INSTITUT DE FRANCE
Académie des sciences

Comptes Rendus
Biologies

Margaret Buckingham

Messenger RNA in differentiating muscle cells—my experience in François Gros' lab in the 1970s and 80s

Volume 346, Special Issue S2 (2023), p. 27-35

Online since: 21 December 2023
Issue date: 29 March 2024

Part of Special Issue: A tribute to François Gros, a founding father of molecular biology

Guest editors: Margaret Buckingham (Professeur émérite et directeur honoraire du département Biologie du développement et cellules souches à l'Institut Pasteur - Membre de l'Académie des sciences) and Moshe Yaniv (Professeur honoraire à l'Institut Pasteur - Directeur de recherche émérite au CNRS - Membre de l'Académie des sciences)

https://doi.org/10.5802/crbiol.132

The Comptes Rendus. Biologies *are a member of the Mersenne Center for open scientific publishing*
www.centre-mersenne.org — e-ISSN : 1768-3238

Comptes Rendus
Biologies
2023, Vol. 346, Special Issue S2, p. 27-35
https://doi.org/10.5802/crbiol.132

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

Messenger RNA in differentiating muscle cells—my experience in François Gros' lab in the 1970s and 80s

Margaret Buckingham [a]

[a] Department of Developmental and Stem Cell Biology, CNRS UMR 3738, Institut Pasteur, 25 rue du Dr Roux, 75015 Paris, France
E-mail: margaret.buckingham@pasteur.fr

Abstract. I joined François Gros' laboratory as a postdoc at the end of 1971 and continued working with him as a research scientist until 1987, when I became an independent group leader at the Institut Pasteur. In the early 1970s, it was the beginning of research in his lab on muscle cell differentiation, as a model eukaryotic system for studying mRNAs and gene regulation. In this article, I recount our work on myogenesis and mention the other research themes in his lab and the people concerned. I remained in close contact with François and pay tribute to him as a major figure in French science and as my personal mentor who provided me with constant support.

Keywords. François Gros, RNA, Myogenesis.

Note. This article follows a symposium held on 25 April 2023 at the Institut Pasteur in tribute to François Gros.

Published online: 21 December 2023, Issue date: 29 March 2024

1. My arrival in France

I joined the laboratory of François Gros as a postdoc in October, 1971. Having just completed a D. Phil. (Ph.D.) on histone modifications with M. Ord in the Biochemistry department of Oxford University, I wanted to work on messenger RNA (mRNA), the discovery of which had inspired us as students. I heard François, who had played an important role in this discovery, speak at a FEBS meeting in Montreux and with some trepidation had asked him if there would be a possibility of working in his lab. With his habitual kindness, he encouraged me to come. Alas, subsequently, there was no reply from François to my letter(s). Finally after a phone call, Geneviève Antolini, who was already his efficient assistant, activated a formal letter of acceptance and, with a postdoctoral fellowship from the Royal Society, I came to Paris with my husband Richard who joined the lab of Marianne Grunberg-Manago at the Institut de Biologie Physico-Chimique (IBPC).

François had left the IBPC in 1968 and had his laboratory in the science faculty of the University of Paris where he was professor of the new discipline of molecular biology. The lab was located in a wing of the huge new concrete complex at Jussieu which housed the faculty. The site was not a very welcoming environment and François' lab at that time was also not a very convivial place for a newly arrived postdoc. It was difficult to find out who was doing what in the absence of joint group meetings. François proposed that I work with Daniel Caput, a Ph.D. student. The Ph.D. cursus involved a thèse de troisième cycle and then a thèse d'État, which could last for many years, unlike the three-year Ph.D. that I had just completed in the UK. François Gros, like others who had characterised mRNA in bacterial systems, wanted to explore mRNA function and regulation in eukaryotes. Through his close contacts with the Weizmann Institute, he knew of David Yaffé's work on muscle cells. Myoblasts can be separated from large multinucleated muscle fibres and will proliferate in culture

at low density. When the culture becomes confluent, they will fuse together, accumulate muscle proteins and form fibres, thus providing a model system for the *in vitro* study of cell differentiation. When I arrived, François had recently decided to adopt this model. Daniel had begun work on muscle primary cultures and Denise Luzzati, in a separate effort, was studying rat L6 and L8 muscle cell lines isolated by D. Yaffé.

