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

Memories of Professor François Gros

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Abstract. I joined the laboratory of Professor Francois Gros in 1987 and worked there as a postdoc with Robert Whalen until 1992. I recount the research we carried out and mention that of the other scientists also working on skeletal muscle on the 6th floor of the Molecular Biology Department of the Institut Pasteur at that time. I then present my subsequent research when I returned to Japan. I pay tribute to the influence of Professor Gros and to his support in establishing Japanese/French meetings on muscle biology and muscular dystrophy. I also invoke personal memories of Robert Whalen and Margaret Buckingham and remember the occasions when I returned to Paris to honour François Gros.

Keywords. François Gros, Memories, Muscle biology, Muscular dystrophy.

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From the beginning of August 1987 to the end of August 1992, I was with Professor François Gros at the Institut Pasteur (IP) in Paris as a postdoctoral fellow. My study abroad was made possible through the recommendations of Professor Nobuo Yanagisawa, of the Third Department of Internal Medicine (Neurology) at Shinshu University, School of Medicine, the late Professor Setsuro Ebashi, and the late Professor Yoshiaki Nonomura of the Department of Pharmacology at the University of Tokyo, School of Medicine, who taught me during my graduate school days.

1. On the 6th floor of the Molecular Biology Building in IP

I wonder what muscle biology looked like at the Institut Pasteur when I studied there. Professor François Gros, a disciple of Nobel Prize winner Dr. Jacques Monod, contributed to the discovery of mRNA in the 1960s, then in the 1970s he became interested in higher-order biological functions and decided to focus his research on muscle development and

differentiation. He set up a laboratory, Unité de Biochimie, on the 6th floor of the Molecular Biology Building in IP. Professor F. Gros served as the director general of the Pasteur Institute in the early 1980s, and in the late 1980s he served as a special scientific advisor to President François Mitterrand. When I was in IP, there were three principal researchers on the 6th floor of the building. One was Dr. Margaret Buckingham, who was already independent and had her own laboratory. While also a professor at the Collège de France with a laboratory there, Professor F. Gros worked with two principal investigators, Dr. Robert G. Whalen and Dr. Marc Fiszman. At that time, Margaret focused on the development and differentiation of skeletal muscle and mainly dealt with myosin light chain (MLC) genes, Robert (Bob) dealt with postnatal muscle development and myosin heavy chain (MyHC) genes, and Marc focused on the relationship between muscle differentiation and splicing, especially of Tropomyosin genes. Therefore, there was considerable complementary between the scientific interests of the groups sharing the 6th floor.

The biggest advances in skeletal muscle biology that occurred during my five-year stay were the discovery of muscle regulatory factors (MRFs), centered on the MyoD gene, and the discovery of dystrophin, which is the cause of Duchenne muscular dystrophy (DMD). The former subject was pursued on the 6th floor of the Molecular Biology Building, which was recognized as one of the centers of research on the expression and role of these factors. MRFs consist of four transcription factors (MyoD, Myogenin, Myf5, and Mrf4), and Myogenin was in part discovered through collaborative research with Dr. Woodring Wright [1], working on the 6th floor. An *in situ* hybridization study on the expression of MRFs was published in *Nature* by Dr. David Sassoon, Margaret's postdoctoral fellow [2], and Shahragim Tajbakhsh, another postdoc in her lab, created a Myf5 null mouse [3]. Heterozygous Myf5 mice, in which the *lacZ* reporter gene has been inserted, are frequently used in muscle development research to this day. Members of the laboratory of Dr. Thomas Braun, who created the Myf5 null mouse one step ahead of Shahragim, Dr. Giulio Cossu, who succeeded in identifying mesoangioblast/pericyte stem cells, and Dr. Stefano Schiaffino, who created the MyHC monoclonal antibodies that are still used around the world, often visited Margaret or stayed in her lab for longer periods of time.

2. Unité de Biochimie directed by Dr. François Gros

Under these favorable circumstances, I was able to participate in Bob's research group with the support of Professor François Gros. Bob achieved a number of groundbreaking results from the late 1970s to the 1980s. These included the discovery of an embryonic myosin heavy chain [4], and the discovery of the expression of a skeletal muscle-type myosin light chain in the fetal heart [5]. Then, he found sequential expression of embryonic, neonatal, and adult types of myosin heavy chains during developmental stages of rat skeletal muscle [6]. Furthermore, innervation, thought to be a key regulatory mechanism controlling the expression of these isozymes, was investigated in his lab, by using denervation procedures. He observed that denervation does not interfere with the expression of the adult type of myosin heavy chain [7]. I will never forget that Professor François

