CYTODIFFERENTIATION IN CULTURED CELLS AND TISSUES

John G. Torrey
Cabot Foundation, Harvard University, Petersham, MA 01366

Plant tissue culture as a useful technique became available about 1930 with the successful research efforts of Gautheret in France and White in the U.S. For the past 45 years a considerable amount of experimental and empirical information has accumulated on plant tissue and cell culture. Only in the past 5 to 10 years has the potential of this technique been widely recognized and applied to the variety of problems for which it is suited. Plant tissue culture has become recognized as a tremendously potent research tool, having also a wide-ranging importance for practical application as well.

I want to concentrate my attention on the major remaining barriers to the general usefulness of plant tissue culture procedures in studying the problems of plant biochemistry, plant physiology, genetics, and horticulture as well as the practical applications such as plant propagation. To a remarkable extent these problems center around our lack of understanding of control mechanisms of genetic expression by cells and tissues.

The first, obvious barrier which, however, appears to have become less and less important, is the failure to establish proliferating callus or cell suspension populations from a given species of plant or from a specific tissue. The list of successfully cultured tissues is impressive indeed, ranging across the spectrum of the land plants, including bryophytes, ferns, gymnosperms, monocots and dicots (cf. 9, 16). Tissues from almost every morphological structure in the higher plants have been used as sources for establishing callus tissues in sterile culture, including explants from shoot tips, root cortical tissue, hypocotyl, cotyledon, leaf, axillary bud, floral parts including anthers and ovules, and fruit parts, including ovary wall, nucellus and embryo. Potentially any plant tissue comprised of living cells can be stimulated to proliferate as an unorganized callus tissue and be maintained indefinitely on a sterile nutrient culture medium of the appropriate constitution. The basis for such success is understood, involving as it does the provision to the explant of essential inorganic ions, energy sources usually in the form of sugar or other metabolizable carbohydrate, growth factors which the tissue is unable to synthesize itself such as vitamins, organic nitrogen compounds and sometimes purines or pyrimidines and finally the appropriate balanced mixture of plant hormones which perpetuate a meristematic state in the cultured tissues. The proper conditions must be worked out empirically although published media formulations such as that by Murashige and Skoog (10) are a good place to start. Lack of success in culturing a tissue is usually attributable to lack of trying.

Establishment of stable plant tissue cultures

A more subtle and more important barrier to effective use of plant tissue cultures is the failure to achieve a *stable* proliferating cell population. By stable I refer to a number of important parameters: genetic stability, stability in chromosome number, and stability in physiological characteristics including rate of growth, dependence on exogenous hormones, and biosynthetic activities. Each of these presents a problem and deserves discussion. The problem of genetic stability was discussed recently by D'Amato (2). Ideally, one would like a proliferating cell population which does not change with time so that one always has available a stable cell line ready to use for study, experimentation, propagation and the like.

Any rapidly reproducing cell population is subject to mutation and some inevitable low rate of change by mutation must be accepted. Some aspects of the tissue culture procedure increase this rate. Thus, for example, synthetic hormones such as the auxin 2,4-D, selected because it is not readily destroyed by most plant tissues, may not be as favorable as the natural auxin, indoleacetic acid. Choice of the appropriate hormones and hormone concentrations for stimulating cell division may help to reduce chromosomal changes. The same may be true of other components of the medium or even of the means used to prepare the medium [refer to the debate started by Holsten et al., (5)]. The longer the tissue is maintained in continuous subculture, the greater the load of chromosomal abnormalities and anomalous nuclear types, and thus the number of genetic abnormalities. Tissue culture media, especially if complex, containing undefined supplements such as coconut milk, yeast extract or other rich mixtures of nutrients, may sustain many kinds of defective cells in culture, even in a proliferating state and thus exaggerate the accumulated genetic variability [see work of Mitra et al., (8) on carrot tissues grown in complex media]. The accumulation of genetic mutations in tissue cultures and their expression in regenerated plants have been dramatically shown by studies of Melchers and his associates (14). To minimize these effects of the tissue culture procedure, it would seem that one should define as precisely as possible a minimal synthetic medium devised so as to stimulate most closely the natural nutritional conditions of the apical meristem and retain a continuous meristematic state.

