

Plant biology – Retrospect and prospect

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Over the last century, plant biology has made remarkable progress, reflected in its greatly improved journals and textbooks. This article summarizes some historically important developments in the study of plant photoreceptors, especially the red-absorbing phytochromes and blue-absorbing flavoproteins, in phytohormones, in cell and tissue culture and in circadian rhythms. Attention is drawn to the neglect of polyamines, especially in relation to stress. Some effects of altered patterns of research support are noted, and a few future research trends are suggested as probable; these include increased use of molecular genetic techniques and increased attention to agricultural, ecological and ethical problems.

TAKING the pulse of experimental plant biology at the turn of the millennium and noting some of the changes over recent time is a daunting challenge, undertaken here with trepidation. Since one obviously cannot cover all subjects, what follows must represent a subjective and highly personal selection. It is limited to the 20th century, into which I was born almost eighty years ago, at about the time of the formation of the American Society of Plant Physiologists. My birth also virtually coincided with Garner and Allard's landmark article on photoperiodism¹, and occurred just a few years before Frits Went's discovery of auxin² ushered in the era of phytohormones. Since my own research involved these two areas, I will dwell on some historical developments in both.

How things were back then

In the year 1936, I entered Cornell University as a freshman and began my first studies in Botany. The Cornell Botany Department was at that time one of the best in the world, especially strong in maize genetics and cytology, thanks to the presence of such luminaries as R. A. Emerson and the future Nobelists G. W. Beadle and Barbara McClintock. The Department also boasted great strength in anatomy and comparative morphology (A. J. Eames and L. H. MacDaniels), and in physiology (L. Knudson, O. F. Curtis and D. G. Clark). Yet as I look back on my student notes of those days, I am astounded at how old-fashioned many of those thoughts

and procedures seem today, and as a consequence, what an inadequate preparation such training would be for the biology of today. The training of modern plant biologists is both broader in scope and more rigorous than I ever experienced, auguring well for an enhanced quality of work and a higher status for our field in the future. One possible modern problem is that the intensive training required for proficiency in the molecular areas tends to produce many narrow specialists lacking the historical background that yields a broad perspective of our science.

In the mid-1930s, we botanists paid scant attention to metabolism, or in fact to any aspect of plant biochemistry, and the biochemistry course I took in another department scarcely mentioned plants. The study of plant hormones, which had arisen out of the study of tropisms in grass coleoptiles, was in its infancy, with only impure preparations of chemically uncharacterized 'auxin' available for experimentation and speculation. During my four undergraduate years at Cornell, I took all the plant physiology courses available, including elementary and advanced plant physiology and laboratory techniques; yet during all this time, I never experienced a single lecture or a laboratory exercise dealing with plant hormones. The most frequent lecture subjects revolved around the uptake, transport and transpiration of water, xylem vs phloem transport and mineral absorption and nutrition. Mirroring and perhaps directing this selection of topics was the textbook in general use at that time, *Plant Physiology* by Miller³, a ponderous and rather formidable compendium of a large volume of observations, data and theories. That situation contrasts sharply with a similarly entitled modern text by Taiz and Zeiger⁴, in which selected and restricted data are introduced only if relevant to clearly delineated theories or phenomena. Similarly, considered against the standards of our modern research journals, the plant biological journals at the start of the century seem primitive and unpolished.

It was not until I began graduate study at the University of Illinois in 1940 that I received my first lectures on such subjects as plant hormones, photoperiodism and vernalization from H. J. Fuller, and some laboratory training in biochemistry under H. E. Carter of the eminent University of Illinois Chemistry Department. As I took a joint major in botany and biochemistry, my dissertation committee consisted of representatives of both

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groups. As a result, my defence of thesis on the physiology of flowering was almost ridiculously easy, since the botanists were convinced that my biochemistry was quite sophisticated and the biochemists, essentially innocent of training in botany, thought the same about my botanical expertise. One prominent biochemist on the committee, William C. Rose, confessed that he had not known whether the word 'photoperiodism' referred to effects of light periodicity or to light effects on iodine metabolism (photo-per-iodism)!

Since biochemical training was rarely offered to the plant physiologists of that day, there existed considerable tension between plant physiologists and plant biochemists, tension of the sort that originally separated classical botany from plant physiology and that now partially separates plant molecular biology from the rest of the field. In each instance, I am heartened to note, the original mutual suspicion between specialties led later

to a strengthening of the entire field by incorporation of the newer, more radical discipline into the mainstream of the subject. Perhaps it is not too much to hope that the gap that presently separates plant molecular biology from some of the rest of the field will be similarly closed with time.

