Meetings

Hormonal Regulation of Plant Growth and Morphogenesis

In their endeavors to understand plant development, botanists have available to them knowledge of the chemical identity of an impressive array of naturally occurring plant hormones which are widespread among plants. Although the molecular mechanisms of action of these hormones are not yet known, sufficient evidence has accumulated to indicate their involvement in such basic processes as cell division, enlargement, and differentiation. Plant physiologists interested in understanding plant development continue to focus their attention on the role of these hormones and to exchange ideas on their regulation of growth and morphogenesis.

In Kyoto, Japan, on 21 to 25 August 1972, a U.S.–Japan Seminar on plant growth regulation was held. Thirty-six scientific papers were presented, 28 of them by Japanese scientists and 8 by visiting scientists from the United States. This was the third seminar of a series under the auspices of the U.S.–Japan Cooperative Science Program sponsored jointly by the National Science Foundation and the Japan Society for the Promotion of Science.

During the course of the meetings, all known major plant hormones—auxins, gibberellins, cytokinins, abscisic acid, and ethylene—were discussed, and proposals for hormone-like activities in plants were made for cyclic adenosine monophosphate (AMP), for dormancy-inducing inhibitors called batatasins, and for several new substances in yeast cells regulating cell enlargement and the mating reaction.

The theme of the conference was reflected in its title "Hormonal regulation of growth and morphogenesis." In planning the program, the organizers from Japan and United States placed emphasis on the varied physiological roles played by plant hormones during development. Groups of papers were concerned with the processes of seed germination, dormancy in both seeds and bulbs, the regulation of cell division, cell enlargement and cell-wall extension, the reproductive physiology of yeasts and of flowering plants, and recent work with isolated plant protoplasts.

During the meetings considerable interest was aroused by reports of new plant hormones or new chemical structures for known classes of hormones. For the past number of years, Japanese workers have studied hormones in the yeast *Saccharomyces cerevisiae*. N. Yanagishima (Osaka City University) described a hormone system, including at least four substances, that controls the mating reaction of yeast; these substances are active in affecting agglutinability, cell expansion, and conjugation. S. Tamura (University of Tokyo) reported studies on the chemical nature of the yeast hormones. One of the cell enlargement stimulators is an auxin-like compound related to indoleacetic acid; one of the agglutinating substances has characteristics of a peptide. C. Shimoda (University of Tokyo) described macromolecular metabolism of yeast cells during mating. Hormones from one mating type elicited inhibition of DNA synthesis in the opposite mating type as a consequence of mixing of the two strains with no parallel inhibition of RNA or protein synthesis.

In a completely different category of not-yet-characterized plant hormones are substances active in controlling dormancy in the bulbils of the yam *Dioscorea batatas*. T. Hashimoto (Institute of Physical and Chemical Research, Wako-shi) isolated three inhibitors, which he called batatasins I, II, and III, from mature dormant yam bulbils. The compounds are believed to be aromatic phenols but distinct from any reported growth inhibitors. These compounds effectively suppress sprouting in stratified bulbils and they show augmented action in the presence of GA_3 (gibberellic acid A_3). Changes in endogenous levels of batatasins were consistent with a natural role in controlling dormancy in yams.

Japanese workers continue their interest in the structure and function of the gibberellins. N. Takahashi (University of Tokyo) reported the isolation and chemical characterization from mature seeds of *Phaseolus* of two new gibberellins, GA_{37} and GA_{38}. These compounds occurred as the glucosyl esters and represent a neutral fraction extractable from seeds. The GA_1 and GA_4 glucosyl esters were also isolated from the mature seed, this being the first reported case of such gibberellin esters.

