zide, ultraviolet light, $\gamma$-irradiation, and x-ray are very efficient at inducing changes (30). A dose of 1600 rad of x-ray increased the frequency of somatic crossing more than 280-fold in Glycine max cells (5).

Conclusion

Tissue-cultured cells spontaneously accumulate many changes in both the number and structure of chromosomes. Such changes could be due to the culture environment or due to naturally occurring events. It appears that cultured cells are more tolerant of chromosomal aberrations than are differentiated cells of the whole plant. Besides the spontaneous changes, many drugs and physical agents can be used to induce specific chromosome changes.

Literature Cited


**Summary and Future Direction: Chemical Regulation in Tissue Culture**

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This series of papers has expanded upon Skoog's milestone discovery regarding the roles of cytokinins and auxins in morphogenesis of in vitro systems. One of the effects of adenine-based cytokinins in culture systems is the formation of adventitious shoots or the expression of axillary shoots. This symposium reported that a cytokinin response also can be observed by using phenylurea-based cytokinins. Two phenyleurea compounds, thidiazuron and 4PU-CI, were illustrated as having strong cytokinin activity, equivalent to or surpassing the adenine-based compounds. One of the effects of these diphenyleureas is the formation of autonomous callus. These observations were used to speculate on the mode of action of the phenyleureas as being stimulation of endogenous cytokinin biosynthesis (or metabolism), resulting in altered cytokinin levels. Other studies indicate that the phenyleureas and adenine cytokinins may have similar action sites, hence similar effects.

In addition to documenting the effects of cytokinin in culture, Skoog showed that low auxin levels enhance root formation and high levels promote callus formation. This symposium suggested that the naturally occurring indole auxin 1H-indole–3-acetic acid (IAA) can have several forms in culture systems. It can be left in its free state, which is thought to be its biologically active form, and initiate its hormonal response. It can be oxidized and possibly removed from any biological effects. Finally, IAA can be conjugated through ester or amide linkages, protecting it from oxidation and allowing transport through tissues. Hydrolysis of the conjugates would free IAA, causing a biological response. The conjugation–hydrolysis balance may be a means to regulate auxin levels and responses of tissues.

Information reported in the preceding papers dealt briefly with the biochemistry of the auxin–cytokinin response. However, progress has not been rapid since the early work of Skoog and associates. Our information contributes to answering the puzzle of cytokinin–
auxin roles; however, we lack knowledge of the main event(s) or site(s) of action of auxin–cytokinin, eventually concluding in gene action.

In the future, the advancement of nucleic acid chemistry or molecular biology should make it possible to determine the effects of cytokinin and auxin at the gene expression level. Whether auxin or cytokinin has direct action at the gene level or through a series of intermediates should be an area of interest for the nucleic acid chemists. The more relevant question to be addressed is that of control of organogenesis in vitro. This question presumably is linked to auxin–cytokinin roles in cell biochemistry and gene action. The difficulty in investigating this line of research is lack of a synchronous experimental system. There is some work progressing in the areas of somatic embryogenesis. However, most work deals with effects after the induction stage, and usually with developmentally associated events rather than events leading to competent tissue. Only recently have workers begun to look at events associated with induction of competent cells.

As basic work on mechanisms of action of growth regulators continue, there should still be a balance of efforts with a more applied nature. Empirical investigations on growth regulator effects on in vitro systems have resulted in regeneration in formerly recalcitrant species such as corn, soybean, and woody crops. It has been suggested that workers consider the use of compounds other than the traditional auxin and cytokinin. The use of diphenylurea derivatives, a more stable compound than the naturally occurring cytokinins, may be useful in culture systems requiring long-term cytokinin treatments. In parallel, the conjugated indole auxins, which are more stable than free indole, may be useful in long-term auxin requiring systems.

In addition to the influence of growth regulators on morphogenesis in tissue culture, there are confounding problems associated with the culture procedure itself. The morphogenetic capacities of cell cultures reduce over time and have been associated with changes in ploidy level. Media components, especially 2,4-D, have been associated with an increase in culture ploidy level and somatic crossing over and may have some effect on the reduced morphogenesis. Obviously, when formulating a project that uses a cell culture procedure, the genetic stability of the end product should be considered and the correct procedure adapted for the project.

The complexity of differentiation, growth, and developmental biology is reflected by the slow progress made in understanding these causal events. However, with development of nucleic acid chemistry and experimental systems, progress at the gene expression level may proceed at a faster pace.