

What history tells us XXIX. Transfers from plant biology: From cross protection to RNA interference and DNA vaccination

MICHEL MORANGE

Centre Cavallès, CIRPHLES USR 3308, Ecole normale supérieure, 29 rue d'Ulm, 75230 Paris Cedex 05, France

(Fax, +33-144-323941; Email, morange@biologie.ens.fr)

1. Introduction

The award in 2006 of the Nobel Prize in Physiology or Medicine to Andrew Fire and Craig Mello immediately aroused a controversy. They were rewarded for their discovery and characterization in *C. elegans* of the mechanisms of RNA interference (Fire 2006; Mello 2006). A small group of young researchers protested because plant scientists had not been associated with the Nobel Prize (Bots *et al.* 2006). They argued that numerous characteristics of the phenomenon – its occurrence at the post-transcriptional level, its sequence specificity, its systemic character, its function in the protection against viruses – had been demonstrated earlier in plants (Voynet and Baulcombe 1997; Ratcliff *et al.* 1997). Gene silencing (co-suppression) was described for the first time in *Petunia* in 1990 (Napoli *et al.* 1990; van der Krol *et al.* 1990), long before the famous experiment carried out by Fire and Mello in *C. elegans* (Fire *et al.* 1998). The Nobel Committee's ignorance of the work done on plants was not surprising: studies on plants have always suffered from a certain form of invisibility to other biologists.

My objective is not to enter into this controversy. The discovery of RNA interference was such a complex process that attributing the main merit to one or other researcher is a nearly impossible task. But when I tried to understand the origin of the controversy, I discovered that the work on plants that finally led to the discovery of RNA interference took place within a broader scientific context that has been largely omitted from the historical records. It is not true that the work on plants was ignored. Its influence was important,

and many exchanges occurred between plant science and other branches of biology. But certain specific reasons prevented acknowledgement of this debt to plant science.

2. Cross protection in plants: Immunity without an immune system

In his Nobel Lecture, Fire recalled earlier events that were important for the discovery of RNA interference (Fire 2006). He mentioned the discovery of the protective role of interferon against viruses in animals, and a similar protective mechanism against viruses in plants that was discovered far earlier, at the end of the 1920s (McKinney 1929). What Fire did not say is that the latter phenomenon of cross protection was extensively studied in the following years and decades, and major reviews devoted to it were written. More importantly, plants were successfully protected against a pathogenic virus by pre-infection with a mild strain of the same virus. An early essay was done on the Isle of Wight (UK) in 1964 (Broadbent 1976) with the tobacco mosaic virus. Diverse plants – tomato, citrus, cacao, papaya – were later used for these tests, which were practiced on a large scale in Brazil, West Africa, Taiwan, etc. Sometimes even growers unwittingly and unconsciously made use of this method of control (Sequeira 1984)

This practice developed rapidly in the 1970s and 1980s, although doubts persisted about whether it was completely effective and harmless (Fulton 1986). Reading the scientific literature of this time, an observer unfamiliar with plant physiology might have concluded that cross protection in plants

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and immunization in animals were one and the same phenomenon. The same words were used to designate these practices (for instance, immunization, attenuation), and the questions raised in these studies – on the best ‘immunization’ protocol or on the possibility that the mild strain would revert to a pathogenic form – were identical. But for most plant biologists, it was obvious that the two phenomena had nothing in common at the mechanistic level. The rare observations in plants of molecules similar to antibodies were never confirmed.

However, there was no consensus on the mechanisms explaining cross protection in plants (de Zoeten and Fulton 1975; Kosuge and Nester 1984; Horsfall and Cowling 1986). The first hypothesis was that there was a substance present in plants required for the growth of the viruses and that, by depleting cells in this substance, the mild attenuated strain prevented the subsequent development of the pathogenic strain. This hypothesis was not new: it was the hypothesis favoured by Pasteur and his followers to explain the success of vaccination before the discovery of the mechanisms of immunity at the end of the 19th century made it obsolete.

The second hypothesis was that there was a unique cellular site for viral reproduction, and that there was competition for this site between the mild and pathogenic viral strains. This hypothesis was also not original. It had been proposed by biologists working on the phenomenon of lysogeny – the capacity of a bacterium to harbour a silent temperate phage. How was it possible to explain that a bacterium bearing a silent phage was immune to a new infection by the same phage? The hypothesis put forward by Elie Wollman and François Jacob was that there was a unique specific site for phage production that was occupied by the temperate phage – which prevented the replication of any incoming phage. This hypothesis was abruptly replaced at the end of the 1950s by a radically new model: there was a repressor produced by the temperate phage that prevented its own replication, and that of any super-infecting phage (Jacob and Wollman 1961).

A third hypothesis was that the challenge virus was somehow trapped by the mild form of the virus that had preceded it and already initiated its replication: this trapping effect could be the result of non-specific adsorption, or of the specific action of the viral coat protein preventing the uncoating of the challenge virus (Sherwood and Fulton 1982).

Let me add one last word on the term ‘interference’ introduced by Craig Mello to designate the new phenomenon (Fire 2006). Tracing the origin of a word is also an impossible task. But it is clear that as early as 1951, the word ‘interference’ was widely used by plant biologists to describe the phenomenon of cross protection (Bennett 1951).