Two papers were presented on the possible role of cyclic AMP in plant growth responses. Y. Oota (Nagoya University) discussed the possibility that cyclic AMP functioned as a second messenger to florigen in flower formation. He found that $10^{-5}M$ cyclic AMP counteracted the inhibition of flowering in *Lemna gibba* produced by sucrose or by NH_4NO_3. He reported that labeled adenine was incorporated into cyclic AMP at an enhanced rate in photoperiodically induced fronds. Critical discussion of this paper centered around the problem of specificity in floral induction in *Lemna*. This point was emphasized by the report of A. Takimoto (Kyoto University) on flowering in *Lemna perpusilla*. A broad spectrum of agents or conditions (or both) including sulfhydryl inhibitors, electron acceptors, EDTA, NH_4^+, sugar, *p*H, and light stimulated flowering to some degree under long-day conditions. S. Kamisaka (Osaka City University) studied the stimulatory effect of cyclic AMP on hypocotyl cell elongation in lettuce seedlings. In different responses (dark-germination of lettuce seeds and cell expansion in artichoke tissue disks) cyclic AMP was found to be inactive alone, but was reported to increase the response elicited by GA_3 alone or combined with 2,4-D. Considerable debate ensued concerning the role of cyclic AMP as a plant hormone. A controlling role for gibberellins in the elongation of floral parts was described in *Pharbitis* by Y. Murakami (National Institute of Agricultural Science, Tokyo). He found that GA_1 and GA_3 in petals and stamens increased rapidly during floral development and excised segments responded to exogenous gibberellin.

The pursuit of answers to the question of the mechanism of action of plant hormones still centers on relatively simple systems. The action of auxin on the plant cell wall during cell enlargement continues to occupy the attention of many hormone physiologists. R. Yamamoto and Y. Masuda (Osaka City University), M. Igari (Nagoya

City University), S. Wada (Tohoku University), and K. Kasamo (University of Tokyo all reported on physical or metabolic changes in elongating tissues in response to auxin treatment.

T. Nakamura (Japan Women's University) described the effects of GA treatment on nucleic acid metabolism in elongating pea seedling epicotyls, and concluded that GA may bind in some way to DNA, increasing its capacity for further DNA or RNA synthesis. H. Shibaoka (University of Tokyo) exhibited electron microscopic evidence purporting to show that gibberellins stimulate elongation of Azuki bean epicotyls by changing microtubule orientation. Colchicine, which depolymerizes microtubules, appears to inhibit specifically the GA_3-induced longitudinal expansion of these cells while not influencing auxin-induced elongation.

Other reports on the nature of cell elongation involved analyses of external environmental influences. Red-light inhibition of *Avena* mesocotyl elongation was studied by T. Yamaki (University of Tokyo). He presented a theory of light inhibition which related concentrations of tissue nicotinamide adenine dinucleotide phosphate (NADP) to reduced auxin transport into the mesocotyl as well as a more direct inhibitory effect of red light on cell-wall extensibility. Such light effects are complex, compounded of a series of multiple interacting systems. M. Furuya (University of Tokyo) examined the NADP content of bean hypocotyl tissue after red and far-red illumination. He found that a reversible phytochrome-dependent reduction of NADP occurred in the particulate fraction of these cells. This light-mediated reduction of NADP was coupled specifically to glucose-6-phosphate dehydrogenase.

Another cell process that received attention was the control of mitosis and cell division. Y. Yamada (Kyoto University) reported both qualitative and quantitative changes in proteins specifically associated with tissue proliferation, which resulted in bud formation in cultured callus tissues of *Nicotiana*. Kinetin treatment of unorganized callus elicted the formation of new proteins and accompanying shoot initiation. K. Syōno (Kitasato University) also working with tobacco callus tissue, developed different strains of tissue with respect to exogenous hormone requirements. A tissue requiring both exogenous auxin and cytokinin was established from stem pith tissue. Later a tissue strain developed which required only auxin. When this strain was cultured in the presence of high concentrations of cytokinin (1 part per million of kinetin) it grew without added auxin. Analysis showed that this tissue was able to form its own auxin which was shown to be indoleacetic acid and two other auxins not characterized.

