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Adrian Minty

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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)

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

On learning molecular biology in François Gros' lab in the late 1970s and early 1980s

Adrian Minty^a

^a Sanofi Recherche, Toulouse, France (retired)
E-mail: adrianjohnminty@gmail.com

Abstract. Although reflection is obviously crucial in molecular biology, experimentation is nonetheless the basis of most major advances. I was lucky to begin my research career at a particularly interesting time, and privileged to have spent a number of years in François Gros' laboratory at the Institut Pasteur. His influence, and that of his lab, were crucial in shaping my early career.

Keywords. mRNA, Molecular biology, Gene cloning, Gene expression.

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1. On the discovery of mRNA and my interest in it

The idea of an unstable messenger RNA taking information from the genome to the sites of protein synthesis now seems to us to be obvious. To see that this was not the case at the time of the experiments of François Gros and colleagues in 1961 [1], readers are encouraged to have a look at the second edition of the book *Nucleus and Cytoplasm*, published by Professor Henry Harris of the University of Oxford in 1970 [2]. In this book, Professor Harris re-examines in detail the critical experiments of Gros *et al.* [1] and others, and continues to conclude that it is, in fact, ribosomal RNA which is the messenger!

When I was completing my biochemistry degree at Cambridge in 1974, the subject of mRNA was still a hot one, and I was lucky to do an undergraduate project on RNA translation in reticulocyte lysates with Tim Hunt and Richard Jackson. This persuaded me to continue in the field of mRNA for my PhD, at the Beatson Institute in Glasgow, and subsequently in my postdoc with François at the Institut Pasteur.

2. On RNA populations in animal cells and gene number

Now that RNA could be isolated, our work at the Beatson Institute, and parallel studies by Nabeel Affara in François' lab in Pasteur, by John Bishop's lab in Edinburgh, Eric Davidson's lab in LA and others, was to attempt to devise experimental ways of studying RNA populations in animal cells. We used the newly discovered reverse transcriptase enzyme to make copies of all polyadenylated mRNAs. In highly miniaturised experimental "containers" with microlitre volumes, we mixed the RNA and radioactively labelled DNA copies and then studied the formation of RNA–DNA duplexes over times up to one week.

The results of these experiments allowed us to estimate the number of genes expressed in an animal cell, and the number of these genes in common with a second cell type. Given all the uncertainties in the calculations, the results were surprisingly consistent from one study to another, with most animal cells and tissues expressing between 10,000 and 20,000 genes [3]. This is in the same range as the figure deduced twenty years later for the number of human genes by genome sequencing [4].

3. On non-polyadenylated mRNA

In 1978, incited by my friend Nabeel Affara, I moved to the Institut Pasteur to work with François. I still remember one of my early exchanges with François, who was a very good letter writer, which took place while I was still in Glasgow. He expressed the hope that an “excellent articulation” would be established between us. I like to think that this was the case!

At the Institut Pasteur, I continued the study of mRNA. In all the previously mentioned studies on mRNA, we had experimentally defined mRNA as being polyadenylated, since either oligo(dT)-cellulose or poly(U)-Sephadex chromatography were used to isolate it. However, a significant fraction of mRNA (defined by its translation in a reticulocyte lysate) did not bind to oligo(dT) or poly(U). We therefore attempted to see if this fraction encoded different proteins, and thus represented a different type of messenger RNA.

Analysing translation products of highly purified poly(A)⁺ and poly(A)⁻ preparations on two-dimensional gel electrophoresis, we showed that the two populations encoded similar proteins, but in very different abundancies [5]. One protein class which was almost entirely poly(A)⁻ was that encoding the histones. Our studies showed that, apart from the histones, poly(A)-mRNA does not encode another, particular, subset of mRNAs. This work, and the critical 2D-gel figure, were cited in the Molecular Cell Biology book by Darnell *et al.* [6]. Few other studies have been done since on this question, but those that have been performed support our conclusion [7].

