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Series

What history tells us XLV. The 'instability' of messenger RNA

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1. Introduction

The characterization of messenger RNAs is one of the most beautiful discoveries in the history of modern biology. This discovery, the fruit of a perfect mixture of serendipity and patient experimental work, can easily be described in a limited number of steps.

At the end of the 1950s, more and more biologists were convinced that the synthesis of RNA was controlled by DNA, and that RNAs were involved in the synthesis of proteins. The latter process had been shown to take place on microsomes, now called ribosomes, particles formed of RNAs and proteins. Ribosomal RNAs (rRNAs) were considered to be responsible for protein synthesis, but there were major puzzling issues: the size of rRNAs was well defined, and their composition in bases was similar in different organisms, whereas that of DNA was highly variable between organisms. How to explain these observations if rRNAs were the intermediates between DNA (genes) and proteins of highly different sizes?

The solution came in four steps. The first step was a series of experiments initiated in 1957 by Arthur Pardee, François Jacob and Jacques Monod, showing that during bacterial conjugation the β-galactosidase gene (*z*) was rapidly expressed (in less than two minutes) at its maximal rate after its passage from the donor bacterium to a recipient one. This occurred without any possible transfer of ribosomes (Pardee *et al.* 1959; for a personal description, see Jacob 1988 and Pardee 2002). One year after the first publication, Arthur Pardee did the second step by performing a complementary experiment in which he showed that the synthesis of β-galactosidase stopped after inactivation of the *z* gene (resulting from the disintegration of the radioactive phosphorus that had been incorporated within it) (Riley *et al.* 1960). These two experiments led to the hypothesis that between DNA and proteins there was a short-lived intermediate, which was dubbed 'X'.

On Good Friday 1960, at a meeting between, among others, François Jacob, Sydney Brenner and Francis Crick, took place the third step. Crick and Brenner suddenly realized that the characteristics of 'X' were those of RNAs that had been described by Volkin and Astrachan years before during the infection of bacteria by bacteriophages (Volkin and Astrachan 1956). Even if the precise chemical nature of 'X' had not been fully discussed before, its identification with an RNA was easily accepted: the role of RNA as an intermediate between DNA and proteins had been recurrently proposed in previous years.

In a fourth and last step, the existence of these unstable RNAs, called by Jacob and Monod 'messenger RNAs' in 1961 (Jacob and Monod 1961), was demonstrated in the following months. Brenner, Jacob and Matthew Meselson showed that, during bacteriophage infection, new rapidly synthesized RNAs associated with pre-existing 'old' ribosomes (distinguished by their labelling with heavy isotopes before the addition of bacteriophages) (Brenner *et al.* 1961; see also Meselson 2014). In an independent experiment, François Gros and his colleagues demonstrated in Jim Watson's lab that in uninfected bacteria there was also the constant production of (rapidly labelled) labile RNAs of different sizes that associated with ribosomes (Gros *et al.* 1961).

Our narrative so far is identical to those that can be found in most articles and books, including the most recent ones (Judson 1979; Jacob 1988, Friedberg 2010; Fry 2016; for a

different point of view, see Cobb 2015), a narrative probably familiar to many readers of the *Journal of Biosciences*. There is, nonetheless, a problem with this narrative. When these characteristics of mRNAs were proposed, it was already known through the experiments on enucleated cells initiated by Joachim Hammerling and pursued by Jean Brachet in Brussels that some mRNAs had to be very stable in eukaryotes (Brachet *et al.* 1955; Thieffry and Burian 1996). Today, it is well known that some mRNAs may be unstable, but that most of them are stable in eukaryotes, but also in bacteria and archaea. The issue which I want to address in this contribution, is: Why was this property of instability so important in characterizing mRNAs when they were discovered? I will successively show that it was in part a consequence of the experimental systems that were used. But some of the experimental 'proofs' of this instability appear today far less compelling. I will present other reasons that also justified this attribute of 'instability' given to mRNAs. The excessive emphasis put on instability explains some of the opposition encountered by molecular biologists in the following years when expanding their models, in particular to eukaryotes.

2. The weight of experiments

PaJaMo was the most convincing experiment, not only because β-galactosidase was rapidly produced after the introduction of the *z* gene, but because it was immediately synthesized at the maximal rate, a result unexplainable if the 'X' intermediate was produced permanently and was even minimally stable (Pardee *et al.* 1959): it would have accumulated, and the rate of synthesis of β-galactosidase would have increased with time.