Stability with respect to DNA content per cell is a special case. The development of polyploid cell populations from initially diploid tissue explants is a common phenomenon in plant tissue culture (17). Libbenga and Torrey (7) showed that DNA doubling by endoreduplication was a function of the hormonal supply to tissues in pea root cortical explants with auxin and cytokinin interacting to stimulate initially diploid cells into tetraploid, octoploid or higher DNA levels. Torrey (20) reported obtaining tetraploid root tips from such pea root callus tissue and Nitsch (11) suggested this technique as a means of producing and propagating polyploid plants from initially diploid populations. It seems likely that careful control of balanced hormone levels in synthetic media may offer the means of controlling and stabilizing the ploidy level of tissues in culture.

Related to these changes induced by manipulation of the external medium and culture conditions are what appear to be spontaneous internal changes in physiological and/or biochemical states within cultured tissues and cells. Gautheret (4) early reported a progressive change in the auxin-requirement of plant tissues cultured for prolonged periods, a change leading to auxin-independence or autotrophy which has been termed "habituation." More recently similar changes with respect to the cytokinin requirement of cultured tissues have been reported (3). In these cases, the habituated tissues have been shown to have increased their capacity to synthesize the hormone and thus become independent of an external supply. These changes in physiological state have been attributed to exposure to high auxin concentrations (1, 19), or to temperature shock (18) or to influences from other aspects of the culture environment. Understanding of these changes will help us to design better cultural methods to minimize or prevent them. These changes in physiological state are interesting to study in their own right and bear on a number of related problems in plant morphogenesis – root nodule formation in legumes and nonlegumes, for example, and on the problem of tumor formation in plants caused by bacterial pathogens or other pathological states in which excessive or abnormal plant cell proliferation occurs.

Loss of developmental capacities in cultured tissues

One of the great concerns in the application of plant tissue culture to a variety of practical purposes is centered in the fact that, upon explanting, tissues change their characteristics and properties. Thus, for example, tissues from a drug-producing plant will not synthesize the drug in culture at significant rates. Or, another example, a cultured tissue which initially regenerates buds or roots or even embryos progressively loses this capacity. These changes are losses in expression of the genetic potential and such losses seriously interfere with the usefulness of the technique. The evidence is good that these changes do not represent losses in genetic information. Rather it appears the changes are losses in genetic expression. In order to fully utilize the potentialities of plant tissue culture we must better understand the biochemical control of gene expression. When does a cell synthesize a special product? What controls when a cell can undergo a special course in cytodifferentiation? What mechanisms relate intercellular controls so that organized structures such as roots, buds or embryos emerge from an undifferentiated tissue mass? These are problems of great theoretical interest which directly affect the practical uses of the technique.

There has been a continuing interest in the possibility that plant tissue cultures of certain species could be used for the commercial production of drugs, alkaloids, and other secondary plant products of importance to man. Some successes in these attempts have been report-

ed [c.f. recent review by Yeoman and Aitchison (23)]. For the most part, empirical manipulations of culture medium and environmental conditions have been used in attempts to turn genes on.

Essentially the same problem exists in the study of cytodifferentiation in cultured cells. Cultured explants can be induced to undergo dedifferentiation under the stimulus of hormones and nutrients and to produce a proliferating cell population. In many cases, cell division precedes the process of differentiation and seems to be an obligatory event. On the other hand, single isolated parenchyma cells in culture can be observed to undergo differentiation directly as single cells (6, 21). There are examples of cultured tissues which appear to have lost their capacity for specific cytodifferentiation e.g., cell suspensions of *Acer* (22). Cell cultures offer excellent material for the analysis of this problem. Tracheary element formation in tissue culture has been studied most extensively (13) but we still do not understand the mechanism whereby a cell becomes committed to a new course of cytodifferentiation. Research on the hormonal regulation of cell differentiation may offer insights useful in understanding gene expression in other contexts.

Finally, organogenesis remains a puzzle. Since 1957, with the work of Skoog and Miller (15), we have known that an unorganized callus tissue can be induced to form either more callus or organized roots or organized buds simply by effecting minute changes in the auxin-cytokinin concentrations to which the tissue is exposed in the culture medium. In the past two decades, this type of manipulation has been applied to a wide variety of cultured tissues with varying degrees of success. It is the basis for propagating a wide variety of plants from cultured cells, explanted from apical meristems, mature parenchyma tissue, embryonic tissues or other tissue sources. We do not understand at the molecular level the mechanism of action of these hormonal stimuli. Their action concerns the development of specialized biochemical activities in restricted cell populations which become organized through the formation of ordered cell lineages into apical meristems characterized as root or shoot. We are getting some insight into the cellular events that precede the establishment of such organization and much research focusses on the internal biochemistry of the cells which regulates their behavior. But clearly much more knowledge must be gained at the level of such biochemical regulation if we are to achieve the full use of the potential available from the technique of plant tissue and cell culture. The block to progress in practical application is our lack of understanding of basic cellular plant biology. Our backlog of information is running out and, regretably, financial support for this needed basic research is alarmingly inadequate. Only recognizing where the barriers lie will allow us to face realistically the logistics of knocking them down.

