INTRODUCTION TO THE CELL CULTURE SYMPOSIUM

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In many ways the first bloom is off the rosy image of the new cell culture with its promise for early development of new interspecific plants through somatic hybridization, incorporation of useful genes through protoplast fusion and gene transformation, and selection of mutant cells with desirable genetic traits for transmission to regenerated plants. If progress in cell culture has been slower than anticipated, a little reflection will help us to realize that this is not unusual in science. We might examine the satisfaction we feel in our knowledge of the field of intermediary metabolism, but only a little reminiscence leads one to the realization that this satisfaction is the product of leaps of creativity and technological advance, but also of many false starts and a great deal of painstaking effort.

Despite the pace of forward movement in the field, cell culture research is a "growth industry" today. In part, this is due to the remarkable progress being made in the more traditional aspects of regeneration of plants from organs, especially apices. Some species that had been refractory have now yielded to manipulation of the culture medium and can be regenerated reproducibly. Included among these are economic plants, and the ability to regenerate has stimulated interest in recently developed approaches to cell selection and genetic manipulation. For a limited number of species, this activity has provided us with techniques for mass isolation and fusion of protoplasts, and regeneration of the hybrids into plants, the production of haploid plants from microspores and anthers, regeneration of embryoids and plantlets of recalcitrant species from callus and cell suspension cultures, and initial successes in obtaining desirable cell mutations which are expressed in the regenerated plant.

These methodologies have laid the basis for a new "cell genetics" as well as for studies of the physiology and biochemistry of plant cell behavior. This cell culture must be viewed as a field in its infancy which in part derives conceptually from the experience and remarkable success in the area of microbial genetics. Today, workers in the plant cell culture field face many of the same barriers that confront the microbiological workers, and a great many more, because we are dealing with species higher in the evolutionary scale and, therefore, of extraordinary complexity. Moreover, we want not only to learn basic biology of somatic cells, but we expect to have useful results.

My personal view is that major new advances in the field can come with extensive basic research aimed at analyzing each of the rate limiting processes involved. Thus, cell selection for desirable agricultural characteristics is possible, but for broad scale application, penetrating study of each of the barriers in somatic cell selection work – producing haploids, tendency toward polyploidization, problems of cell selection, regeneration of selected cells into plants – is essential. The same applies to other aspects of somatic cell research and the symposium participants were invited because of their high standing in fields of significance to such developments.

It is most appropriate that a symposium on "Cell Culture" is sponsored jointly by the American Society for Horticultural Science and the Plant Growth Regulator Working Group. The subject bridges current knowledge and the possibilities of new advances. It deals with an emerging field which holds promise both of major understanding of plant regulation and, ultimately, of advances in crop improvement, subjects of major interest to both organizations.

CURRENT STATUS OF PLANT CELL AND ORGAN CULTURES

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In 1902, Haberlandt published on his unsuccessful but pioneering attempt to cultivate cells from leaves of several angiosperms (5). Today, nearly 75 years later, plant cell and organ cultures are established as potent research tools and economical agricultural practices. Cell cultures enabled the demonstration of totipotentiality of plant cells as Haberlandt first suggested (22). Furthermore, they played a critical role in the discovery of cytokinins, an important group of plant hormones (8). Cell and organ cultures provide unique systems to explore organ initiation (17), cell and tissue differentiation (21), and other morphogenetic processes. They furnish new opportunities in somatic cell genetics (2). Organ growth and development can be studied with minimum interference by correlative influences (1, 14, 18, 19, 24).

Perhaps most significant has been the increasing utility of cell and organ cultures in economic applications. They are new sources of pharmaceuticals and other plant constituents. They serve as tools in hybridization and new cultivar development. They are employed to establish pathogen-free stocks and are becoming a standard method of rapid clonal multiplication.

Kinds of plant cultures

The callus is perhaps the most widely cultivated plant tissue (Fig. 1). Its successful culture was first reported in 1939, separately but nearly simultaneously, by Gautheret (4), Nobecourt (15) and White (25). The callus has been used primarily as experimental material in morphogenesis, biochemistry, disease etiology, and other biological studies. After many years' exploration they are now emerging as economic sources of certain plant substances. They are sometimes used as an intermediary in clonal multiplication procedures. Callus cultures can be established quite readily for most plants; indeed, examples are known in various species, from mosses through monocotyledonous angiosperms, and of virtually any plant tissue or organ. One needs only to provide a balanced salt mixture, sugar, one or more vitamins, and the phytohormones auxin and cytokinin. The auxin alone may be sufficient as hormonal addendum, especially when 2,4-D is employed. Sometimes preparations of natural complexes, such as coconut endosperm, yeast or malt extract, fruit pulp or juice, and protein hydrolysates, are provided to obtain additional stimulation. The tissue can be maintained indefinitely through repeated subcultures, but this practice is not always advisable, inasmuch as predominance by polyploids and other genetically modified cells usually results. The callus is simply a wound tissue, and grows as a whitish or cream-colored mass on an agar-gelled medium. It can be transferred to a liquid nutrient and transformed into a suspension of free-living cells and few-celled aggregates (Fig. 2). The dissociation of cells is enhanced by first rendering the tissue friable, by pre-culturing in an agar medium containing high salts, relatively high auxin and casein hydrolysate. The liquid must be agitated vigorously and constantly to maintain the dissociated state. Even then, cultures constituted by predominantly single-cell units are rare.

If desired, callus clones of single cell origin are obtainable. One method involves agar-plating of cell suspension cultures (Fig. 3). It may be necessary to separate the single cells from aggregates by filtering the suspension through a suitable screen. The plating is accomplished by mixing aliquots of the screened suspension with sterile, fluid nutrient agar, the temperature of which should be about 40°C to prevent premature gelling, then pouring the suspension into culture dishes. Other methods of single-cell cloning include the use of microchambers (7) and nurse callus (10).

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