Although not noted as an experimentalist, Harry Fuller was an eloquent and inspirational teacher, who opened my eyes to the fields of flowering physiology as related to hormones, circadian rhythms and in general, the influence of light on plant behaviour. Although frequently absent from the campus during the hectic years of World War II, when he was exploring South America for stands of *Hevea brasiliensis* that might ameliorate the serious shortage of rubber resulting from the Japanese conquest of the Malaysian peninsula, he conscientiously and devotedly directed my thesis on the physiology of flowering in soybeans. (Incidentally in

Arthur W. Galston

Arthur W. Galston is among the most distinguished and respected plant biologists of our times, who has become a legend in his own life time. He has been an outstanding teacher who held generations of students spell-bound through his most wonderful, lively and thought-provoking lectures. His landmark books have changed the course of many careers. Written in simple and engaging style, these books have been translated in many languages and students from all over the world, and over the years have thronged to his laboratory to carve out a career in plant biology. However, books comprise a small part of his total contribution – his research programmes have had a profound influence on the growth of plant biology.

Galston had humble beginnings. The lives of his parents were catapulted as they had to escape the anti-semitic excesses of Czarist Russia and flee to USA at the turn of the last century. He was born in 1920 in Brooklyn, New York where he had his schooling. In his own words: 'I aspired to study medicine but that entailed expenses that were far beyond the capacity of my family. So I chose veterinary medicine instead, since there was a tuition-free college for New York State residents on the campus of Cornell University. However, this career was de-railed by a charismatic professor, Loren C. Petry, who taught me elementary botany.' Galston majored in plant physiology, receiving his BS in 1940. Readers would be interested to know that utilizing his musical talents the young Galston earned his way through college, playing the saxophone! He was awarded the Ph D degree in 1943 for his seminal work on the physiology of flowering. Again, in his own words: 'Expecting to enter military service immediately after completion of my thesis, I was surprised to receive an offer of a scientific job, and thus yielding a deferment of service. I went to work for James Bonner at CalTech on the Emergency Rubber Project, designed to produce rubber from the Mexican shrub, guayule (*Parthenium argentatum* Gray). This respite lasted not quite one year, after which I entered the navy as an enlisted man; later on I became a commissioned officer and saw my major duty on a Military Government team on Okinawa. I was released from service after two years, and returned to New York City.' But only briefly. After one year's instructorship at Yale, Galston again went back to CalTech and this time for nine years. These according to him were the best years of his life as he had an opportunity to interact with the intellectual giants of our time, Max Delbruck, Richard Feynman, Linus Pauling and also outstanding biologists – George Beadle, James Bonner, Frits Went. The next 45 years of his very productive life were spent at Yale. His latest interest is bioethics, the beneficiaries of his interest are Yale undergraduates. Because of the great relevance of bioethics today, it is hoped that Galston's wisdom would spread far and wide.

In the accompanying article Galston traces the growth of plant biology and this he does in the first person.

(SCM and SKS)



1940, when I began my studies, this crop, now our major agricultural export, was planted only experimentally, and on only a few Illinois farms!). Fuller had recently returned from a sabbatical spent at CalTech with Frits Went (my future colleague), and introduced me to the intricacies of the *Avena* bioassay, then the standard method for estimating auxin quantitatively. I performed hundreds of *Avena* test bioassays in the course of my thesis research. The interest in such subjects generated by this early work has remained with me all of my life, manifesting itself in much of my research on photoreactions in plants and on hormone physiology.

Support of scientific research: A changing scene

When I began my graduate work at the University of Illinois in September 1940, the country was still trying to pull itself out of a prolonged economic depression which had started a little over a decade earlier. Unemployment was still a problem, and graduate assistantships and fellowships were greatly prized and highly competitive. Science was usually done on a 'shoestring' budget, with extremely simple equipment. World War II changed all that in the United States; suddenly there was no significant unemployment, support of young scientists became more common and organized governmental support of research science at universities became regularized, at first through military agencies like the Office of Naval Research, later through the National Science Foundation, the National Institutes of Health, the Department of Energy, the Department of Agriculture, the National Aeronautics and Space Agency, and numerous smaller sources of funds. At the same time, private foundations like Rockefeller and Ford added their tremendous assets to the support of scientific research.

From about 1950 to 1990, financial support was available to almost all able investigators in the United States, leading to a veritable flood of scientific findings. Soon, agencies in Europe, Japan and elsewhere enlarged their activities, providing a similar stimulus in their own countries: the medical and agricultural research groups (MRC and ARC) in the UK, the Deutsche Forschungsgemeinschaft and the Max Planck Institute in Germany, the CNRS in France, RIKEN in Japan and a host of similar organizations in other countries arose to support scientific research, which was perceived as an investment that 'paid off' in the future. Private industry, also sensing the advantages of sponsored research, added to its own in-house efforts, and also subsidized relevant activities in academe. For the academic researcher, this new money was a boon, permitting the planning and execution of many scientific projects which would otherwise have remained in limbo.

