

**Nagata T & Takebe I.** Plating of isolated tobacco mesophyll protoplasts on agar medium. *Planta* 99:12-20, 1971.

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A method was developed to obtain cell clones from tobacco mesophyll protoplasts on agar medium at high frequency. From these clones, regeneration of whole plants was shown. This study made possible the idea of regenerating whole plants from genetically manipulated protoplasts. [The SCI® indicates that this paper has been cited in over 260 publications since 1971.]

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After more than six months of considerable but vain effort to culture tobacco mesophyll protoplasts, I first observed cell division in these cells in November 1969. Our progress began soon after we learned that the most important point was careful preparation of the starting plant materials, which is still true today. The preliminary results were published in 1970,<sup>1</sup> but the culture conditions had not been optimized at that time. Thus, in this paper, we extended our work to find a suitable medium and to improve the culture methods.

By the late summer of 1970, we had found that a modified version of the Murashige and Skoog medium was suitable. As for culture methods, it would be ideal if one could plate protoplasts directly on agar medium to form colonies, because protoplasts are single cells in the strict sense. The original method of Bergmann,<sup>2</sup> which was suggested

by G. Melchers, Tübingen, Federal Republic of Germany, was not successful. But once modified, it produced up to 80 percent colony formation under optimal conditions. From these results arose a more realistic idea: the regeneration of whole plants from genetically manipulated protoplasts. These plants could then be utilized as starting materials for plant breeding. In this context, this paper may have played a role in the application of protoplasts to the genetic manipulation of plants. I think this is why the paper has been cited so frequently.

However, in this paper we confined ourselves to describing the methodological part of the study. We avoided a discussion that, we thought later, should definitely have been included. It was well known at that time that whole plants could be regenerated from carrot root cells in culture. The totipotency of somatic cells of plants has been an unquestioned doctrine in plant science since the study by Steward *et al.*<sup>3</sup> But if one carefully reads their original paper,<sup>4</sup> the mitotic percentage of the initial tissue was only 5-8 percent. What was the fate of the remaining 92-95 percent of the cells? According to our results, more than 80 percent of mesophyll cells formed colonies that could be regenerated to whole plants. Thus, in our paper it was shown unambiguously that almost all mesophyll cells are totipotent. We deleted this discussion to facilitate earlier publication. Therefore, I am lucky to have an opportunity to mention this point 14 years after publication!

The main part of this work was done at the Institute for Plant Virus Research, which was located temporarily in Chiba. Later it moved to Tsukuba, but it does not exist anymore because of a reorganization of the research institutions of the Ministry of Agriculture, Forestry, and Fisheries. Takebe is now professor of biology at Nagoya University. I am now associate professor of cell biology at the newly established interuniversity facility of the National Institute for Basic Biology. For a recent review, see *Protoplasts* 1983.<sup>5</sup>

1. Nagata T & Takebe I. Cell wall regeneration and cell division in isolated tobacco mesophyll protoplasts. *Planta* 92:301-8, 1970. (Cited 235 times.)
2. Bergmann L. Growth and division of single cells of higher plants *in vitro*. *J. Gen. Physiol.* 43:841-51, 1960. (Cited 130 times.)
3. Steward F C, Blakely L M, Kent A E & Mapes M O. Growth and organization in free cell cultures. *Brookhaven Symp. Biol.* 16:73-88, 1963. (Cited 30 times.)
4. Israel H W & Steward F C. The fine structure of quiescent and growing carrot cells: its relation to growth induction. *Ann. Bot.* 30:63-79, 1966. (Cited 40 times.)
5. Potrykus I, ed. *Protoplasts 1983*. Basel: Birkhauser Verlag, 1983. 366 p.