

1 When I Encountered Tobacco BY-2 Cells!

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1 Introduction

The tobacco BY-2 cell line has attained a unique position in the plant science community. Although it is one of many plant cell lines, it has spread to at least 27 countries, as far as I am aware, and more than 500 papers in which the BY-2 cell line is handled as experimental material have been published from 1990–1999. Since then, publications on this cell line are steadily increasing. It seems that this cell line has been brought to much wider locations than the above-mentioned 27 countries as well without my notice. Thus, the basic knowledge on the molecular and cellular biology of plant cells is most extensively and intensively accumulated on this cell line (Nagata et al. 1992; Geelen and Inzé 2001). This is one of the strong reasons why this volume is included in this series of *Biotechnology in Agriculture and Forestry* (BAF). The accumulated knowledge can be shared with scientists of applied as well as basic fields. In this situation, it is worthwhile to reflect what happened in the earlier stage of the culture of tobacco BY-2 cells.

2 Encounter

It was 1981 when I first came across the tobacco BY-2 cell line. In fact, there was a successive series of coincidences, without which this cell line would not have become so popular. Shortly before that time, I was asked to move to Nagoya University from the University of Tokyo by the late Professor Itaru Takebe. Strictly speaking, this had been asked a year before his acceptance of the professorship proposed by the Faculty of Science, Nagoya University. He told me that his prerequisite for accepting the proposal depended on whether I would accept his wish and go to Nagoya with him. When I accepted his proposal, I moved to Nagoya in May 1979 and started to work with him. From previous discussions, we decided to try to establish systems in which genes can be introduced into plant protoplasts by any means. Soon we succeeded in

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establishing a reproducible system to introduce nucleic acids into protoplasts, in which tobacco mosaic virus (TMV) RNA that was encapsulated in liposomes was efficiently introduced into protoplasts with the aid of polyethylene glycol or polyvinylalcohol (Fukunaga et al. 1981). In these trials the protoplasts were prepared from *Catharanthus roseus* cell cultures. Shortly after the acceptance of this paper by the journal, I was asked to demonstrate experimental details by Professor Yoshimi Okada at a kind of technical workshop at the Department of Biochemistry and Biophysics at the University of Tokyo. When I arrived at the laboratory, I was shown a certain tobacco cell line. Professor Okada asked me to conduct the same experiments on the liposome-mediated delivery of viral RNA into protoplasts prepared from this cell line. Although the demonstration to introduce viral RNA into protoplasts prepared from *C. roseus* was satisfactory, only some limited success was obtained with the tobacco cell line. After we finished the technical workshop in Tokyo, I came back to Nagoya the following Monday. At that time, as my family was living in Tokyo, I came back to Nagoya with this cell line after spending the weekend with my family. Then I started to culture the cells by myself and immediately noted that this cell was quite different from what I knew of plant cell cultures. Before this I had had experience with *C. roseus* cells and the tobacco XD6 line. Both were said to have rather higher growth rates than others. Furthermore, due to some careless mistakes in preparing the stock solution of the culture media, the growth rate of the tobacco cell line became faster. On inspection, the mistake that was made by one of our graduate students was in the phosphate concentration. Immediately, I learned that the increase in the phosphate concentration accelerated the growth rate of this cell line. Soon we succeeded in getting an efficient delivery of TMV RNA encapsulated in liposomes to protoplasts from the tobacco cells. The results were much better than that shown using *C. roseus* cells. Somewhat later, the Annual Meeting of the Japanese Plant Physiologist Meeting was held in Sapporo. After I came back from the meeting, I was told by a graduate student that he could not reproduce the successful delivery of TMV (virus particles) into tobacco protoplasts with the aid of a polycationic, polyethyleneimine (Kikkawa et al. 1982). Initially, I did not understand what had happened. After I inspected his experiment, it turned out that the enzyme (cellulase) that had been used for his successful experiments was delivered as a sample by a company (Yakult Biochemicals which was located at Nishinomiya-shi, Hyogo Prefecture at that time and then moved to Tokyo) and it had been prepared according to a slightly different procedure from the previous one of Cellulase Onozuka R10 which has been available since 1968. Then I found that successful results were only obtained with the new type of cellulase. Therefore, we immediately ordered a new cellulase which was later named Cellulase Onozuka RS, and turned out to retain stronger cell wall degrading activity. With this small, but useful finding, we were confident with this system and our experimental results. Then we prepared a manuscript (Nagata et al. 1981). Before submission of this paper, however, we had to solve one more problem. After our success, I asked Dr.

Takeshi Ohno, who later became a professor of Plant Molecular Biology at Hokkaido University and passed away in 1992, what was the source of this tobacco cell line. Although he told me that it had been brought from the Japan Tobacco and Salt Public Corporation and had the name of BY-2 cell line, people at the Corporation told me that this cell line should not have been brought to the laboratory of Professor Okada when I called an employee of the Corporation. Apparently, it had been brought to the laboratory without permission. At that moment, I learned that this cell line was called BY-2 cell line and was derived from a seedling of tobacco cultivar Bright Yellow-2 (Kato et al. 1972). Thus, this was my first encounter with this cell line. After negotiation, they finally allowed me to use this cell line, but did not allow me further distribution of the cells to a third party. On this occasion, Professor Nobutaka Takahashi of the University of Tokyo helped me very much. This was the initial stage. After the publication of papers in journals, however, other people made requests to us for the cell line. Then, after several negotiations with the company, they finally allowed other people to use the cell line, if they underwrote the requested formula. At that time, the Japan Tobacco and Salt Public Corporation became a company of the private sector, called Japan Tobacco Company. In May 1999, the Japan Tobacco Company finally decided to allow anybody to use the cell line without restrictions and I am responsible for distributing the cell line.