2. François Gros' lab in the Institut Pasteur

In 1972, François moved most of his lab to the Institut Pasteur. In retrospect, this move cast a shadow over the lab during my first year as a postdoc, because of uncertainty about who would move with him. Jacques Monod had just been appointed Director of the Institute and had decided, with admirable realism, that the effort required to re-establish the failing financial situation was incompatible with directing his own research. He therefore proposed to François to return as Director of the Laboratory of Biochemistry, located on the 6th floor of the new Molecular Biology building, now called the Monod building. This floor had been planned for Monod's laboratory and included a large office, with elegant light wood fittings. François Jacob, and other previous members (J.-P. Changeux, H. Buc, M. Schwartz, etc.) of the Monod team had laboratories located on other floors, constituting the molecular biology department of the Institut Pasteur. It was a stimulating scientific environment with exciting internal and external seminars. In François' lab, we now had memorable weekly meetings when the different groups would present their work. This ranged from transcriptional regulation of phage lambda (P. Kourilsky, finishing his thèse d'État), of the SV40 virus (M. Yaniv, just returned from a postdoc with Paul Berg) to mRNA translation (J. Thibault, J.-C. Lelong, D. Lazar), and to muscle cell differentiation worked on initially in the small group constituted by D. Caput and myself. François presided and would ask questions and construct possible theories, leading to lively discussion. His scientific insights were valuable and his encouragement precious. These were convivial occasions with coffee and croissants from the best patisserie in the nearby rue de Vaugirard. I remember that François, who spoke and wrote elegantly, did not like anglicisms even in scientific French and would construct a phrase to avoid such pollution, for example a whole sentence for "Southern blot", a technique developed by Ed. Southern for characterising DNA. François' mastery of English included erudite words; he explained that as a student he had decided it was essential to know English and therefore memorised several pages of the dictionary every day. Among his many attributes he had a remarkable memory! A few years later M. Yaniv became director of his own research unit in the building and P. Kourilsky left for a postdoc with B. Mach in Switzerland. After François Gros' election to the chair of cellular biochemistry at the Collège de France in 1973, he established a second laboratory there with a focus on neural differentiation. Some of his collaborators, including those working on mRNA translation in Pasteur, moved to the Collège to help establish this new laboratory. M. Crépin formed a research group in the Pasteur lab, working on the initiation of mRNA transcription, with an interest in the MMTV virus. M. Jacquet joined François' laboratory in Pasteur, with a group working on the function of chromatin in the transcriptional regulation of eukaryotic cells, thus complementing the interest of J. Yaniv in the bacterial chromosomal protein HU. D. Caput left the lab when he had finished his thesis and joined the pharmaceutical company of Sanofi.

3. The expansion of research on the muscle model of cell differentiation

Gradually François recruited more researchers working on muscle differentiation, notably Bob Whalen, a postdoc who had worked with Paul Doty in Harvard. Bob and I shared a small office with another American postdoc, John Merlie, who worked with J.-P. Changeux, in collaboration with François, on the synthesis of the acetylcholine receptor in muscle cell cultures. We had fun discussing everything from science to politics. The office was opposite François' secretariat and it was impressive how many people, from the most eminent to the most humble, came to seek his advice. Bob Whalen carried out important research on muscle protein isoforms, notably actins and myosin heavy and light chains. He, like me, was able to form a research group in François' lab, with postdocs such as Gill Butler-Browne and Shin-ichi Takeda, as well as Lev Ovchinikov from A. Spirin's institute in the then Soviet Union. We

both benefitted from very good people who had applied to work with François, in my case Sataro Goto who had worked on muscle cell hybrids with Nils Ringertz, and then Adrian Minty. Woodring (Woody) Wright was another remarkable American postdoc who came to work with François, developing his original idea of using heterokaryons, rather than hybrid cells, to look at the dominance of the muscle phenotype on other cell backgrounds. Woody was a fervent collector of "junk" from the pavements and flea markets of Paris which accumulated in his small lab space, viewed by François with amused tolerance. Another muscle researcher, recruited later by François, was Marc Fiszman who had developed a model system for regulating muscle cell behaviour, using thermosensitive retroviral infection in chick cell cultures. Domenico Libri, Didier Montarras and Vincent Mouly carried out research with him on muscle cell differentiation and on the muscle isoforms of tropomyosin.

