

Nagata T & Takebe I. Cell wall regeneration and cell division in isolated tobacco mesophyll protoplasts. *Planta* 92:301-8, 1970.
[Institute for Plant Virus Research, Chiba, Japan]

This study showed that protoplasts isolated enzymatically from tobacco leaves regenerated cell walls and divided to form cell clusters under suitable conditions. Consequently, the use of protoplasts as a means of somatic cell genetics and genetic engineering became realistic. [The *SCI*® indicates that this paper has been cited in over 280 publications.]

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Initially, I wanted to use protoplasts to examine the effect of plant growth regulators on single cells, since cultured cells in suspension are composed mostly of several cell clusters, making them unfit for this purpose. However, suitable culture conditions for protoplasts had not been established, although mass isolation of protoplasts from tobacco mesophyll had been reported by I. Takebe and coauthors.¹ Thus, I devoted considerable effort to finding a suitable condition for the culture of protoplasts, which was soon found to be extremely difficult. When I cultured protoplasts in media conjectured from previous publications in combination with several environmental conditions, I observed only brown-colored dead protoplasts the next day, and I saw only sporadic nuclear division of protoplasts, which did not continue at all.

I could not find a solution to this problem for more than six months. Then one day we decided to try another cultivar, although we felt there would not be many major differences among cultivars of the same species. Contrary to our expectations, however, on the fifth of November 1969, I observed very active cell division of protoplasts derived from the tobacco cultivar Xanthi nc.

We later learned that physiological conditions of the initial cultivar Bright Yellow, which is a common tobacco cultivar in Japan, showed seasonal fluctuation, and I had conducted experiments during rather bad seasons. Still, this paper became the first reproducible report on the high-frequency cell division of plant protoplasts. We soon found more suitable conditions for protoplast culture and regenerated whole plants from the mesophyll protoplasts of tobacco that we prepared.²

In 1982, when protoplasts became realistic weapons for somatic hybridization and genetic engineering of plants, I had an unusual experience. One day a journalist called me and explained that he had found an interesting article in a newly arrived issue of *Current Contents*®. The paper dealt with the informational analysis of the field of plant protoplasts and mentioned our article as one of the core papers in this field.³ I realized then that the field of plant protoplasts had become more popular and was affecting society, even though it had started as a modest topic in plant science.

This paper also contributed significantly to the study of protoplasts by introducing a fluorescent brightener, Calcofluor White ST, as a means for the removal of cell walls from cell surfaces and for the detection of regenerating cell walls on the protoplast surface. Although electron microscopy can be used for the same purpose, it is time-consuming and laborious, a sharp contrast to the procedure that uses this fluorescent dye. Now this procedure is a standard method for the detection, removal, and regeneration of cell walls on the protoplast surface, and it is used rather frequently without citation to the original paper.

I am now working in the field of cell biology, using plant protoplasts in a continuation of this work. One recent subject of study was the production of the four cell-cycle phases in protoplasts from highly synchronized tobacco cells in liquid culture. The delivery of plasmid DNA into respective phases of the cell cycle by an electric impulse showed that M phase protoplasts accepted more DNA than the other phases.⁴ This system can be used for the search of a cell-cycle dependent plant promoter.⁵

1. Takebe I, Otsuki Y & Aoki S. Isolation of tobacco mesophyll cells in intact and active state. *Plant Cell Physiol.* 9:115-24, 1968. (Cited 230 times.)
2. Nagata T & Takebe I. Plating of isolated tobacco mesophyll protoplasts on agar medium. *Planta* 9:12-20, 1971. (Cited 300 times.)
3. Garfield E. Computer-aided historiography—how ISI uses cluster tracking to monitor the "vital signs" of science. *Essays of an information scientist.* Philadelphia: ISI Press, 1983. Vol. 5, p. 473-83. (Reprinted from: *Current Contents* (14):5-15, 5 April 1982.)
4. Okada K, Takebe I & Nagata T. Expression and integration of genes introduced into highly synchronized plant protoplasts. *Mol. Gen. Genet.* 205:398-403, 1986.
5. Nagata T, Okada K, Kawazu T & Takebe I. Cauliflower mosaic virus 35 S promoter directs S phase specific expression in plant cells. *Mol. Gen. Genet.* 207:242-4, 1987.