Literature Cited

1. Bennici, A., M. Buiatti, F. Tognoni, D. Rosellini and L. Giorgi. 1972. Habituation in *Nicotiana bigelovii* tissue cultures: different behaviour of two varienties. *Plant & Cell Physiol.* 13:1-6.
2. D'Amato, F. 1975. The problem of genetic stability in plant tissue and cell cultures. p. 333-348 *In* Crop genetic resources for today and tomorrow. Cambridge Univ. Press, Cambridge, England.
3. Fox, J. E. 1963. Growth factor requirements and chromosome number in tobacco tissue cultures. *Physiol. Plant.* 16:793-803.
4. Gautheret, R. 1946. Comparison entre l'actions de l'acide indoleacetique et celle du *Phytomonas tumefasciens* sur la croissance des tissus vegetaux. *C. R. Soc. Biol.* 140:169-171.
5. Holsten, R. D., M. Sugii and F. C. Steward. 1965. Direct and indirect effects of radiation on plant cells: their relation to growth induction. *Nature* 208:850-856.
6. Kohlenbach, H. W. and B. Schmidt. 1975. Cytodifferenzierung in Form einer direkten Umwandlung isolierter Mesophyllzellen zu Tracheiden. *Z. Pflanzenphysiol.* 75-364-374.
7. Libbenga, K. R. and J. G. Torrey. 1973. Hormone-induced endoreduplication prior to mitosis in cultured pea root cortex cells. *Amer. J. Bot.* 60: 293-299.
8. Mitra, J., M. O. Mapes and F. C. Steward. 1960. Growth and organized development of cultured cells. IV. The behaviour of the nucleus. *Amer. J. Bot.* 47:357-368.
9. Murashige, T. 1974. Plant propagation through tissue cultures. *Ann. Rev. Plant Physiol.* 25:135-166.
10. ———and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15:473-497.
11. Nitsch, J. P. 1971. La production *in vitro* d'embryons haploides: resultats et perspectives. *Coll. Intern. C.N.R.S.* 193:281-294.
12. Phillips, R. and J. G. Torrey. 1973. DNA synthesis, cell division and specific cytodifferentiation in cultured pea root cortical explants. *Develop. Biol.* 31:336-347.
13. Roberts, L. W. 1976. Cytodifferentiation in plants. Cambridge Univ. Press, Cambridge, England.
14. Sacristan, M. D. and G. Melchers. 1970. Agrobacterium-induzierte Pflanzentumoren und durch sie entstehende entwicklungsphysiologie und cytogenetische Probleme. *Die Kulturpflanze* 6:111-126.
15. Skoog, F. and C. O. Miller. 1957. Chemical regulation of growth and organ formation in plant tissues cultured *in vitro*. *Symp. Soc. Expt. Biol.* 11:118-130.
16. Street, H. E. (Ed.). 1973. Plant tissue and cell culture. Blackwell Scientific Publications, Oxford, England.
17. Sunderland, N. 1973. Nuclear cytology. p. 161-190 *In* H. E. Street (ed.) Plant tissue and cell culture. Blackwell Sci. Publ., Oxford, England.
18. Syōno, K. and T. Furuya. 1971. Effects of temperature on the cytokinin requirement of tobacco calluses. *Plant & Cell Physiol.* 12:61-71.
19. ———and ———. 1974. Induction of auxin-nonrequiring tobacco calluses and its reversal by treatments with auxins. *Plant & Cell Physiol.* 15:7-17.
20. Torrey, J. G. 1967. Morphogenesis in relation to chromosomal constitution in long-term plant tissue cultures. *Physiol. Plant.* 20:265-275.
21. ———. 1975. Tracheary element formation from single isolated cells in culture. *Physiol. Plant.* 35:158-165.
22. Wright, K. and D. H. Northcote. 1972. Induced root differentiation in sycamore callus. *J. Cell Sci.* 11:319-337.
23. Yeoman, M. M. and P. A. Aitchison. 1973. Growth patterns in tissue (callus) cultures. p. 240-268 *In* H. E. Street (ed.) Plant tissue and cell culture. Blackwell Sci. Publ., Oxford, England.