4. Professional women in research

From the beginning of my time in Pasteur, thanks to François, I benefitted from the collaboration of Arlette Cohen who worked with me for 30 years, first as a technician and latterly as a research engineer. In addition to valuable practical help at the bench, she also was my mentor for Parisian fashion—disapproving of my Scottish clothes—, and has remained a close friend. She is François' second cousin. His wife, Danièle, also worked as a technician in the lab, first with P. Kourilsky, then with M. Crépin and later with M. Jacquet and then Marc Fiszman. She said, laughingly, that this was the only way to see more of François and to have some contact with his professional life. He did indeed work non-stop. For him, French holidays provided a peaceful time to concentrate on writing—reports and also books, mainly about the major advances of molecular biology and also his own experiences in the development of this new discipline. I remember an occasion when the lift stopped on the 6th floor and three small boys rushed out down the corridor, escaping their child minder, with cries of "maman". They did not go the other way towards François' office, having been taught that their father should not be disturbed. This said, although François did not spend much time playing with them, he was clearly a benevolent father. In Parisian bourgeois society, that a daughter should work as a technician or as a scientist was totally acceptable. Educated women were encouraged to pursue their career as well as having a family. This was in contrast to other countries at that time where this was frowned upon. I remember when my first son was born in 1980, François sent me congratulations and a large bunch of flowers!

5. Early experiments on mRNAs during muscle cell differentiation

My research as a postdoc, initiated with Daniel Caput, involved pulse labelling RNA as muscle cells differentiated. Our experiments at that time were rather reminiscent of those carried out by François using *E. coli* in Jim Watson's lab in Harvard [1], when he was sent there as a biochemist to explore unstable RNA (mRNA) by Jacques Monod. Daniel had decided to make use of a source of muscle from foetal calves, available from the Vaugirard slaughter house, not far from Pasteur, which still existed in the first half of the 1970s. The advantage of course was the quantity of the material even if the stage of development was not precisely controlled. Many eukaryotic mRNAs contain poly(A) at the three prime end, and we could isolate them from bulk ribosomal RNA using poly(U)-sepharose columns. We also fractionated cytoplasmic extracts on sucrose gradients to separate the heavier polysomal fraction from the so-called ribonucleoprotein (RNP) fraction nearer the top of the gradient. These kinds of experiments led us to describe increased mRNA stability in cultures where proliferation was lower and cell fusion to form myotubes had been initiated. With Sataro Goto we explored a possible role of the length of poly(A) tails on mRNA stability. We also noted that certain mRNAs could be chased from the RNP compartment into polysomes [2]. An echo of these results came 38 years later when we showed that adult muscle satellite cells sequester the transcript of the myogenic determination gene *myf5* in RNP particles prior to their activation and differentiation when the mRNA is translated [3]. At the time we did not have a way of identifying individual mRNAs. A major step forward came with the availability of systems—wheat germ or reticulocyte lysates—for *in vitro* translation of mRNA populations, followed by identification of

radioactively labelled protein products. Separation of denatured proteins by two-dimensional gel electrophoresis, made it possible to distinguish different isoforms of the contractile proteins and thus plot the appearance of mRNAs encoding muscle isoforms as cells differentiated [4]. This work was carried out with Philippe Daubas, my first Ph.D. student, who later returned to my lab with a CNRS position, after postdoctoral experience elsewhere. By the end of the 1970s, the synthesis of complementary DNA (cDNA) sequences, obtained by reverse transcription of mRNA populations, made it possible to perform experiments of differential hybridisation. Nabeel Affara, a postdoc in François' lab, working with a teratocarcinoma derived myoblast cell line isolated by members of François Jacob's lab, documented changes in mRNA complexity with a newly transcribed group of mRNAs now present in polysomes, as these cells formed muscle fibres [5].