Like almost all desirable innovations, this change in the support of science has also had some unfortunate consequences. One serious side-effect has been that many able senior investigators have been effectively removed from direct participation in active research, spending their time mainly in the preparation of successful grant applications, the hiring of effective post-doctoral fellows, the recruiting of able graduate students and the writing of papers worthy of publication in respected journals. After a decade or so of such isolation from the laboratory, some investigators may well have run out of useful ideas or lost the desire or ability to do research.

Important research trends

In my view, there has been no more important or unique series of investigations in 20th century plant physiology than those leading to the discovery of phytochrome and subsequent exposition of its physiological significance. I shall thus start with this subject, then move on to the related subjects of phototropism and blue light effects, then to tissue culture, plant hormones and circadian rhythms.

Photoreceptor pigments

Although the receptor pigments for photosynthesis have long been known as chlorophylls, carotenoids and other pigments capable of transferring their excitation energy to the photochemical centres of the chloroplast, knowledge of the photoreceptors for processes directly linked to growth and development have remained mysterious until comparatively recently. However, spectacular successes have now been achieved in identifying the pigments responsible for photon absorption for mainly red-light-sensitive (photoperiodism, deetiolation, etc.) and blue-light-sensitive (phototropism, photoreactivation) reactions in plants. In fact, the discoveries of phytochrome and of photoreactive flavoproteins have radically transformed our understanding of many different processes studied in modern plant physiology.

Phytochrome: Phytochrome was discovered in 1959 thanks to the genius of a physical chemist, Sterling B. Hendricks, working in conjunction with several botanists, including Harry A. Borthwick, at the United States Department of Agriculture (USDA) Station in Beltsville, Maryland, near Washington DC. Planning to extend the discoveries of their USDA predecessors W. W. Garner and H. A. Allard, who had uncovered major facts about the photoperiodic control of flowering¹, and utilizing the finding of Hamner and Bonner that photoperiodically sensitive plants measure the length of the dark, rather than of the light period⁵,

Hendricks *et al.*⁶ constructed a large monochromator, using parts scavenged from other laboratories and defunct theatres. With this apparatus, they were able to expose sets of photoperiodically determinate plants to selected energies and wavelengths in the middle of their dark period, thus favouring the flowering of long-day plants and inhibiting the flowering of short-day plants.

By producing dose-response curves for each selected wavelength, they were able to determine an action spectrum for the interruption of flowering in a short-day plant (soybean) and the promotion of flowering in a long-day plant (barley). These two virtually identical action spectra⁷, showing major peaks in the red and smaller peaks in the blue, resembled the absorption spectrum of a known pigment, allo-phycoyanin, an open-chain, metal-free tetrapyrrole⁸. But this pigment, found readily in the cyanobacteria, could not be shown to exist in higher plants. It was here that serendipity intervened. A previous experiment by Flint and McAlister⁹ at the nearby Smithsonian Institution Radiation Laboratory in Washington DC. on the photoactivation of lettuce-seed germination, had produced a similar action spectrum, peaking at about 660 nm. A surprising added feature of this action spectrum, discovered because the 'controls' had been poised at about 50% germination, was a marked inhibition of germination by 'far-red' light, peaking at about 730 nm. Hendricks and colleagues then showed that far-red given after red annulled the promotive effects of red, and that red given after far-red exposures would similarly reverse the latter's inhibitory action. This mutual photoreversibility led to the prediction that there were two forms of photochromic pigment, and that the peaks corresponded to the shifting absorption peaks of the two forms of the pigment.

With mutual photoreversibility available as an assay criterion, and with the aid of an instrument produced by Karl Norris for the detection of small absorbancy changes in optically dense material such as apple fruits¹⁰, it was possible to demonstrate the physical existence of the pigment *in vivo* and *in vitro*, and ultimately to isolate a highly purified blue-green protein, appropriately named phytochrome¹¹. We now know that there is a family of phytochromes consisting of at least five members differing in cellular location, absorption properties and physiological action. Collectively, they control an impressive array of developmental processes, including seed germination, seedling deetiolation, formation of chlorophylls, anthocyanins and other pigments, circadian rhythms and flowering.