3. The entrance of molecular biologists into the arena

The last hypothesis already represented a first step towards a molecular explanation of the phenomenon of cross

protection (Sequeira 1984). The solution to the uncertainties and risks linked to the practice of cross protection, as well as the hope of reaching a mechanistic understanding of this phenomenon, was looked for by the new rapidly growing group of plant molecular biologists through the application of the new technology of transgenesis (Weising *et al.* 1988).

After an early successful attempt at transfection (Bevan *et al.* 1985), it was demonstrated that it was possible to protect tobacco against tobacco mosaic virus by transfecting plants with the gene encoding the coat protein (Powell Abel *et al.* 1986). Similar results were rapidly obtained on other plants infected by different viruses (Beachy *et al.* 1990). A new strategy, parallel to and different from the traditional isolation of resistant plant strains, had emerged.

More precise scenarios to explain the phenomenon that had been observed were proposed: the coat protein might bind directly to the viral RNA at a specific regulatory site, and prevent its replication. Other ways to obtain plants resistant to viruses were also proposed (Baulcombe 1989). David Baulcombe showed that the expression in transgenic plants of an inactive form of the viral replicase had an inhibitory effect (Longstaff *et al.* 1993).

These results were generalized through the concept of ‘pathogen-derived resistance’ elaborated by John C Sanford and Stephen A Johnston: protecting organisms by injecting them with genes from the pathogen. After a value-of-proof experiment on the Q β phage and its host *E. coli* (Grumet *et al.* 1987), a theoretical model was proposed to apply this concept to AIDS (Sanford 1988). Five years later, Michael Wilson could say that ‘pathogen-derived resistance’ blossomed as a strategy to protect crop plants against viruses (Wilson 1993).

However, difficulties rapidly appeared. Cross protection was already known to work for viroids that are devoid of capsid proteins (Niblett *et al.* 1978). Protection also occurred when the coat protein sequence introduced was untranslatable (Lindbo and Dougherty 1992). The mechanism appeared more complex, and the simple scenarios disappeared. RNA progressively acquired a pre-eminent place in the explanation of the phenomenon. The road to the final explanation, the production of small interfering RNAs, was open but nevertheless full of obstacles: in particular because another hypothesis, an inhibition by the production of inhibitory antisense RNA, seemed highly attractive (Ecker and Davis 1986; Morange 2008).

4. From plants to animals: DNA vaccination

Later work on RNA interference was in part done by biologists other than plant scientists, but the studies on cross protection had nonetheless another sequel in the practices of animal immunology.

Interestingly, some of the main players in this new episode were the same as those who pushed the concept of pathogen-derived resistance. Sanford designed a new technique to introduce genes into plants by bombardment with microprojectiles. It allowed transfer of genes in plants without removing cell walls, and also the introduction of genes into mitochondria and chloroplasts. In 1988, he demonstrated the efficiency of the new methodology on maize (Klein *et al.* 1988), yeasts (Johnston *et al.* 1988) and later mice (Williams *et al.* 1991). This new method to introduce genes into organisms was immediately adopted for the development of a new project: vaccination through the direct introduction of DNA into recipient organisms.

From 1990, a series of tests were performed (Donnelly *et al.* 1997). Gene-gun immunization was not the only technique used (Fynan *et al.* 1993), but it had an important place in the tests of these DNA vaccines (Tang *et al.* 1992).

Because of their simplicity, viruses – influenza virus (Ulmer *et al.*, 1993) and HIV (Wang *et al.* 1993) – were the preferred targets for this new strategy of vaccination. In the case of HIV, the microinjected DNA contained the sequences for the envelope protein, establishing a striking parallel with the work performed some years earlier on plants.

In this case, the transfer of technology from plants to animals is well demonstrated, and the transfer of models seems obvious. But no acknowledgements of this transfer are found in the articles on DNA (gene) immunization despite the fact that some authors, such as John Sanford, crossed the border between the two disciplines.

5. Discussion

What happened in the years that preceded the characterization of the mechanisms of RNA interference cannot be reduced to a simple neglect of the work done on plants, and especially of the discovery of the co-suppression effects in *Petunia* in 1990 (Napoli *et al.* 1990; van der Krol *et al.* 1990). There was a very active field of research, both applied and fundamental, on cross protection in plants that preceded the discovery of gene silencing in *Petunia*. And there was an active exchange of techniques and concepts between the two branches of biology: the language and approaches of immunology were used to describe the phenomenon of cross protection in plants, and techniques and concepts that emerged from the study of cross protection in plants were used for the development of DNA vaccination in animals at the beginning of the 1990s.

Nonetheless, it is true that these exchanges were not visible and acknowledged. The language of immunology was used to describe cross protection and its application, but the parallel implicitly established with the mechanisms of immunity by this transfer of language was not discussed.

And the fact that the work done on plants led to DNA immunization is never mentioned in articles on the latter.

I would like to propose two hypotheses to explain this paradox. The first is that cross protection in plants was mostly a practice often developed in ‘peripheral’ countries. This probably explains why these studies easily disappeared from the historical records of the work that led to the discovery and characterization of RNA interference. The second reason was the absence of a solid scientific justification for these practices. It was obvious in the work on cross protection, but it was also rapidly the case with the results obtained by transgenesis.

Maybe the origin of the difficulties is to be found in a cultural difference between plant biologists and other biologists. The former are more likely than the latter to accept and use a practice that has not yet received a precise mechanistic explanation; with good reason, because these practices have often played a major role in crop production. This cultural difference does not prevent exchanges, but it does reduce their visibility.

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