6. The advent of specific cDNA probes for single mRNAs—myogenesis *in vivo*

After the development of DNA reverse transcription and its application to RNA, a major technological breakthrough came with the cloning of cDNAs. When introduced into bacteria in a plasmid vector, characterisation of the bacterial colonies permitted the isolation of single cDNA sequences which could then be used as molecular probes for specific mRNA sequences. Such DNA cloning of potentially pathological sequences was a subject of concern and in 1974 a moratorium was introduced by the scientific community. This was lifted in 1975 after an important conference at Asilomar in California had laid down security guidelines for such experimentation [6]. In my group, we began to clone cDNAs of mRNAs for muscle actins and myosins. This was not easy, partly because the technology was new and partly because we had to follow strict security. The experiments took place in a P2 facility in the second basement (not accessible with the usual lift) and all the equipment and solutions used had to be autoclaved before being taken back to the lab. Experiments had to receive prior approval from a scientific committee set up by the Ministry of Research. We decided to use mouse skeletal muscle as a source of mRNA. Cloned probes would make it possible to look at skeletal muscle formation *in vivo* as well as in cultured cells and the mouse is a suitable animal model. We adopted the mouse C2 muscle cell line, isolated by David Yaffé, as our *in vitro* mammalian system. The first muscle mRNA to be cloned, by Adrian Minty, was a muscle α-actin sequence [7]. The similarities between actin isoforms meant that the coding sequences cross-hybridised, but fortunately the $3'$ non-coding sequences of these and other mRNAs are distinct. With cloned cDNA probes, we could now look, by hybridisation to Northern blots, at specific mRNAs during C2 cell differentiation. This approach, backed up by two-dimensional gel analysis of the products of *in vitro* mRNA translation, gave precise information about the presence of contractile protein mRNAs, showing, for example, the accumulation of the mRNA for the embryonic myosin isoform, MLC1emb, at the onset of cell fusion, before that of the fast isoforms MLC1F and MLC3F [8]. *In vivo*, in fetal mouse skeletal muscle, two α-actin type sequences co-accumulate, coding the cardiac isoform of the adult heart as well as the predominant isoform of adult skeletal muscle [9]. In the C2 muscle cell line, cardiac actin mRNA is also expressed as soon as the cells begin to differentiate. This co-expression of actin genes is also seen in developing heart muscle. Expression of the same isoforms in the two types of striated muscle applies also to myosins, for example, the so-called MLC1emb isoform of developing skeletal muscle is also present in the atria of the adult heart (MLC1A) [10]. Instead of co-expression, sequential expression of developmental and adult isoforms is also a strategy, as documented for the myosin heavy chain mRNAs by André Weydert in my group [11]. Interestingly this is reflected in the organisation of the corresponding genes. Using interspecific mouse lines for genetic mapping, in collaboration with J.-L. Guenet in Pasteur, we could show that actin and myosin light chain genes are dispersed in the genome [12], while sequentially expressed myosin heavy chain genes are present in the same locus [13].

7. Characterisation of muscle genes

In addition to characterising modes of expression, cDNA probes also made it possible to isolate genes and examine their structure. Thus Benoit Robert, who had joined my group as a Ph.D. (thèse d'État)

student, after working with M. Jacquet, and had cloned the first myosin cDNAs, isolated the locus that encompasses exons encoding the fast myosin MLC1 and MLC3 proteins. He showed how these are generated by the use of distinct promoters with differential splicing [14], thus providing one of the first examples of this phenomenon which leads to the generation of isoforms of many contractile proteins. On my first visit to Japan, when I spoke in place of P. Kourilsky at an international conference, I had learned that Yo-ichi Nabeshima was working on the same gene in chicken. I visited his rather obscure lab in Nigata and, after the obligatory polite exchanges with his professor, we had an exciting time comparing results which showed the same gene structure. We agreed to try to synchronise publication of our papers which were accepted by *Cell* and *Nature*. Y. Nabeshima subsequently discovered the myogenic regulatory factor, Myogenin, and became a leading Japanese scientist, with a professorship in Kyoto University. My second Ph.D. student, Serge Alonso and a British postdoc, Ian Garner, continued the work on actin coding sequences and their evolution [15]. As a result of a genetic analysis of actin genes, a mutation in the cardiac actin locus of *BALB/c* mice led to new insights into an upstream regulatory region of the cardiac actin gene [16]. Later, we isolated distinct skeletal and cardiac muscle enhancer sequences located at the 5′-end of the gene [17]. In the 1980s, initial studies on the transcriptional regulation of muscle genes depended on transcriptional "run on" experiments in the C2 cell line and then transfection of differentiating cultured cells with reporter plasmids controlled by candidate regulatory sequences. *In vivo* experiments with transgenes only came later. In addition to Ian, two other able British postdocs joined my group in the 1980s, Roger Cox who did "run on" experiments [18] and also worked on MHC genes and Paul Barton, who was briefly a permanent Inserm researcher, working on myosin light chain genes, notably coding for MLC1emb [19].

