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Le "Grand" François☆

Agnes Ullmann

Institut Pasteur, 28 rue du Dr. Roux, 750724 Paris Cedex 15, France

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When I arrived in 1958 from Hungary at the Institut Pasteur in Jacques Monod's laboratory, I started to work with François Gros, known by everyone as "François". In the lab, there was a strict ritual: at 11.45 we had seminars in the library also attended by the Lwoff and Jacob teams that came down from the "attic". After the seminar, we had lunch in a long glass-roofed room (la verriére); we bought food from a nearby grocery. At the beginning I didn't know who was who. Nevertheless I noticed that a gentleman in a white laboratory coat had everyday, on his plate, two slices of ham, an apple and an orange. He was also called François: François Jacob. I asked, when people were talking how did they made the distinction between the two? It's simple, was I told: François Gros is simply "François" while François Jacob is "le Grand François", because he's taller and older. At lunchtime, there were animated discussions in French or English, including interpretations of the – now famous – PaJaMo (Pardee, Jacob, Monod) conjugation experiment [4]. I did not understand much of the discussions because, in Hungary, teaching of genetics was prohibited; only Lyssenko's theories were allowed. Fortunately, Leo Szilard, who was spending some time at Pasteur and was happy to speak some Hungarian after so many years, was kind enough to reveal some clues as to what those people were talking about. Only later did I realize that Szilard played an important role in the interpretation of the PaJaMo experiment in terms of a "general repression model" instead of the former hypothesis of a "general induction model". The PaJaMo experiment served as a starting point for proposing the model of negative regulation by a repressor, the concept of messenger RNA and of the operon model.

During my first two months stay at the Pasteur, I had practically no contact with François Jacob. I worked a lot in the lab and since it was my first visit beyond the "iron curtain", I spent all my free time visiting Paris and reading books that were prohibited in Hungary. When I came back to Pasteur in 1959 for six additional months, still working with François Gros, I had more opportunities to see "le Grand François". He invited me to his home, where I met his wife Lise who was a pianist. She had often tickets for concerts but François rarely kept her company: because of his war injuries he could not sit for a very long time without moving. So Lise invited me to concerts and we became good friends. When I had to go back to Budapest, Lise and François accompanied me to the railway station, where Lise bought me a bunch of books by Camus, Sartre, Simone de Beauvoir, Orwell and others. I spent the 20 h it took to reach the border reading those books that I had to discard before arriving there because they were banned in Communist Hungary. I left Paris with a heavy heart. I had to return home otherwise I would have endangered my husband, my colleagues and my friends. But hope kept me alive: Jacques Monod had promised that he would try to help to smuggle my husband and me out of Hungary. Indeed, he did organize our escape, which turned out to be an unbelievable adventure (described in [6]).

By the end of 1960, I was back for good at Pasteur and started working immediately. The operon model had been settled but the Lac repressor had not yet been identified. At that time, the repressor was thought to be an RNA that would bind directly to the DNA stretch of the operator and would be

☆ In an Obituary for François Jacob published a few months earlier [7]. I had shortly described his remarkable career as a scientist, as a writer and as a hero during World War II. The present paper relates only to a few fond remembrances, snapshots of friendly collaborations and discussions I had with him over the last 50 years.

E-mail address: ullmann@pasteur.fr.

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recognized by the inducer. Jacques Monod suggested that I should work on this project. To start, I needed specific mutants that could serve as suitable controls, and François Jacob provided me with them. François Gros had left the Pasteur and hence, Jacob remained the only François. The start of the repressor project allowed us to have prolonged discussions. After a few months, the results convinced me that the repressor couldn't be RNA: it might be a protein. Indeed, in 1962, Jacob with Raquel Sussman obtained thermosensitive and suppressible nonsense mutants of the λ repressor [5]. Others had obtained similar mutants in the Lac system; therefore the Lac-repressor should be a protein. But all the purification procedures I had set up to isolate the repressor consistently failed. With Jacques, using a genetic approach, we obtained a positive but most disappointing result suggesting that the number of repressor molecules was no higher than 10 per cell. This was so discouraging that I asked François if it were possible for us to obtain a strain that would make at least ten times more repressor? He said that this was hopeless. Well, a few years later, Wally Gilbert and Benno Müller-Hill did obtain such mutants, which allowed them to purify the Lac repressor and show that it was indeed a protein [1]. Many years later, at a meeting where I was chairing a session on regulation with Benno, I introduced him as my best competitor friend because he was the person who had isolated the Lac repressor, which I missed. François Jacob who was the first speaker started his talk by saying "Agnes, you were wrong: we were the ones who missed the repressor".

