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Working with François Gros at the Institut Pasteur: allostery, the nicotinic receptor and the biology of the future

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Abstract: Working with François Gros was a privileged moment in my scientific life, enabling me to appreciate a scientific personality whose generosity knew no bounds and whose vision of science was far ahead of its time.

Keywords: François Gros, Allostery, Nicotinic receptor

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We have just lost a great scientist, a colleague whose generosity knew no bounds, and a faithful friend. More than that, François Gros was for me a kind of scientific father, a role model. I met François in 1959 when I joined the department of cellular biochemistry set up by Jacques Monod at the Institut Pasteur. François himself had joined the department in 1954 from the Macheboeuf biochemistry laboratory at Pasteur. I was then a young student, certainly willing, but inexperienced in biochemistry. Jacques Monod undertook my training and managed to teach me the basics of molecular biology in a few weeks. Nevertheless, I felt quite isolated. One day, I bumped into François in the corridor of the department of cellular biochemistry.

It's important to realise that corridors play a critical role in the life of a research laboratory. It's a little known but essential fact, like tea at five o'clock, except that it doesn't involve celebrating the Queen of England. The corridor allows scientists who know little or nothing about each other to meet unexpectedly. The meeting is often of major importance, even – and especially – when the partners are in total disagreement, trapped in a catastrophic lack of dialogue…

1. Allostery

This was not the case with François, even though there was a significant difference in age and, more importantly, experience between us. Jacob and Monod were then involved in the genesis of the operon model for the regulation of protein biosynthesis (1961), which was to become famous. I wanted a close and competent partner to share and discuss my PhD project on the regulation of bacterial biosynthetic enzyme activity by negative feedback. I had chosen this topic among others (mainly on the operon), hoping for a more personal project. I was passionate about it, but sadly it didn't attract my supervisors' interest as I'd hoped. One evening, Jacques Monod even made it clear to me that this project was of lesser scientific importance than the one he was still working on himself (fortunately, he was to change his mind as my PhD work progressed). The encounter with François was therefore very timely. From the very first words we exchanged, he understood the importance of my research and encouraged me strongly to succeed in a project that was so difficult for me at the time. His interest never waned, and I kept him regularly informed of both my progress and my difficulties. Never the slightest sign of jealousy, as we all too often see between scientists, even those who are close to each other. The time came when Jacques Monod took a closer interest in my PhD work. This gave him the opportunity to distance himself from François Jacob, who for his part was getting closer to Sydney Brenner with a replicon that fizzled out. François Gros always followed with genuine and incredibly generous interest the developments that, starting with my PhD work, led to the highly popular Monod-Wyman-Changeux (MWC) model of 1965. Such was its success that, even under André Lwoff's pen, the discovery of allostery was attributed to Jacques Monod. François had followed things closely. In June 1964, my PhD work was publicly defended in front of André Wurmser, chairman, and Jacques Monod and Piotr Slonimski, examiners. Writing the dissertation had taken a lot of effort. Meanwhile, the MWC model had been submitted to the Journal of Molecular Biology by Jacques Monod. Initially, the model was to be the conclusion of my dissertation. This was no longer the case. Instead, I summarized my most personal contributions to the overall work of my PhD and the MWC model in particular. The oral presentation was well received and crowned with congratulations from the jury. Things could have ended there. However, back at Pasteur I met François in the corridor, as usual. He had attended my defense. I was very surprised when he offered to publish, in extenso, my dissertation as a series of six articles under my name in the Bulletin de la Société de Chimie Biologique – an incredible and totally unexpected token of esteem! Naturally, I accepted. In a gesture of generosity, François even offered me his secretary's help with the necessary formatting. This publication was of capital importance to me. Not only for the recognition of my work, but also because of a remark inserted in the last concluding chapter, which I've mentioned many times since. In this last part, I wanted to distinguish myself from the immediate Pasteurian environment and give a more personal tone to my text. While studying marine biology at the Arago Laboratory in Banyuls-sur-Mer, I had become interested in the behavior of a curious copepod crustacean, a parasite of Holothurians, which I had discovered, and in the organisation of its nervous system. Keeping this first experience in mind, I took the risk of suggesting in the last article the extension of allostery to synaptic transmission: "One day, we should try

to recognize in membrane phenomena that give rise both to the recognition of stereospecific metabolic signals and to their transmission – synaptic transmission, for example – mechanisms analogous to those described in connection with allosteric proteins". A program that I carried out in the decades that followed, and which is still relevant today!

Thank you François for your clear-sightedness and your generous benevolence.

