated in meeting by Renato Baserga,

an Italian scientist that worked for a long time in Philadelphia (Baserga 2006). Now we have the complete sequence of the genome of many relevant organisms, and among them the human genome. These data are freely available in the Genomic Data Banks (GenBank, Ensemble, etc.) but these sequences tell us that something of relevance is lacking in order to achieve a real understanding of the information presents in these genomes. For example consider two relevant mammals, human and mouse. The genome of both was completely sequenced, both genomes encode for a similar number of proteins (about 20,000 protein coding genes), the human and mouse proteins are very similar (more than 95% of similarity in most cases), both genomes contain a high proportion of repetitive sequences (LINE, SINE, ERV, DNA transposons), the size of the two genomes is comparable (around 3 Giga-bases), but where is the difference? Why similar genomes generate so different organisms? What are the key differences in terms of genome encoded information between a mouse and a human being that are causally linked to the final outcome of two distinct species? We don't know but the deep understanding of the genome information and how it is decoded is still a frontier of our knowledge!

4 Advice to the Next Generation of Scientists

Accelerate a path of independence and seek for a position in an Institution (University or Research Center) well equipped with shared instruments and up to date facilities. This will allows a young Ph.D. student and/or post-doc to carry out good research without having high budgets. This can also be available in Italy, but the real frontier is the world and therefore it may be useful or even necessary to move abroad where the selection of proposals and personnel is more based on merit and where perhaps there are more opportunities to make a good start to a career in prestigious laboratories, perhaps under the mentorship of established scientists (not necessarily a Nobel Prize winner). This would allow you to gain good experience, learn new techniques and obviously have good publications in journals with a high impact factor that will also be useful in view of a possible return at home.

However, do not be a slave to the Impact Factor when striving to publish a good work. It is important to publish in international journals, with peer review (https://www.nobelprize.org/impact-factors/), and try not to follow fashion, but your inspirations and ideas that initially might be considered extravagant or not "fashionable" can at the end be a passport for new discoveries. Think of now famous scientists who have been refused the publication of their work in prestigious journals: just to give an example to Hans Krebs who saw his fundamental work on the discovery of the citric acid cycle (later called the Krebs Cycle) rejected by *Nature* and then published in *Enzymologia* (Krebs and Johnson 1937). Or to scientists who have had the recognition of their discoveries only many years later like Barbara McClintock (McClintock 1951), (https://www.nobelprize.org/womenwhochangedscience/stories/barbara-mcclintock). The publication in an international journal guarantees you the authorship of an original idea that, if valid, sooner or later will be recognized. For additional information on this point see also the paper of Stephan et al. (2017).

Another important point for a researcher is the ability to see what others do not see, often new discoveries or new phenomena are available but few are able to grasp the meaning, just think of Alexander Fleming, many researchers had plates polluted by molds, but they were usually thrown away without further investigation, (Fleming 1929) (https://www.nobelprize.org/prizes/medicine/1945/fleming/facts/) or of Watson and Crick, the double helix was there, there were the diffraction images and biochemical data, but only they could see it (Watson and Crick 1953). It is important to know how to seize the opportunities that are offered to us while maintaining a great curiosity and an open mind on wide horizons. Research activity often forces us to overspecialize in a specific sector and topic, but it is necessary to expand our knowledge even on fields apparently very distant from the specific topic we are working on. This will allow us great flexibility and the possibility to imagine and understand interesting events and phenomena that might otherwise escape our appreciation or be underestimated.

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Enzo Martegani Born just at the half of past century (1950): graduated in Biology at the University of Milan, Italy in 1973 and then acquired a Specialization in Biological Research (University of Milan, 1975). Working activity: Fellowship from National Council of Research (1974–75), then Assistant professor, Associate professor and Full Professor of Molecular Biology (1994–2021, University of Milano Bicocca), retired in 2022. As researcher initially studied macromolecular syntheses and growth regulation in *Neurospora crassa*, then regulation of cell cycle and signal transduction in budding yeast and in mammalian cells using biochemistry and molecular biology. He taught Molecular Biology for almost 40 years to graduate students.