A most elegant system for the study of cell division in plant cells was described by M. Wada (University of Tokyo) in the gametophytes of the fern *Adianum*. Protonemata cultured under continuous red light when transferred to dark divide transversely within 36 hours. Far-red light before dark delayed the division several hours, and the effect was reversed by red light. Brief exposure to blue light before the dark treatment induced cell division earlier. Transfer to continuous white light after continuous red light caused two transverse divisions followed by a longitudinal division. The plane of the longitudinal division was parallel to the direction of the incident light. Thus, using appropriate irradiation treatments, it was possible to control precisely the orientation and timing of cell division in the apical cells of the fern protonemata.

One of the highlights of the meeting was a comprehensive review by I. Takebe and T. Nagata (Institute for Plant Virus Research, Chiba) of the recent developments in the techniques of isolation and culture of protoplasts from tobacco leaves. Both the biochemical and developmental capabilities of the chloroplast-containing protoplasts derived by enzymatic treatment from leaf mesophyll tissue were studied. The RNA precursors were readily incorporated, first into the nucleus; protein synthesis proceeds in both the cytoplasm and in the chloroplasts. Virus production by isolated, infected protoplasts was analyzed by bioassay, immunological methods, and electron microscopy. Cell-wall synthesis was followed by means of a cellulose-specific fluorescent wall stain and light and electron microscopy. Early wall formation was observed by 3 days, mitoses on day 4, with daughter cell formation. Continued proliferation of cell colonies from protoplasts depended on appropriate hormonal conditions in the medium. Wall formation and cell division were dependent on the kind and concentration of auxin; naphthaleneacetic acid served better than 2,4-D and indoleacetic acid was ineffective. The auxin antagonist, 2,4,6-trichlorophenoxyacetic acid antagonized the wall-promoting effect of 2,4-D. Under appropriate hormonal conditions cell colonies gave rise to callus tissues in which shoots and roots were found which could be transplanted and brought to flowering and seed formation, showing normal genetic constitution. High plating efficiencies (up to 67 percent) for cell colony formation make these protoplasts a useful tool for genetic work.*

American participants included R. Cleland (University of Washington, Seattle), M. Crispeels (University of California at La Jolla), L. Dure (University of Georgia, Athens), R. Jones (University of California at Berkeley), J. Shen-Miller (Argonne National Laboratory), J. Torrey (Harvard University), and J. Zeevaart (Michigan State University, East Lansing).

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* A more detailed summary of the meeting is available upon request.

Forthcoming Events

October

14–17. American **Chemical** Soc., 5th Northeast regional, Rochester, N.Y. (P. Tingue, Bldg. 81, Room 254, Research Labs., Eastman Kodak Co., Rochester 14650)

14–17. Association of **Life Insurance Medical Directors**, New York, N.Y. (A. E. Brown, ALIMD, 501 Boylston St., Boston, Mass. 02117)

14–18. American Inst. of **Ultrasound in Medicine**, Ann Arbor, Mich. (M. A. Wainstock, Dept. of Ophthalmology, Univ. of Michigan Medical Center, Ann Arbor 48105)

14–19. Society of **Motion Picture and Television Engineers**, New York, N.Y. (D. A. Courtney, SMPTE, 9 E. 41 St., New York 10017)

14–20. **Allergology**, 8th intern. congr., Tokyo, Japan. (Japanese Soc. of Allergology, c/o Dept. of Microbiology and Immunology, Nippon Medical School, 1-1 Sendagi, Bunkyo-ku, Tokyo)

14–20. World **Medical** Assoc., Munich, Germany. (A. Z. Romualdez, WMA, 10 Columbus Circle, New York 10019)

14–21. International Congr. on **Tropical Medicine and Malaria**, 9th, Hellenic Ministry of Social Services and Hellenic Ministry of Culture and Sciences, Athens, Greece. (J. Papavassilious, ICTMM, P.O. Box 1373, Athens)

15–16. **Environmental Geologic Mapping Colloquium**, Austin, Tex. (E. G. Wermund, Bureau of Economic Geology, Univ. of Texas, Box X, University Station, Austin 78712)

15–17. National **Electronics** Conf., Inst. of Electrical and Electronics Engineers,