8. Transcription factors that activate myogenesis

Little by little we obtained clues about the regulation of myogenesis. Characterisation of the muscle cell *in vitro* system and of the onset of muscle gene expression when cells begin to differentiate was a necessary first step. However, the ultimate aim was to understand how this is regulated, how a differentiated tissue phenotype is acquired during development. One approach was to identify sequences that control muscle gene expression and systematically progress upstream from there. Another was to identify potential candidate upstream regulators by a shot in the dark approach. During the formation of skeletal muscle in the fruit fly *Drosophila*, a muscle specific homeobox factor had been described as an upstream regulator of myogenesis. With Benoit Robert, we therefore decided to look for the mammalian homologue and cloned a sequence for what we called Hox7 [20]. This, and its homologue Hox8, are now known as Msx1 and Msx2. Msx1 is expressed at a low level in some myogenic progenitors in the mouse embryo, however these factors are mainly important in patterning the limb mesenchyme and at other non-myogenic sites during development. Benoit subsequently explored their functions in his own lab. A major breakthrough came in 1987 when Hal Weintraub and colleagues identified a cDNA sequence present in muscle cells, which, when transfected into fibroblasts, would convert them to myogenesis. They described the bHLH transcription factor encoded by this sequence as myogenic determination factor MyoD1 [21]. This approach would not have worked for most tissue types where more than one "pioneer" transcription factor is required for tissue differentiation. In the case of skeletal muscle, other factors are also implicated in the activation of muscle genes, however MyoD plays an over-riding role. We now know that there are four members of the MyoD family. They are specific to skeletal muscle cells and all have the capacity, when overexpressed, to force many cell types into myogenesis. Myogenin and Mrf4, as well as MyoD, are implicated in muscle cell differentiation, whereas MyoD and Myf5 (with Mrf4 in the early embryo) expressed in myoblasts act as myogenic determination factors before muscle tissue forms. Towards the end of the 1980s, David Sassoon, an American postdoc, joined my lab and established the newly developed technique of *in situ* hybridisation on tissue sections to analyse gene expression during embryonic development. At that time, we had a bet with Hal Weintraub who was convinced that MyoD would be expressed first at the

onset of myogenesis in the mouse embryo. David showed that this was not the case and that the gene for another member of the family, Myogenin, was expressed earlier [22]. Using this technique, David and Gary Lyons, another American postdoc in my group, mapped out the temporal-spatial expression of muscle genes during cardiac and skeletal muscle development (ex. [23]). At that time this was a new technical approach, and we organised an EMBO course on *in situ* hybridisation in 1990.

9. Subsequent research in my lab

In my own laboratory, we subsequently showed that Myf5 is expressed prior to the first formation of skeletal muscle [24] and much of our subsequent work focussed on the function and regulation of the gene for this myogenic determination factor (ex. [25]). We also studied the role of Pax3 (ex. [26]), a transcription factor which is not specific to myogenic progenitor cells, but which orchestrates the entry of cells into the myogenic programme. It controls cell fate choices in the mesodermal structure of the somite, myogenic progenitor cell proliferation and migration from the somites to the limbs, and directly activates sequences regulating the transcription of *myf5*. Most of our research on the regulatory hierarchy that controls the onset of myogenesis concerned the embryo, but we also, with Didier Montarras, worked with muscle stem cells in the adult, so called satellite cells which are responsible for muscle regeneration [27] and which are governed by a similar genetic hierarchy. As a result of our interest in the expression of myosin genes, initiated in François' lab, and due to an insertion site effect on a myosin transgene, we discovered the second heart field [28]. This is an important source of cardiac progenitors which we showed constituted a second myocardial cell lineage [29]. Cardiogenesis, as well as myogenesis, became a major theme of my lab. From the 1980s, as a result of the tools of molecular biology and later of molecular genetics also, it became possible to examine gene expression, regulation and function during the development of the organism. It was an exciting time. More recently the centre of interest has shifted to stem cell biology, with less emphasis on the developmental aspect which underlies stem cell behaviour and more on adult tissue regeneration, with the potential for therapeutic applications.