The second experiment, done by Monica Riley and Pardee after his departure from the Pasteur Institute, is often presented as symmetrical to the PaJaMo experiment (Riley *et al.* 1960). The PaJaMo experiment showed that 'X' was rapidly synthesized, and Pardee and Riley's experiment showed that 'X' is rapidly degraded, the only way to explain why the protein synthesis stopped soon after degradation of the gene. In fact, Riley's experiment was not intended to show this, and did not! The ranges of times in the two experiments were very different. In the PaJaMo experiment, the results showed that 'X' was produced in less than two minutes. In the second experiment, β-galactosidase synthesis (and therefore 'X' necessary for this synthesis) decreased at a rate similar to the rate of gene degradation: the result of the experiment would have been different if 'X' had been stable over days; it would have been the same if the half-life of 'X' had been in the range of hours! Riley and colleagues were very clear in their discussion: the results eliminated another model, which was that 'X' was rapidly produced during a short lapse of time and remained fully stable thereafter.

The two last experiments provided evidence for the existence of mRNAs and demonstrated the rapid synthesis of a heterogeneous ensemble of high-molecular-weight RNAs, and their rapid association with ribosomes. In addition, the Brenner-Jacob experiment demonstrated that the newly synthesized RNAs associated with 'old' pre-existing ribosomes.

The demonstration of the instability of mRNAs was less convincing. The chase experiments were limited in both papers to one time. A possibility was that the observed instability of mRNAs reflected, not the expected physiological instability, but an artefact generated by the experimental conditions. One observation, made by François Gros and his collaborators, suggested that this was the case: when mRNAs bind to ribosomes, it was expected that the sedimentation coefficient of the latter would change, but such a change was not observed (Gros *et al.* 1961). Retrospectively, it is difficult to propose an explanation other than the rapid artefactual degradation of the labelled mRNAs: only short fragments bound to ribosomes.

The rapid and transient synthesis of the inducible enzyme β-galactosidase and of viral proteins during infection was not surprising, since a rapid change in protein synthesis had to occur in the two systems. These two experimental systems were considered as models for the study of protein synthesis, and historically they played this role. But can the results obtained in situations of rapid changes in protein synthesis be extrapolated to all cases, in particular to the synthesis of what were later called 'housekeeping' proteins and enzymes? The only experiment in this crucial period that might have provided an answer was the Gros's experiment. As we have seen, experimentally it was the least convincing!

The instability of mRNAs was less a characteristic closely linked to their function than a condition that allowed them to be seen and to be distinguished from rRNAs. The article published in 1960 by Martynas Ycas and Walter Vincent is often cited as one of the earliest pieces of evidence for the existence of unstable RNAs (Ycas and Vincent 1960). A good reason why this article did not receive due credit is the complexity of the techniques that were used, which required the help of an 'electronic computer' to analyze the data. What is most interesting in this article is that the authors do not say that they have isolated a category of labile RNAs, but simply that they were able to distinguish by their 'unequal rates of turnover' different categories of RNAs, including one with a base composition similar to that of DNA. They clearly point to what was essential: not that mRNAs were naturally unstable, but that they were globally less stable that other RNAs, and in particular less stable than rRNAs. Their conclusion remains valid today for prokaryotes and eukaryotes – they used yeasts in their experiments – whereas the supposed natural instability of mRNAs no longer has any place in biology.

3. Other reasons to favour the hypothesis of mRNA instability

Behind the experimental observations, there were additional reasons to support the hypothesis that mRNAs were unstable that were rooted in the way the new vision of molecular biology had been built.

The obstacle to the discovery of mRNA that made it 'postmature' was the structural (and catalytic) role of rRNAs in protein synthesis. The characteristics of mRNAs had to be different from those of rRNAs. Since rRNAs were stable, mRNAs had to be unstable. In addition, this instability of mRNAs was the perfect explanation for the fact that they had not been observed before: maybe too perfect an explanation to be considered fully valid!

There was another opposition that has not been sufficiently emphasized, that between the instability of mRNAs, on the one hand, and the stability of DNA and proteins, on the other. By using heavy isotopes, Rudolph Schoenheimer observed at the end of the 1930s that the constituents of the body, and in particular proteins, were in a dynamic state, and therefore unstable (Schoenheimer 1942). These results were widely accepted. But in the 1950s, molecular biologists, with Monod at their head, demonstrated that this was not true for β-galactosidase. In absence of any strong experimental evidence, it was nevertheless admitted that it was also not true for proteins in general (Monod 1966; see also Morange 2016 in this series). To emphasize the unstable, transient nature of mRNA was a way to confine this molecule in a unique role, that of an intermediate. It was often, at least initially, admitted that each molecule of mRNA permitted the synthesis of only one molecule of protein (a hypothesis seriously considered in the two articles of 1961 (Brenner *et al.* 1961; Gros *et al.* 1961).

In the new informational vision of molecular biology, DNA contained the genetic information, and proteins were the functional realization of this information. Between the two, mRNA was initially described as a simple transporter of information, not an informational molecule itself. It was a signal that transferred to the ribosome the information needed to synthesize a protein. It has recently been argued that this vision has been an obstacle to the discovery of other functions for mRNAs (and RNAs in general), catalytic or regulatory, and of their probable role in early forms of life (Sharp 2009; Darnell 2011).