2. 2. The nicotinic receptor

Towards the end of the 1960s, the scientific landscape was changing at the Institut Pasteur. *E. coli* had made a major contribution to molecular biology. Jacques Monod had proclaimed that "what's true for *E. coli* is also true for the elephant", but the reverse is obviously not true. It's hard to imagine *E. coli* growing a trunk. It was becoming urgent to switch to eukaryotes, to convert to higher organisms. After unsuccessful attempts with the nematode *Caenorhabditis* – dear to Sydney Brenner – François Jacob now turned to embryonic development and the mouse. In 1970, François Gros also chose development, but mainly cell differentiation. After a visit from Isaac Harary of UCLA and Denise Luzzati, François carried out his first work on skeletal muscle development.

Contact with François was resumed, fruitfully. It led to a genuine collaboration. A new encounter occurred through John Merlie, a dynamic young American postdoctoral fellow who was working at Pasteur in a bacteriology laboratory. He was bored. He had other ambitions. So one day, he came to see me and asked me straight away to welcome him into my laboratory. The very strict regulations of the Institut Pasteur regarding the number of researchers per unit did not allow it. So what could I do? Other than to physically accommodate John in another laboratory with which a collaboration could be established. With his ongoing work on muscle in mind, I turned to François Gros to see if we could work together. His response was, to my delight, positive. It was to be the starting point of a vast body of work devoted to the molecular biology of the synapse, both in France and in the USA.

In this collaboration, François brought his experience of muscle protein biosynthesis. I brought my knowledge of the acetylcholine receptor, present on skeletal muscle at the neuromuscular junction, and John did the experimental work.

The nicotinic acetylcholine receptor was the first neurotransmitter receptor identified. By the 1960s, the most experienced electrophysiologists and pharmacologists believed that, given the small number and diversity of molecules involved, this was not materially possible. The solution was to turn to an extremely rich organ containing a single type of receptor, the electrical organ of Torpedo or electric eel fish [1]. The electroplaques that make up this organ are transformed muscle cells. They have lost their contractility but retained their excitability. Their receptor is therefore a muscle receptor. On the other hand, the Taiwanese pharmacologist Chen Yuan Lee, in his studies of local snake venoms, had discovered that they contained a paralyzing toxin, the a-bungarotoxin, which binds selectively to the neuromuscular junction receptor. This is how the snake suffocates its prey. In association with the electric organ, it enabled the receptor to be isolated, purified and even observed by electron microscopy [2, 3].

The higher vertebrate skeletal muscle receptor itself had not been identified. By mutual agreement, we turned to the nicotinic receptor in cultured muscle cells, opening up the prospect of studying its evolution during synapse formation.

This was John's first work on the nicotinic receptor. Benefiting from François' experience with muscle protein biosynthesis, the first demonstration of receptor synthesis – labelled with a-bungarotoxin – by cultured muscle cells was achieved even in the absence of the motor nerve [4]. This validated the in vitro system as a possible experimental model for understanding the early development stages of the neuromuscular junction. This also provided an opportunity to measure the metabolic lifetime of this receptor, which is present on the muscle cell when the motor nerve terminal first arrives. The method employed was based on the incorporation of a radioactive amino acid into the receptor protein for a short time, terminated by the addition of a massive dose of this non-radioactive amino acid (pulse-chase labelling) [5, 6]. The measured lifetime is 17 hours, much

shorter than the 11-day lifetime of the sub-synaptic receptor at the adult neuromuscular junction. During the genesis of the junction, the muscle receptor aggregates below the motor terminal and, in parallel, its lifespan is considerably extended. Outside the junction, the non-junctional extrasynaptic receptor is eliminated. This work opened an important first window into synapse biogenesis, and had the distinction of being published in the English journal *Nature*.

A few years later, work on the nicotinic muscle receptor continued in François' laboratory with Christian Pinset and Didier Montarras. In the meantime, it had been shown by other groups that receptors below the synapse had a different subunit composition from the extra-synaptic receptors: a2bed for the adult synapse, a2bgd or the embryonic non-synaptic. In the embryo, the g subunit replaces the e subunit initially identified in the adult synapse. Pinset and Montarras had succeeded in culturing Sol8 myogenic cells on a mesenchymal feeder layer, which had the property of contracting spontaneously for 2 weeks. This activity brought us closer to the situation in vivo, where the junction is functional from the earliest stages and the muscle cell is active very early on. Interestingly, while the g embryonic subunit is present after 1–2 days in culture, after 5 days the e ubunit appears and is maintained over the following days, while the g subunit decreases [7]. In other words, the changes seen in vivo in the presence of motor nerves occur in culture with Sol8 cells in the absence of motor innervation. This indicates a major role for muscle cell activity – described as spontaneous – in regulating receptor gene expression during synapse formation – below the synapse – and outside the synapse. There is therefore a powerful "epigenetic" regulation of synapse formation. A major conclusion with regard to the search for evidence of learning in our brains [8, 9]. The work that followed, inspired by this first collaboration with François, enabled us to analyse the process of synapse morphogenesis in detail and at the molecular level.