10. External recognition and scientific meetings with François

Thanks to François' support, I had obtained a permanent position in the CNRS in 1975. There was less competition then in the small community of molecular biologists and the recommendation of a "grand patron" counted a lot. I had acquired an international reputation and had also been successful in gaining grants, notably at that time financial support from the American Muscular Dystrophy Association (MDA), which benefitted his lab. Recognition of my scientific contribution also owed a lot to François. He introduced young scientists from his lab to the French community and would on occasion drive us to meetings in Paris. To be a passenger in François' car was not without risk. I remember an occasion when he was driving round the Etoile and had entered the inside lane nearest the Arc de Triomphe. He was so concentrated on a scientific discussion that he did not move towards an outside lane to exit and we continued going round and round until an alarmed passenger reminded him of our destination! From 1982, he did not co-sign many of my publications and before that he encouraged me to present my work at international meetings, such as the big FEBS meeting in Paris in 1975. I remember that David Yaffé, also speaking in the session on cell differentiation, noticed my nervousness and offered me a piece of chocolate, his remedy for stress before speaking. David played a major role in the development of the field of myogenesis, organising EMBO workshops that continue today. He organised a first EMBO workshop on the subject at the Shoresh kibbutz in Israel in 1975 and then again in 1980. I attended both meetings with François. At that time, we were a small number of researchers in the world working on muscle cell differentiation. Clashes of opinion at the meetings were frequent between the Americans Irv Konigsberg and Howard Holtzer, both strong personalities, who, together with David Yaffé, were the great men of muscle cell culture, primary cultures from chick in the case of the former and mouse cell lines for David. Molecular regulation, which interested François, was in its infancy. His quiet interventions at the meeting had a calming effect on the cell biologists! In general, François was always courteous and very rarely expressed anger. After the 1980 meeting in Shoresh, I had planned to

stay in Jerusalem to explore the old city. I remember François' concern at leaving me—expecting my first child in a few months' time—, in a rather down at heel hotel run by Palestinians beside the Jaffa gate. Later, in 1988, we organised an EMBO meeting on the island of Bendor near Bandol in the South of France. By that time the community working on myogenesis was bigger and the focus of interest was muscle gene regulation. During my time in François' lab, other memorable meetings that we attended were organised by his colleague and friend Marianne Grunberg-Manago in the then Soviet Union, as part of Russian-French scientific collaboration, and also on the Greek island of Spetsai where regular summer schools were held with leading international figures giving lectures on molecular biology. I remember Francis Crick sitting at the back of the lecture theatre in the school asking lethal questions in a piping voice "But my dear boy, what about…". The Spetsai summer schools continue today.

In 1987, I was made laboratory head in Pasteur with an independent research Unit. I had decided it was time to move on and was thinking of applying for my own group in CNRS centres in the Paris region. When I discussed this with François he was reluctant that I leave. François Jacob had just decided to give part of his space on the 4th floor to his ex-pupil Jean-François Nicolas and advised that I be allocated half of the 6th floor. François generously decided to follow this advice. The Scientific Council of Pasteur agreed and I thus became officially independent while benefitting from the continuing proximity of my 6th floor colleagues.

11. François Gros' responsibilities in French science, in addition to those as lab director

In 1975, Jacques Monod became fatally ill and asked François to take over as director of the Institut Pasteur. He occupied the director's offices on the other side of the Pasteur campus and was less present in the lab. However, he continued to attend lab meetings and would come to the 6th floor at the end of the day when we could discuss science with him. During this period, he wrote an important report with François Jacob and Pierre Royer, commissioned by the French president V. Giscard d'Estaing, on the impact that the new discoveries of molecular biology were likely to have on the community. After the election of François Mitterrand as president, François was appointed scientific advisor to the prime ministers, Pierre Mauroy and then Laurent Fabius, a post that he occupied from 1981 to 1985. During this period, he played an important political role in the organisation of scientific research in France and recognition of the scientific and medical implications of molecular biology and emergent biotechnologies. In addition to his work for the government, in the context of muscle research, François raised awareness of the importance of this with the directors of the French AFM (Association Française contre les Myopathies) and became the first president of their scientific council from 1986. The AFM continues to provide precious financial support to research labs in France today. Despite all the demands on his time during the 1980s, François still came to the lab regularly, while the group leaders ensured its functioning and maintained the scientific life that François had instituted.

The end of the 1980s was a difficult time for François because of the propagation of the AIDS virus and the disastrous contamination of blood banks. The government was blamed for not taking action sooner and François, as scientific advisor, was also accused in the subsequent legal proceedings. Many scientific colleagues around the world wrote letters in his support and indeed little was known about the virus at the time. In the end, François was cleared of any mis-doing. In 1991, he was elected Secretary of the French Académie des Sciences of which he had been a member since 1979. Together with the President, two "Secretaries", one for biological sciences and one for physical sciences, run the Academy. It is an important position and his election demonstrated the esteem in which he was held by his fellow scientists in all disciplines. He now had his main office in the Academy and occupied the position until 2000, continuing to be active in the Academy until the end of his life. He continued as head of his lab in Pasteur and also as professor at the Collège de France, until he reached retirement age (70) in 1995. He still came to the lab on the 6th floor of the Monod building and enjoyed hearing about the experiments that were ongoing. He also always remained very supportive of those who had worked with him. I greatly benefitted from his thoughtful interest in my research, his kindness and the valuable advice and practical assistance

that he generously gave. For example, my election to the Académie des Sciences in 2005 owed much to his discrete support. At the end of his life, he still maintained his interest in research and indeed, even after the Covid-19 epidemic had meant that he could no longer go to the Academy and communication was limited to phone calls, he wanted to hear about new scientific results.