3 Synchronization

Subsequently, it would also be worthwhile to relate how I attained higher synchrony using this cell line with aphidicolin, a specific inhibitor for DNA polymerase α , as the BY-2 cell line is indispensable in the cell biology of higher plants because of its high synchrony using this drug. Since then, there has been no alternative cell line that is comparable to the BY-2 cell line. As mentioned above, I noted an exceptionally higher growth rate of BY-2 cells and the idea to synchronize the cell line fell upon me. It was in early spring of 1981 when I first read an interesting paper in which aphidicolin was implemented for cell synchrony in animal cell culture (Pedrali-Noy et al. 1980). I thought immediately that aphidicolin was one possible candidate for this purpose. Then I wrote to Dr. A. Todd of the Imperial Chemical Industries, Cheshire, UK, in order to purchase the drug and he was kind enough to send me around 20 mg of aphidicolin. In total, he gave me ca. 200 mg of aphidicolin free of charge, as he sent me the drug (ca. 50 mg) every time I sent the papers published on this issue until the drug became commercially available. On this occasion, I wish to thank him and the company for their extraordinary courtesy. It really took a few months to start to examine the effect of aphidicolin upon the cell cycle synchrony of tobacco BY-2 cells. Then towards the end of 1981, I remembered I had the drug in my drawer and I started to

examine its effect upon the induction of cell cycle synchronization. Already in the first experiment, I obtained a very high level of cell synchrony, reaching the mitotic index of 70%. It was also highly reproducible in our hands. Until that time, regarding cell synchrony in plant cells, if one could get mitotic indices of ca. 10%, they stated it was synchronized. Such papers were innumerable. Then I examined the preparation of protoplasts from four cell cycle stages and examined whether there is cell cycle dependency upon the acceptance of liposome-encapsulated viral RNA. We obtained a positive result and compiled an article (Nagata et al. 1982). Just at that moment, I received an invitation to give a talk at the 14th Mile International Symposium on Cell Fusion, which was held from 7–9 June 1982 at the Johns Hopkins University in Baltimore, Maryland, USA. Hence, my first public talk on the success of cell cycle synchrony of tobacco BY-2 cells was not given at a domestic meeting, but at an international meeting, although this outcome was put in the last part of my talk (Nagata 1984). Nonetheless, it seemed that the significance of this success was not immediately appreciated. Using the highly synchronized cells, we published a series of papers (Okada et al. 1986; Nagata et al. 1987; Nagata 1989).

4 Distribution

It took some time for this system to spread to other laboratories, as from the beginning, I was told several times that our protocol was not easy to reproduce. In this context, it would be worthwhile to mention how the synchronization protocol was brought to Professor Hiroh Shibaoka's laboratory at Osaka University. At that time, he was trying to work phragmoplasts at biochemical levels and for this aim he needed significant amounts of phragmoplasts to work with. Until that time, this organelle had been only observed under a microscope. He asked me to teach them how to handle the cells and how to synchronize the cells. Then Dr. Tatsuo Kakimoto came to our laboratory at Okazaki as I was working at the National Institute for Basic Biology at that time. He came to me toward the end of 1986 and I showed him how to handle the whole procedure and he brought the cells back to Osaka. After a few weeks, however, I was told that the synchrony was no longer reproducible in their hands at Osaka and I was asked to take the cell line which functioned again only for a while. During these experiences, I learned that when the cells were brought to other laboratories, the cells were not necessarily treated in the proper way and, as a consequence, cells tended to become sick. It took almost half a year before the people of Shibaoka's laboratory at Osaka learned how to handle the cell line. Later, Professor Shibaoka confessed to me that he would not give up learning these procedures, as he trusted me when we first knew each other at the Botanical Gardens of the University of Tokyo in 1968. In this context, I add that they did make significant contributions to the cell synchrony

as well. Although there are some other contributions to this issue, I will mention only two in this place. One is the introduction of a microtubule-destroying drug, propyzamide, with which in combination with aphidicolin one could obtain very high synchrony starting from M phase (Kakimoto et al. 1988; Nagata et al. 1992). The other is that they finally succeeded in isolating significant amounts of phragmoplasts, with which they demonstrated the dynamic nature of phragmoplasts (Asada et al. 1991). From the latter publication, people worldwide recognized that the high synchrony using BY-2 cells is really true. A similar situation happened when the cell line was brought to Strasbourg. Anne-Marie Lambert and Claude Gigot were enthusiastic about this. Therefore, I tried to show them how to handle the cell line and we sent fresh cell cultures of BY-2 cells several times. Finally, they could manage to do this. Then the cell line gradually started to be distributed to other laboratories, such as Ghent, Cambridge, and Gif-sur-Yvette in Europe.

5 Concluding Remarks

As subsequent stories and technical know-how can be referred to in my previous papers (Nagata et al. 1992; Nagata and Kumagai 1999), I will not repeat them here. Instead, I would like to add a few sentences. I should probably consider myself very fortunate, as over time I interacted with many people who helped me in various ways to accomplish high synchrony using tobacco BY-2 cells. In particular, special thanks should be given to those who tried to establish rapidly growing plant cells (Kato et al. 1972). If there is anything for me to be proud of, it is that I was the first person who noted this cell line to be so useful for basic studies on plant cell and molecular biology and tried to disclose the outcome of my experience using tobacco BY-2 cells, when I noted that the tobacco BY-2 cells have unique characteristics among cultured plant cells. In fact, some of the results from these studies are included in this volume of BAF.

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