Gros gave a detailed summary of these studies upon the retirement of Dr. Gillian Butler-Browne, who was a co-researcher on many of them. As a postdoctoral fellow under Dr. Robert G. Whalen, I worked hard on cloning the mouse MyHC IIB gene promoter and elucidating its transcriptional regulatory mechanism, and published several papers [8–10]. Subsequent research did not sufficiently clarify the transcriptional regulatory mechanism of MyHC IIB gene, but in recent years we have been able to shed some light on it through research on mice living on the space station. It involves a group of transcription factors called Maf. There are three types of Maf expressed in skeletal muscle, and when we created triple knockout mice in which all of them were knocked out, the expression of MyHC IIB disappeared, which revealed the regulatory mechanism from the transcriptional factor side for the first time [11]. Meanwhile, Bob then ventured into a completely new field of biology. This was an important attempt to create a vaccine by injecting into skeletal muscle, as a plasmid, a part of the genes that make up the virus [12]. The 2023 Nobel Prize in Physiology and Medicine was awarded to two researchers who developed an mRNA vaccine against the new type of coronavirus, but, although there is a difference between DNA and RNA, Bob's attempt largely preceded their work. Therefore, I think it deserves more recognition. The insight of Professor François Gros in realising the potential of Bob as a young researcher is also to be praised.

3. What I have done after I went back to Japan

I returned to Japan in 1992 and worked at the National Institute of Neuroscience, National Center of Neurology and Psychiatry, NCNP, in Kodaira City, Tokyo. What kind of work have I been able to accomplish at the National Institute of Neuroscience since then? It is broadly divided into four fields. The first was research to develop treatments for muscular dystrophy, which I began after returning to Japan [13, 14]. I have also focused my efforts on researching the pathology of muscular dystrophy, with the development of a mouse model of muscular dystrophy using a gene knockout approach [15, 16]. As a further development, we were able to establish a colony of muscular dystrophy dogs, which are medium-sized and have a more severe form of the disease, thus providing a good animal model [17]. The third category of

my research was in muscle cell biology, which can be seen as a direct consequence of the research on skeletal muscle differentiation conducted at IP. In 2007, we published an expression analysis study using microarrays on muscle satellite cells, which are one of the tissue stem cells of skeletal muscle [18]. Furthermore, in 2010, we showed that PDGFR α -positive mesenchymal progenitors exist in the mesenchyme of skeletal muscle, and that they not only positively control muscle regeneration but also are the origin of adipogenic cells and fibrosis [19]. Based on these studies, we are currently conducting research to induce muscle lineage stem cells using iPS cells and apply them to regenerative medicine, but this research has a long way to go. Finally, I gradually began researching signal transduction in skeletal muscle. We were able to demonstrate that neuronal nitric oxide synthase (nNOS) and nitric oxide play an important role not only in muscle atrophy [20], but also in muscle hypertrophy [21]. In particular, the latter research has uncovered the calcium signal transduction mechanism centered on the sarcoplasmic reticulum (SR), making it possible to reconsider the pathology of muscular dystrophy from the perspective of the calcium control mechanism [22].

4. The influence of Professor François Gros on me

After returning to Japan, I began therapeutic research for muscular dystrophy, but in carrying out this translational research, I needed to study not only basic and clinical medicine, but also biochemistry, cell biology, molecular genetics, and molecular biology. An integrated understanding was necessary. At that time, unlike Japan, where research tends to be carried out vertically in each field, France, where cross-disciplinary exchanges are encouraged in a free atmosphere, was an unforgettable experience for me, especially in the IP environment. I recall that there was a visit from Japan to Professor François Gros' laboratory in the context of fostering a Center of Excellence (a nationally representative research facility like IP), which later became one of the pillars of scientific research policy in Japan. Therefore, I came up with the idea of holding a workshop to connect Japan and France, and on March 20, 1995, I was able to hold an initial research meeting. The key participants in the meeting at IP (Figure 1) were Dr. Robert

<i>Association Thérapie Génique Franco-Japonaise</i>	
Institut Pasteur 25, rue du Docteur Roux 75724 Paris cedex 15, France	
Monday, March 20, 1995 Salle de Presse, Centre d'Information Scientifique	
Programme	
9:30 AM	Meet at entrance to Pasteur Institute
9:45 AM	Welcome and General Remarks Robert WHALEN
10:00 AM	<i>Cellular Therapy and Human Myoblast Immortalization</i> Dr. Gillian BUTLER-BROWNE Chargée de Recherche, INSERM, Paris
10:30 AM	Discussion
10:45 AM	<i>Adenovirus and Gene Therapy</i> Dr. Shin'ichi TAKEDA National Institute of Neuroscience, NCNP, Tokyo
11:15 AM	Discussion Comments: Dr. Thierry DIAGANA Pasteur Institute, Paris
11:30 AM	<i>Plasmid DNA and Use for Gene Transfer Studies</i> Dr. Robert WHALEN Directeur de Recherche, CNRS, Paris
12:00 PM	Discussion and Summary
12:30 PM	Lunch: Restaurant Hardel 8, avenue du Maine, 75015 Paris Tel: 45.44.39.41
2:15 PM	Visit to the Museum of Pasteur Institute: Scientific Museum and Apartments of Louis Pasteur
3:00 PM	Free Time