4. On the advent of gene cloning

The previous studies on mRNA populations represented the last in the “pre-cloning era”. The advent of recombinant DNA technology was to revolutionise our experimental approaches in molecular biology, and François’ laboratory was no exception (see his chapter on *Le “boum” du génie génétique* [8]). A gene cloning project was set up in Margaret Buckingham’s group, and I took the responsibility for cloning the actin genes of the mouse. I still remember crossing the rue du Dr. Roux to discuss with François Rougeon, one of the world specialists in gene cloning, who warned me that this might

be complicated because the actin mRNAs were less abundant than the globin mRNA and the other mRNAs that had been cloned up to then! Consequently, we subjected the RNA to sucrose-gradient separation to enrich for the actin mRNA(s) before cloning!

Gene cloning was something of a challenge for someone who had not previously done experimental work with bacterial plasmids, nor indeed with bacteria! Nonetheless, the abundance of actin mRNAs (particularly in the size-purified RNA!) meant that a hundred or so recombinant bacteria were largely sufficient to isolate several different actin cDNAs, including two different muscle actin cDNAs [9]. This work enabled us to characterise, for the first time, the expression of both skeletal muscle and cardiac actins in skeletal muscle [10].

5. On the retro-transcription of polyadenylated mRNA and integration of DNA copies in the genome

One of the first subjects to be addressed with the newly cloned actin cDNAs, was the number of actin genes in mammalian genomes. We found that in mouse DNA, in addition to the six actin genes expected from protein sequencing work, there was also a family of weakly-related sequences, some of which had recently been amplified in the genome [11]. Although these had not been sequenced, the length of these actin-like genes in genomic DNA led us to predict that they could represent intron-less pseudogenes, generated by reverse transcription of actin mRNAs and insertion in the genome. This process has since been confirmed, with the sequencing of over 8000 retropseudogenes in the human genome [12].

It is somewhat surprising that studies on mRNA-copy reintegration into the genome were largely ignored during the recent debate on the possibility of Covid-19 RNA vaccine reintegration in the genome. Alex Whiting, writing in 2020 [13], quoted Professor Michel Goldman of the Université Libre de Bruxelles as saying “mRNA vaccines do not alter your DNA. A concern that some have had about mRNA vaccines is that they could change people’s DNA. But that idea is ‘completely false’ and has ‘no scientific basis.’”

It would seem, in fact, that it is stating the impossibility of re-integration into the genome which is completely false [14]. When we combat conspiracy

theories on the potential dangers of RNA vaccines, this should not involve ignoring any potentially contradictory facts!

6. On the nature gene regulatory sequences

The “grail” of molecular biologists in the 1980s was the identification of the sequences regulating gene expression in mammalian cells. In pursuit of this, I was to continue the work on actin genes in the laboratory of Larry Kedes at Stanford University. Apart from the 9000 km that separated Paris from Palo Alto, the main difference between the two labs was that the Kedes lab worked on human actin genes rather than mouse actin genes. After some months of rather painstaking experimental work identifying the transcription start site for the human cardiac actin gene, I was to have some luck.

This was the time in 1984 when Apple was launching the first Apple Macintosh, and a special offer was made to workers at Stanford. It was thus on my own Macintosh, using a very rudimentary DNA comparison programme, that the “CCArichGG” regulatory sequence was identified at my house in Mountain View [15]. These CCArGG DNA sequences have since been shown to be more widespread than we originally anticipated, and to have more diverse roles. Indeed, the molecular mechanisms of the binding of transcription factors to these sequences are still in the process of being elucidated thirty-seven years later [16]. I feel extremely lucky to have been able to participate, thanks to François’ help, in this very exciting period of research.

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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It will be clear from the above, that the early years of my research career were greatly shaped by my time in François Gros’ lab. I will always be grateful to François for allowing me to come and work in his lab, for allowing me to choose my research subjects and

for offering me so often some of his precious time and advice. Among the other people who helped me a lot during my time at Pasteur, I would also like to thank Nabeel Affara, who persuaded me to come to Paris, and Margaret Buckingham with whom I was to work on the gene cloning project. Last, but very decidedly not least, it was also working in François’ lab that I was to meet my wife Catherine, and it was there too that I acquired the nationality of a country that has now been mine for the last forty-five years!

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