In 1963, Jacob and Monod had elucidated most of the major concepts regarding the structure of the Lac operon. One of the questions that remained was the site at which the transcription of the structural genes would start. François had the idea to create gene fusions by constructing deletions on *Flac* episomes, extending at one end over various lengths of the *lacZ* gene, leaving the *lacY* gene intact to select for Lac−permease activity. At the other end, the deletion was expected to extend into another operon with the hope that the permease would be regulated by the control system of that other operon. We analyzed several thousand clones without success until François obtained deletions extending into *purE*. During this period, we proceeded like workers on an assembly line: François would analyze the Petri dishes, his technician would culture the clones under different conditions and I would analyze the cultures to determine whether the Lac-permease would be regulated by a molecule other than a β-galactoside. François was anxious to learn about the results but I would arrive late at the lab and also leave late. Every morning, François would be pacing the corridor, growing increasingly impatient and greeting me with such remarks, as "isn't it rather late to start?". In the evening, he left early to have dinner with his children but before leaving, he always passed by my lab to ask whether anything new had happened. "Not yet" I would always reply. And each time, he would mumble something like "had you started earlier … call me if there is anything new".

One evening I finally got the awaited result: purine repressed the Lac-permease: the *purE-lacZY* fusion had created a new operon [2]. I took my revenge on François' impatience and waited a few hours before calling him. It was around midnight when I called and obviously I woke him up. All he said, curtly, was "merci". Next morning, I arrived even later than usual. François did not make any remarks; just asked to see the results. Then he told me that if I should call him next time, he would prefer that it be done before 10 p.m. But that was the last time I ever heard him comment about my working hours.

While searching for gene fusions, François obtained a large collection of deletion mutants. Some of them, covering the *lac* regulatory region and extending to various sites of the *Z* gene, did complement certain promoter-distal mutants (i.e. restored enzymatic activity). François refined the *in vivo* complementation tests while I set up an *in vitro* system with crude extracts of different deletion mutants. Fortunately, the two approaches converged with the conclusion that all deletions not extending beyond a certain point in the gene (which we called the ω-barrier) would complement all promoter-distal mutants of the *Z* gene. We called this phenomenon ω-complementation [8]. In trying to understand its mechanism by using biochemical and immunological approaches, we arrived at the conclusion that ω-complementation involved the non-covalent association between peptides corresponding to different fragments of the wild-type β-galactosidase, indicating that ω was able to fold itself into the correct wild-type structure.

Once ω-complementation was settled, I wondered whether other combinations of deletion mutants would generate an active enzyme. When I mixed extracts containing partial deletions of the promoter proximal segment of *lacZ* with extracts of β-galactosidase-negative mutants whose promoter-proximal segments were intact, I recovered enzymatic activity: α-complementation was born! Jacques liked the idea but François was somehow reluctant to accept these results because he had not observed those effects previously on EMB-Lactose plates. After a while, by choosing the appropriate heterozygous strains, he eventually confirmed the *in vitro* results but considered that *in vivo* α-complementation was very inefficient [9]. Soon, α-complementation became the basis for the commonly used blue/white screen to identify recombinant DNA. The reasons for the barely positive EMB data became clear later: *Flac* episomes used by François were not multi-copy vectors and EMB was much less sensitive than X-gal.

While the ω-peptide represented about one third of the total length of β-galactosidase (ca 40 kDa), the α-peptide was much smaller (6 kDa) and heat-stable. Surprisingly, it seemed that in α-complementation, the α-peptide acted by eliciting a conformational change of the acceptor polypeptide, leading to enzymatic activity.

François was pleased when he received 30 years later a preprint from Brian Matthews on the three-dimensional structure of β-galactosidase [3] It confirmed our early conclusions regarding both α- and ω-complementation based on genetic and biochemical data.

The last paper I co-signed with Jacob and Monod on complementation appeared in 1968 [10]. Around this time, the paths that François and Jacques had followed led them in