Figure 1. A circular of the scientific meeting at the Institut Pasteur on 20/03/1995. This meeting was the precursor to a series of 12 consecutive Japan-France international workshops for muscular dystrophy.

G. Whalen, who was the head of the team at IP, and Dr. Gillian Butler-Browne, who was about to move from IP to the Institut de Myologie (IdM). On the Japanese side, Dr. Hideo Sugita, President of NCNP (who unfortunately passed away in November 2019) agreed with the project and organised support for the travel and event expenses, together with Dr. Tadayuki Ishihara, who was the vice director of Higashi Saitama National Hospital (and later served as director of Hakone National Hospital). Needless to say, we had the support of Professor François Gros. The day the research meeting was held, the sarin gas attack on the Tokyo subway occurred, making it an unforgettable day. As a result of this initial meeting, we were subsequently able to hold an international Japan-France workshop on muscle biology and muscular dystrophy between the two countries.

To date, we have been able to hold this workshop 12 times, and there are several reasons for this. One was the “joy of being exposed to science”, which allowed us to bring together and present remarkable scientific results. Japan has a long history of biochemistry and cell biology, while France excelled in the fields of molecular biology and molecular genetics, which must have been a source of reciprocal inspiration. Next, it was also important that the workshop provided an opportunity for mutual exchange among researchers. Furthermore, the success of the first workshop held in Tokyo in 1996, followed by the second held in Paris the following year 1997, was a major factor in the decision to continue. On the French side, Dr. Michel Fardeau, Dr. Fernando Tomé, Dr. Pascal Guicheney, Dr. Marc Fiszman, Dr. Thomas Voit, Dr. Gillian Butler-Browne, and Dr. Gisèle Bonne from IdM, and on the Japanese side, the late Dr. Sugita, Dr. Eijiro Ozawa, the late Dr. Kiichi Arahata, Dr. Ikuya Nonaka, Professor Makiko Osawa, and Dr. Ichizo Nishino were major participants in this effort. It was only possible to continue the international workshop to this day due to the great contributions of these researchers and clinicians.

5. Personal memories

I would like to express my gratitude to these two benefactors. The first person is Dr. Robert G. Whalen, who was the head of the IP team. When I was working on transcriptional regulation of the murine MyHC IIB gene, he said to me, “You have directly treated patients with muscular dystrophy as a clinician, and at the University of Tokyo, you studied the biochemistry of muscle proteins. If you could acquire the approach of molecular biology of skeletal muscle at the genetic level, then you would be a unique person in this field of research.” He continued to give me research opportunities. I’ve never met a person as talented as him. When I left IP, I will never forget the tears in his eyes as he bid me farewell.

The second person was Dr. Margaret Buckingham, who was on the same floor but in the laboratory next door and later became a member of the French Academy of Sciences. Margaret created an opportunity for me to get a position in Japan. In 1990, an international symposium entitled “Muscle Development, Maintenance, and Contraction; Recent Developments” was held in Japan, sponsored by

the Uehara Memorial Life Science Foundation. Margaret was invited and gave an excellent lecture; she mentioned to many Japanese researchers that a researcher named Shin'ichi Takeda was studying at IP. I believe that this introduction was passed on to the professors at the National Institute of Neuroscience NCNP, including Dr. Yo-ichi Nabeshima, who was Margaret's colleague, and led to my return to Japan.

Finally, it is with great regret that I lost Professor François Gros on February 18, 2022. I thank him very much for receiving me in his laboratory in IP and supporting my stay in France (his letter of recommendation was required in order to obtain a residence permission in France), and for his letter of recommendation to the National Institute of Neuroscience NCNP, when I returned home. I am also grateful to him for giving me the opportunity to hold the workshop between France and Japan, and having the opportunity to speak at his 90th birthday celebration held at IP in April 2015. On April 24, 2023, a farewell meeting and celebration in his memory was held at IP and the French Academy of Sciences, which I was able to attend, after which I would like to once again offer my sincere prayers for his soul.

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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