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Takebe I, Otsuki Y & Aoki S. Isolation of tobacco mesophyll cells in intact and active state. *Plant Cell Physiol.* 9:115-24, 1968.
[Institute for Plant Virus Research, Aobacho, Chiba, Japan]

A method was developed using pectinase to isolate large numbers of metabolically active mesophyll cells from tobacco leaves Subsequent treatment with cellulase converted the isolated cells into protoplasts [Over 210 citations in the *SCI*® makes this one of the most-cited papers published in this journal]

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Plant virologists have long suffered from the lack of an experimental system that uses single cells. Plant cell walls present another problem since plant viruses cannot penetrate cell walls or inject their nucleic acids through them. When I was appointed laboratory head of the Institute for Plant Virus Research in 1965, I thought of using protoplasts, wall-less single cells of plants, as a host for virus infection. I therefore needed a method to prepare protoplasts in sufficient amounts for experimentation.

I chose tobacco leaves as the starting material because tobacco is a good host for many viruses and its leaves contain the largest number of cells. My plan was first to dissociate leaf tissues into single cells using pectinase and then to digest their walls with cellulase. E.C. Cocking in England had reported small-scale isolation of plant protoplasts using cellulase he had prepared from fungal culture filtrate. But I decided to use commercially manufactured enzymes because they were readily available in my country.

The major obstacle in our project was the damage to leaf cells incurred during tissue dissociation. This was partly overcome by screening a number of pectinase preparations for high activity and low toxicity. In addition, we found that using a hypertonic medium with the addition of dextran sulfate greatly reduced the trauma of tissue dissociation. Dextran sulfate was intended as an inhibitor of RNase, which may be present in the crude cell preparation, since pancreatic RNase was reported to lyse plant protoplasts. However, it was later shown that the effect of RNase is due to its highly positive charge and not to its enzymatic action. The reason that dextran sulfate protects cells is still not known.

Once leaf cells were isolated in intact and active states, they could be readily converted into protoplasts by using a selected preparation of cellulase. The two-step procedure yielded up to 10^8 protoplasts within a couple of hours. The latest version of this procedure is described in a recent article.[1]

The isolated tobacco leaf protoplasts could be efficiently infected *in vitro* with tobacco mosaic virus.[2] The system of synchronous infection thus established was extended to many other plants and viruses and is now widely used for studying plant virus replication at cellular and molecular levels.[3] In 1975, I received the Jakob Eriksson Prize from the Swedish Royal Academy for the development of the protoplast system for plant virus research.

The method of preparing leaf protoplasts also opened up immense possibilities for other facets of plant science, as is indicated by the number of citations to the paper. Removal of walls endowed plant cells with an ability to undergo fusion or to take up macromolecules. Together with their totipotency,[4] these cell properties form the experimental basis for somatic hybridization[5] and transformation[6] of higher plants.

Our proverb says, "The blind are not scared by a snake." I had been trained as a microbiologist. Perhaps, I would have not dared to undertake the project if I had been a botanist.

1 **Takebe I & Nagata T.** Isolation and culture of protoplasts tobacco (Vasil I K, ed) *Cell culture and somatic cell genetics of plants* Orlando, FL Academic Press, 1984 Vol I p 328-39

2 **Takebe I & Otsuki Y.** Infection of tobacco mesophyll protoplasts by tobacco mosaic virus *Proc Nat Acad Sci US* 64 843-8, 1969 (Cited 145 times)

3 **Takebe I.** Protoplasts in plant virus research. *Int Rev Cytol* (Suppl 16) 89-111, 1983

4 **Takebe I, Labib G & Melchers G.** Regeneration of whole plants from isolated mesophyll protoplasts of tobacco *Naturwissenschaften* 58 318-20, 1971 (Cited 160 times)

5 **Harms C T.** Somatic hybridization by plant protoplast fusion (Potrykus I, Harms C T, Hinnen A, Hütter R, King P J & Shillito R D, eds) *Protoplasts 1983* Basel· Birkhäuser Verlag, 1983 p 69-84

6 **Schilperoort R A & Wullems G J.** Protoplast transformation by Ti plasmid—whole plants and progeny *Int Rev Cytol* (Suppl. 16) 169-89, 1983.