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A tribute to François Gros, a founding father of molecular biology

François Gros: from antibiotics to messenger RNA

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Abstract. François Gros was a prominent French Molecular Biologist who made a major contribution to the discovery of messenger RNA in 1961. He pursued outstanding research on bacterial mRNA and its translation into proteins followed by pioneering work on muscle differentiation. I was lucky to be among his graduate students and owe much of my success in science to him. In this short text I will describe how the initial post-war studies of François guided him to discover the existence of short-lived RNA in bacteria, the messenger RNA containing the information for protein synthesis. I will also recount the influence he had on his students and their carrier in science.

Keywords. François Gros, messenger RNA, translation, myogenesis.

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1. Introduction

I was extremely lucky and honoured to be accepted as a PhD student by François 59 years ago. He took the risk to accept a chemistry graduate from Israel with very little background in molecular biology or genetics. The laboratory was located in the Institut de Biologie-Physico Chimie (IBPC) in the Latin Quarter, spread over five floors, very crowded, very international with wonderful science and sense of camaraderie. We had close contact with the laboratories of Monod and Jacob and used to go over to the Institut Pasteur almost twice a week for seminars.

François already had many responsibilities at the national level and his office was open for visitors looking for advice and help from around France and beyond. Still he insisted on doing experiments himself, labelling phage T4 infected cells in the cellar and being interrupted by Genevieve, his loyal secretary, who called him to respond to an urgent phone call. In the meantime, the infected bacteria would lyse and the experiment had to be repeated.

I was lucky to meet my future wife in the laboratory, Josette Rouvière, and we shared long days and evenings working hard in the laboratory. The Latin Quarter had an advantage, we could go to see a movie while the ultracentrifuge was running for two hours to isolate ribosomes or other subcellular fractions.

The Institute was close to the barricades erected by the students in May 1968; we were in the laboratory when the CRS charged the barricades and we opened the door of the Institute to let in young students escaping the police as well as photographers who used our dark room to develop their films and print some pictures. When we called François, he strongly approved our action. Later, Jacques Monod came to help protect the students seeking refuge at the Institute.

Early on during my PhD, François was always available for discussions, encouraged my initiatives and introduced me to the international scientific community. He succeeded in defending my candidature for a CNRS position that I received during my second year of graduate work, an unattainable dream for current day students. François sent me to participate in prestigious scientific meetings and helped me secure short term EMBO fellowships to travel to Fred Sanger's laboratory in Cambridge UK, to sequence two of the first tRNA species.

Since that time, François was my guide and inspiration in science.

Later when we came back from our postdoctoral training in Stanford, he gave Josette and me space and support to start our own groups at the Institut Pasteur where he moved his lab in 1972. This was a launching pad to becoming an independent head of a unit in the Molecular Biology Department at the Institut Pasteur.

His support and encouragements never ceased during all the years we have been together at the Pasteur Institute and at the Academy. A long-standing friendship developed between François and Daniele and Josette and me. We miss François.

2. From antibiotics to mRNA and beyond

In this short text, I would like to demonstrate briefly how François moved from his initial study of the mode of action of antibiotics to discover mRNA.

François joined the Service de Biochimie, as it was called at the time, headed by Professor Michel Macheboeuf in the Pasteur Institute in 1945. A subject "à la mode" after the war was the research on the mode of action of the newly discovered antibiotics and this was the field in which he embarked at the Pasteur laboratory. I will mention several of his early publications from this period. Typical for these early post war years, the publications are in French [1–3].

Studying the action of streptomycin, François and others realized that this antibiotic precipitated nucleic acids in the test tube and that this could explain its antibacterial action. Quite a prediction, many years before the observation by X-ray crystallography that it binds to three RNA helices of the small ribosomal subunit, interferes with the correct recognition of the incoming aminoacyl tRNA and thus inhibits the synthesis of the bacterial proteins.

After completion of a PhD and the premature death of M. Macheboeuf, François joined the laboratory of Jacques Monod for a short period before leaving for a postdoctoral training in the United States. Quite an experience with Sol Spiegelman in Urbana, Illinois and Rollin Hotchkiss at the Rockefeller Institute. With Spiegelman, he started to study the effect of nucleic acid analogues on enzymatic adaptation in yeast.

Coming back to the Institut Pasteur, in the service now directed by Jacques Monod, François pursued the study of the effect of nucleic acid analogues on the synthesis of proteins. Together with Alain Bussard and Shiro Naono, he observed that on the addition of fluorouracil, an analogue of uracil, the induction of an active enzyme, beta-galactosidase, is partially inhibited due to the production of an inactive enzyme.

This provided an indication that RNA may be the template for the synthesis of proteins [4–6]. However, it was still unclear which RNA. In fact, for a number of years it had been observed that the ribosomes are the site of synthesis of proteins in mammalian cells. One hypothesis postulated that the ribosomal RNA is varying among the ribosomal particles and that it may be the template for the biosynthesis of specific proteins.

At the end of the fifties, it remained to establish what is the template for protein synthesis and to prove the mRNA hypothesis that emerged during the genetic studies that preceded the conception of the Operon Theory by Monod and Jacob.

Two distinct experimental approaches were taken. François Gros travelled to Harvard to join forces with scientists in James Watson's laboratory to attempt to label *E. coli* cultures with radioactive phosphate and follow the fate of the label by ultracentrifugation on sucrose gradients. François Jacob together with Sydney Brenner travelled to Caltech to use density isotope labelling to follow the fate of newly synthesized RNA after T4 infection of *E. coli* cells. Both series of experiments confirmed the existence of rapidly labelled unstable RNA and were published back to back in Nature in June 1961 [7, 8]. At the same time the Operon paper appeared in the Journal of Molecular Biology. The discovery of messenger RNA in bacteria was followed by the generalization of this class of RNA to all living cells [9]. Furthermore, it was followed by an intense competition to establish and decipher the genetic code, the match between the nucleotide triplets in the mRNA, the codon, and the corresponding amino acid in the protein.