The collaboration with François – all too brief – was particularly productive, with an important future.

François Gros was a true pioneer in molecular biology, but he also opened up many new fields in the life sciences and medicine, with a genuine multidisciplinary expertise that is so rare these days.

3. François Gros and the biology of the future

Always keen to share his experience of science and advances in knowledge with the general public, François has written several books of general interest. Among them is *Les mondes nouveaux de la biologie*, published in 2012 by Odile Jacob. In it, he demonstrates extraordinary insight into the future of biology. Ten years after it was written, the developments in the discipline have proven François infinitely right, on all the points he tackles.

3.1. *3.1. Biodiversity under threat*

This is a very timely issue, especially in light of global warming. All around us, species are becoming rare or extinct. François proposes to develop a systematic inventory of the molecular species still alive today and to use this knowledge to assess the threats to biodiversity and, above all, to predict the possible changes that may result. It goes without saying that the agri-food conditions to be proposed to reduce hunger in the world become essential.

3.2. *3.2.The first humans*

Nothing is more obsessive for humans than knowing our origins. For a molecular biologist like François, the question is all the more important. It's easy to say that humans are descended from apes, but much more difficult to propose a plausible genetic mechanism. Paradoxically, when the sequences of chimpanzees and Homo sapiens are examined, significant similarities are found, if only in the number of genes. Minor differences in genome sequence exist and their importance is critical, but remains largely unknown. Neanderthal genetics is a work in progress, but still provides us with little information about the hominization process, brain evolution, and especially language acquisition. François shows us the way forward: from genomics to paleoanthropology.

3.3. *Synthetic biology*

Our almost complete knowledge of the molecules that make up everything from the most primitive bacterial cell to the human body and brain should provide us with an answer to the origin of life and, why not, its possible synthesis. At the crossroads of biology and chemistry, François asks the haunting question: where does life come from? Where do we come from? The answer may seem simple, but it isn't.

The artificial creation of biological objects – genes, viruses, genomes... – is possible. But no "living cell", not even a bacterial one, has ever been created. In the past, we would have thought of an imaginary "élan vital". Obviously not! Is some information missing? So what is it? We know all the building materials, but not their three-dimensional organization: the supramolecular structure of the cell wall, for example. Epigenetic inheritance of the "shape" of this wall, and thus of the bacteria themselves, cannot be ruled out.

A new avenue has been opened, but much remains to be done.

3.4. *The Revival of Molecular Biology*

While François was deeply interested in the fundamental implications of research, he was equally passionate about its impact on society. Indeed, this was one of the most striking features of his personality. In the world of RNAs: microRNAs, interference RNAs, etc., François saw the applied importance of messenger RNAs. François' discovery has been updated–if not rediscovered–by the general public with the new RNA vaccines against Covid 19, a worldwide success for his great discovery.

3.5. *Genetics and health*

François' constant concern was to better understand human disease through the prism of molecular biology. He has written extensively on predisposition to genetic diseases, from sickle cell anemia, Huntington's disease, Alzheimer's disease, muscular dystrophies, fragile X, autism, etc., to cancer. In his extensive discussion, he highlights the novelty of "drug genes" and gene therapy. He introduces us to the medicine of the 21st century: towards personalized medicine.

4. Epilogue: a personal address

Dear François, director of the Pasteur Institute, you had a visionary idea to dedicate a new building to neuropharmacology. This desire was accompanied by the wish to relocate the groups working in this field, including my group. This dream came true with the support of the CNRS, but at the cost of a tough battle with your successor, who did not share your vision of science. You foresaw the importance of this research field for the Institut Pasteur. My gratitude is truly deep.

What is even more precious is your commitment to the future of the scientific community as a whole, quite apart from any personal ambition. You have always sought to convince the political authorities of the importance of the role of science in our country, a role that the French political class is still finding very difficult to grasp.

You have put into practice the goal that Louis Pasteur himself set for researchers: to work with all their might "for the good of humanity".

5. Disclosure of interests

The authors do not work, do not advise, do not own shares, do not receive funds from an organization that could benefit from this article, and have not declared any affiliation other than their research organizations.

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