Declaration of interests

The author does not work for, advise, own shares in, or receive funds from any organization that could benefit from this article, and has declared no affiliations other than her research institution.

References

[1] F. Gros, H. Hiatt, W. Gilbert, C. G. Kurland, R. W. Risebrough, J. D. Watson, "Unstable ribonucleic acid revealed by pulse labelling of Escherichia coli", *Nature* **190** (1961), p. 581-585.

[2] M. E. Buckingham, D. Caput, A. Cohen, R. G. Whalen, F. Gros, "The synthesis and stability of cytoplasmic messenger RNA during myoblast differentiation in culture", *Proc. Natl. Acad. Sci. USA* **71** (1974), p. 1466-1470.

[3] C. G. Crist, D. Montarras, M. Buckingham, "Muscle satellite cells are primed for myogenesis but maintain quiescence with sequestration of Myf5 mRNA targeted by microRNA-31 in mRNP granules", *Cell Stem Cell* **11** (2012), p. 118-126.

[4] P. Daubas, D. Caput, M. Buckingham, F. Gros, "A comparison between the synthesis of contractile proteins and the accumulation of their translatable mRNAs during calf myoblast differentiation", *Dev. Biol.* **84** (1981), p. 133-143.

[5] N. A. Affara, B. Robert, M. Jacquet, M. E. Buckingham, F. Gros, "Changes in gene expression during myogenic differentiation. I. Regulation of messenger RNA sequences expressed during myotube formation", *J. Mol. Biol.* **140** (1980), p. 441-458.

[6] P. Berg, D. Baltimore, S. Brenner, R. O. Roblin, M. F. Singer, "Summary statement of the Asilomar conference on recombinant DNA molecules", *Proc. Natl. Acad. Sci. USA* **72** (1975), p. 1981-1984.

[7] A. J. Minty, M. Caravatti, B. Robert, A. Cohen, P. Daubas, A. Weydert, F. Gros, M. E. Buckingham, "Mouse actin messenger RNAs. Construction and characterization of a recombinant plasmid molecule containing a complementary DNA transcript of mouse alpha-actin mRNA", *J. Biol. Chem.* **256** (1981), p. 1008-1014.

[8] M. Caravatti, A. Minty, B. Robert, D. Montarras, A. Weydert, A. Cohen, P. Daubas, M. Buckingham, "Regulation of muscle gene expression. The accumulation of messenger RNAs coding for muscle-specific proteins during myogenesis in a mouse cell line", *J. Mol. Biol.* **160** (1982), p. 59-76.

[9] A. J. Minty, S. Alonso, M. Caravatti, M. E. Buckingham, "A fetal skeletal muscle actin mRNA in the mouse and its identity with cardiac actin mRNA", *Cell* **30** (1982), p. 185-192.

[10] P. J. Barton, B. Robert, M. Y. Fiszman, D. P. Leader, M. E. Buckingham, "The same myosin alkali light chain gene is expressed in adult cardiac atria and in fetal skeletal muscle", *J. Muscle Res. Cell Motil.* **6** (1985), p. 461-475.

[11] A. Weydert, P. Barton, A. J. Harris, C. Pinset, M. Buckingham, "Developmental pattern of mouse skeletal myosin heavy chain gene transcripts in vivo and in vitro", *Cell* **49** (1987), p. 121-129.

[12] B. Robert, P. Barton, A. Minty, P. Daubas, A. Weydert, F. Bonhomme, J. Catalan, D. Chazottes, J. L. Guénet, M. Buckingham, "Investigation of genetic linkage between myosin and actin genes using an interspecific mouse back-cross", *Nature* **314** (1985), p. 181-183.

[13] A. Weydert, P. Daubas, I. Lazaridis, P. Barton, I. Garner, D. P. Leader, F. Bonhomme, J. Catalan, D. Simon, J. L. Guénet, F. Gros, M. E. Buckingham, "Genes for skeletal muscle myosin heavy chains are clustered and are not located on the same mouse chromosome as a cardiac myosin heavy chain gene", *Proc. Natl. Acad. Sci. USA* **82** (1985), p. 7183-7187.