These were certainly the great years of the nascent molecular biology and François was a major actor in this revolution.

3. Transcription and mRNA translation

Following the pioneering studies on mRNA, François was invited to join the IBPC in the Latin Quarter,

where I joined his laboratory in 1964. François began to study the regulation of the synthesis of specific mRNAs, typically the RNA produced during phage Lambda development, those of the lactose operon during induction of beta-galactosidase or during phage T4 infection [10–12]. At this time, Klaus Scherrer in François' laboratory started to study the synthesis of globin mRNA, its precursors and mature RNA in the duck and he will recount these studies in his contribution.

It was certainly the curiosity of François and the desire to expand our understanding of living organisms beyond microorganisms that drove François to initiate studies of RNA synthesis in a model of cell differentiation in which myoblasts undergo the transition to myotubes [13]. Margaret Buckingham will discuss this period in François' trajectory that resulted in his laboratory becoming one of the world leaders in the emerging field of molecular and cell biology.

The existence of mRNA also raised questions about the mechanisms involved in protein synthesis. Upon the suggestion of François, I embarked on the study of *E. coli* temperature sensitive mutants that were defective in protein synthesis at the non-permissive temperature.

I could show that the mutations occurred in genes encoding the enzymes responsible for linking specific amino acids to their cognate tRNA that recognizes the triplet codons on the mRNA [14]. I went on to study the properties of Valyl-tRNA synthetase, the enzyme that links the specific amino acid to its cognate tRNA. I isolated the corresponding three Valine tRNAs and established their primary sequence during several visits to the laboratory of Fred Sanger in Cambridge, UK. Together with Alain Favre, we introduced an intramolecular cross-link following UV irradiation of the Valyl-tRNA and together with Jacques Ninio we built a model that predicted half of the 3D structure of tRNAs [15, 16] as solved later by X-ray crystallography.

Michel Revel in the laboratory isolated the bacterial protein factors that are essential for the positioning of the ribosome at the first codon of the mRNA and for the initiation step of the synthesis of the protein [17, 18].

4. How my science was influenced by François

I continued to study the world of tRNA with Paul Berg at Stanford where I demonstrated the role of the anticodon in its recognition by the cognate aminoacyl synthetase [19].

When we came back from Stanford, Josette and I became part of the service de Biochimie that he directed upon his return to the Pasteur Institute. With the endorsement of François and his support, I started to study small DNA tumour viruses, Polyomavirus and SV40, with a focus on the structure and replication of their genomes and their transcription in the nucleus. I was certainly influenced by the step François had taken in studying the muscle system and his great interest in the structure of chromatin and chromosomal proteins. We could show that the circular viral DNA is associated with nuclear histones as minichromosomes, that newly replicated viral DNA is rapidly assembled into nucleosomes [20] and that the transcription control region is free of nucleosomes [21]. This became a general mark of transcription control regions in eukaryotes. Furthermore, we showed in collaboration with Daniel Blangy, that mutants of Polyomavirus, adapted to grow in embryonic carcinoma cells, harbour mutations and rearrangements in their enhancer sequences included in the nucleosome free region [22, 23]. Josette studied bacterial chromosomal proteins and discovered the HU protein, a small conserved DNA-binding protein that condenses the bacterial DNA [24]. Upon the departure of François Cuzin to Nice, I became the head of the DNA Tumour Viruses Unit, three floors below in the building, but kept close contact with François and his floor.

Several years later I moved to another building at the Institut Pasteur, François was more involved in government work but we still saw each other frequently and discussed science as usual. Like François, we became more and more interested in transcription and cell differentiation, isolating transcription factors that are essential for Polyomavirus and SV40 transcription or for hepatic cell differentiation, in collaboration with Mary Weiss [25–27]. Finally, we came back to chromatin by studying chromatin remodelling complexes essential for opening the chromatin during transcription [28]. Several of the genes encoding for transcription factors or chromatin remodeler subunits were identified as

oncogenes or tumour suppressor genes bringing us closer to the study of cancer [29, 30]. Similarly, two of the liver specific transcription factors were discovered as human disease genes associated with diabetes and kidney polycystic disease bringing us closer to human diseases [31]. Finally, the arrival at the Pasteur of our regretted colleague Gérard Orth, sponsored by François as the director, opened a long-standing collaboration between both our laboratories on the study of the genome of the papilloma group of viruses and on their transcription and replication.

5. Conclusions

In conclusion, this short summary of the first part of the path of François from antibiotics to mRNA and protein synthesis illustrates the constant desire of François to always move forward and investigate new biological horizons, which he succeeded in doing.

He knew how to instil passion for science, for hard work, curiosity and dedication in his collaborators. He encouraged our maturation and development into independent successful scientists, open to the world around us and motivated to improve human well-being. We are grateful to François for the chance to be close to him during so many years. I always admired the enormous knowledge of François, his curiosity and his incomparable memory. I also envied his unlimited capacity to conduct top science, while, in parallel, being involved in advancing science in France and in developing countries, helping friends and colleagues, with his door always open for advice. I admired his capacity to find time to write monographs about the advances of molecular biology and the importance of science for society. He kept his interest and full knowledge of innovations in science many years after closing his laboratory, following the entire field of life sciences.

We miss him.

Declaration of interests

The authors do not work for, advise, own shares in, or receive funds from any organization that could benefit from this article, and have declared no affiliations other than their research organizations.

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