This instability of mRNAs was concomitant with the attribution of a major role in the regulation of gene expression to the control of transcription. It was very clearly stated in 1961 (Brenner *et al.* 1961). If mRNAs had been stable, it would have been necessary to consider the control of mRNA stability, the rate of translation, and the stability of proteins as possible levels of regulation. For Monod, one of the authors of the operon model, nature had to be simple: a single level for the control of gene expression was the best solution. Unfortunately, nature, or more precisely natural selection, is indifferent to such principles.

In summary, the instability of mRNAs was a necessary condition for demonstrating their existence in the early 1960s; it found additional justifications in the simplistic informational vision that dominated at the same period.

4. mRNA instability at the centre of many debates in the next twenty years

The stability of certain mRNAs was rapidly demonstrated after 1961. Jean Brachet, using inhibitors of transcription and protein synthesis, showed the rapid synthesis of new RNAs at certain stages of amphibian development, but also confirmed the results that he had obtained before on the stability of mRNAs in algae (Brachet and Denis 1963; Brachet 1963). The effect of protein synthesis inhibitors on the morphogenesis of algae also demonstrated that morphogenesis was controlled at multiple levels, and not uniquely at the transcriptional level. *A contrario*, he confirmed the relation existing between the instability of mRNAS and the privileged control of gene expression at the transcriptional level.

Embryologists in general confirmed that some mRNAs were stable during development: for instance, mRNAs were prestored in the oocyte in an inactive form and were activated after fertilization to permit the early stages of development (Gross and Cousineau 1963: Spirin and Nemer 1965). These observations supported the conviction shared by most embryologists that many mechanisms of regulation of gene expression existed and took part in development, aside from the mechanisms described in the operon model and taking place at the transcriptional level (see, for instance, Schimke and Doyle 1970 for a possible control of the stability of proteins).

The nature and role of mRNA instability was also questioned by observations made in prokaryotes. The discovery of polar mutations, occurring in the upstream part of the operons and affecting the synthesis of the proteins encoded by distal genes had a major impact (Zipser 1969). The first reason was that these mutations had not been correctly characterized by the inventors of the operon model, and were confused with mutations in the operator. The mechanism that was proposed, detachment of the ribosomes and degradation of the unprotected mRNA, was rapidly suggested to exist independently of the polar mutations, and to regulate gene expression independently of the regulation taking place at the transcriptional level.

In eukaryotes, Henry Harris showed that RNAs were stable in the cytoplasm, but unstable in the nucleus (Harris 1963; Harris *et al.* 1963). His observations remained unexplained during the next twenty years, before the discovery of the splicing process of RNAs. These observations convinced

many biologists that instability was not a characteristic of mRNA, but a complex phenomenon still lacking an explanation. It even pushed Henry Harris and others to deny the existence of mRNAs! (Harris 1994).

It is obvious that the emphasis initially put on the instability of mRNAs in some cases hindered recognition of their major role in the synthesis of proteins. The order of relations between DNA, mRNA and protein was nonetheless generally accepted, even if the expressions 'D-RNA' (Stevens 1963) and 'informational RNAs' (Davidson 1968) were for a while preferred to 'mRNA' by biochemists and embryologists.

5. Conclusions

Two lessons can be learned from a precise description of this historical episode. The first concerns model systems. They are particularly important in biology: both phages and adaptive (inducible) enzymes had a major role in the rise of molecular biology. But any model has limits and advantages. The two previous systems permitted observation of rapid changes in protein synthesis, and for this reason represented a favoured way to access the mechanisms of protein synthesis. But they were not representative of most cases of protein synthesis. The instability attributed to mRNAs was a consequence of the choice of these models; it did not represent a general characteristic of mRNAs.

The second lesson concerns the way these emblematic experiments are often presented. They are simplified, and any aspect of these experiments or of the conclusions that was problematic is eliminated. As a result, it becomes impossible to understand the role that they played, and they may even appear to contradict our present state of knowledge. Historians have already raised these issues in the case of the Hershey-Chase experiments demonstrating that DNA is the genetic material of the bacteriophage (Wyatt 1974). Experiments that provide evidence for mRNAs are another example that has been far less studied by historians. Continuing to present these experiments as if instability was still considered today as a major characteristic of mRNA creates a malaise. Historically, it was important to separate mRNAs from other types of RNAs. It is no longer important and is even wrong if it is presented as a general fact! The path to scientific truth is tortuous, with many contributions, not all of which go in the same direction (Cobb 2015). This does not mean that these historical experiments cannot be used for teaching. But they should not be caricatured and over-simplified. The context in which they were done has to be carefully described, and the biases in the experiments or in the conclusions have to be outlined. This is the only way to give history its proper and useful place in the training of scientists.

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