[14] B. Robert, P. Daubas, M. A. Akimenko, A. Cohen, I. Garner, J. L. Guenet, M. Buckingham, "A single locus in the mouse encodes both myosin light chains 1 and 3, a second locus corresponds to a related pseudogene", *Cell* **39** (1984), p. 129-140.

[15] S. Alonso, A. Minty, Y. Bourlet, M. Buckingham, "Comparison of three actin-coding sequences in the mouse; evolutionary relationships between the actin genes of warm-blooded vertebrates", *J. Mol. Evol.* **23** (1986), p. 11-22.

[16] I. Garner, A. J. Minty, S. Alonso, P. J. Barton, M. E. Buckingham, "A 5′ duplication of the alpha-cardiac actin gene in BALB/c mice is associated with abnormal levels of alpha-cardiac and alpha-skeletal actin mRNAs in adult cardiac tissue", *EMBO J.* **5** (1986), p. 2559-2567.

[17] C. Biben, J. Hadchouel, S. Tajbakhsh, M. Buckingham, "Developmental and tissue-specific regulation of the murine cardiac actin gene in vivo depends on distinct skeletal and cardiac muscle-specific enhancer elements in addition to the proximal promoter", *Dev. Biol.* **173** (1996), p. 200-212.

[18] R. D. Cox, I. Garner, M. E. Buckingham, "Transcriptional regulation of actin and myosin genes during differentiation of a mouse muscle cell line", *Differentiation* **43** (1990), p. 183-191.

[19] P. J. Barton, B. Robert, A. Cohen, I. Garner, D. Sassoon, A. Weydert, M. E. Buckingham, "Structure and sequence of the myosin alkali light chain gene expressed in adult cardiac atria and fetal striated muscle", *J. Biol. Chem.* **263** (1988), p. 12669-12676.

[20] B. Robert, D. Sassoon, B. Jacq, W. Gehring, M. Buckingham, "Hox-7, a mouse homeobox gene with a novel pattern of expression during embryogenesis", *EMBO J.* **8** (1989), p. 91-100.

[21] R. L. Davis, H. Weintraub, A. B. Lassar, "Expression of a single transfected cDNA converts fibroblasts to myoblasts", *Cell* **51** (1987), p. 987-1000.

[22] D. Sassoon, G. Lyons, W. E. Wright, V. Lin, A. Lassar, H. Weintraub, M. Buckingham, "Expression of two myogenic regulatory factors myogenin and MyoD1 during mouse embryogenesis", *Nature* **341** (1989), p. 303-307.

[23] G. E. Lyons, M. Ontell, R. Cox, D. Sassoon, M. Buckingham, "The expression of myosin genes in developing skeletal mus-

cle in the mouse embryo", *J. Cell Biol.* **111** (1990), p. 1465-1476.

[24] M. O. Ott, E. Bober, G. Lyons, H. Arnold, M. Buckingham, "Early expression of the myogenic regulatory gene, myf-5, in precursor cells of skeletal muscle in the mouse embryo", *Development* **111** (1991), p. 1097-1107.

[25] S. Tajbakhsh, D. Rocancourt, M. Buckingham, "Muscle progenitor cells failing to respond to positional cues adopt non-myogenic fates in myf-5 null mice", *Nature* **384** (1996), p. 266-270.

[26] F. Relaix, D. Rocancourt, A. Mansouri, M. Buckingham, "A Pax3/Pax7-dependent population of skeletal muscle progenitor cells", *Nature* **435** (2005), p. 948-953.

[27] D. Montarras, J. Morgan, C. Collins, F. Relaix, S. Zaffran, A. Cumano, T. Partridge, M. Buckingham, "Direct isolation of satellite cells for skeletal muscle regeneration", *Science* **309** (2005), p. 2064-2067.

[28] R. G. Kelly, N. A. Brown, M. E. Buckingham, "The arterial pole of the mouse heart forms from Fgf10-expressing cells in pharyngeal mesoderm", *Dev. Cell* **1** (2001), p. 435-440.

[29] S. M. Meilhac, M. Esner, R. G. Kelly, J.-F. Nicolas, M. E. Buckingham, "The clonal origin of myocardial cells in different regions of the embryonic mouse heart", *Dev. Cell* **6** (2004), p. 685-698.