A detailed account of subsequent progress in elucidating the purification and isolation of phytochrome, the nature and structure of its protein and chromophore moieties, its cellular locations, its possible modes of action and physiological significance is described in a remarkable book by a non-scientist, Linda Sage, entitled

*Pigment of the Imagination*¹². Sage, a writer and resident of St. Louis, was asked by Joseph Varner, then a professor at Washington University in St. Louis, to undertake the writing of the history of phytochrome. With support from the USDA, she travelled around Europe, Japan and the United States for over two years, interviewing and interpreting the contributions of most of the researchers in the field of phytochrome. Included in her narrative are contributions from several prominent individuals whom I had the privilege of introducing to the field of 'phytochromology' while serving as a professor in the Department of Biology at Yale University. These included my former graduate student Masaki Furuya, my former post-doctoral associates Harry Smith, William S. Hillman, Ruth L. Satter and Bruce A. Bonner, and my DuPont (where I was a consultant) colleagues Franklin E. Mumford and Edward L. Jenner. Recent work and interpretations on the mechanism of action of phytochromes are reviewed in a subsequent article of this special section by Sharma.

Flavoprotein photoreceptors: The blue-light receptors, by contrast, have been isolated only during the last two or three years, partly because some early conclusions about their nature were faulty. In the 1930s, it was widely believed that carotenoids were the receptors for phototropism, on the basis of a similarity between their absorption spectra and the action spectrum for phototropism¹³. Because carotenoids function in animal vision, and because of a general belief in the relatedness of functions of similar biochemical compounds, this conclusion was firmly established¹⁴. For example, Erwin Bünning, who had become justly famous for his discoveries in the field of circadian rhythmicity, published a series of papers entitled 'Phototropismus und Carotinoide' over a 20-year period starting from 1937 (refs 15–18). He concluded, on the basis of considerable circumstantial evidence, including action spectra, that the receptor pigments for phototropism and associated blue-light-mediated plant photo-reactions were carotenoids.

In 1949, just fifty years ago, I challenged Bünning's conclusions when I discovered that photo-activated riboflavin could oxidize the phytohormone IAA *in vitro*¹⁹, and proposed that this reaction might explain the phototropic curvature in grass coleoptiles known to be associated with auxin asymmetry. I showed further that action spectra in the blue (made with the Beltsville monochromator under the supervision of Sterling Hendricks) could fit flavoproteins as well as the carotenoids, and reasoned that it would be difficult, on the basis of action spectra in the visible alone, to discriminate between the two putative photoreceptors²⁰. The subsequent discovery of an action peak near 370 nm favoured the flavin type of photoreceptor. When later experiments by Briggs *et al.*²¹ showed that flavin-mediated photo-destruction of IAA could not explain

phototropism, since the amount of auxin in responding organs remained constant, many people rejected the flavin photoreceptor theory entirely. But I had also pointed out that certain amino acids, like tryptophan and histidine, as well as polypeptides and enzymes containing them, as well as bacteriophages, were also substrates for photo-activated flavins²⁰. Thus, flavins might function as photoreceptors even if auxin destruction was not involved in phototropic curvature.

The idea of physiologically significant photoreception by flavins remained unproved until very recently, when the lengthy and elegant experiments of Briggs and co-workers with a nonphototropic mutant of *Arabidopsis* led to isolation of 'phototropin', a flavoprotein which is now accepted as the photoreceptor for this process²². In the same way, a flavoprotein photoreceptor has recently been identified by Cashmore *et al.*²³ as responsible for the blue-light-mediated inhibition of stem growth and for DNA lyase activity in *Arabidopsis*. Both workers referred to my early work suggesting flavin photoreception. Pterins have also been invoked as photoreceptors²⁴ in other blue-light-sensitive systems; since riboflavin may be considered as a benzpteridine, this idea is consonant with the flavin hypothesis. The subject of blue-light-mediated reactions is reviewed in an essay by Khurana in this special section.

I have often been asked why I virtually abandoned research on flavins as photoreceptors almost 50 years ago, when the idea had generated such interest and seemed so attractive. I can only reply that at the time of this discovery, I was a 29-year old untenured Research Fellow, and that those expressing strong opposition to my theory included George Wald (later a Nobelist for work on carotenoid photochemistry during vision), Kenneth Thimann (the eminent auxin researcher), Frits Went (the discoverer of auxin), Folke Skoog (later the discoverer of kinetin) and Erwin Bünning (the distinguished exponent of circadian rhythms). When, after submitting a grant renewal application to the National Science Foundation, I was warned that in view of the strong criticism my work was receiving from respected authorities in the field, future work on this topic would probably have difficulty obtaining funding, I found it expedient to switch to a different line of research. As it turned out, this was a serious error. Young researchers of today should probably take note of this object lesson.

Plant hormones

The cultivation of single cells and of most plant tissues requires greater knowledge of plant growth factors than was generally available before the early 1950s. Went's auxin² had been characterized as 3-indoleacetic acid (IAA) by Kögl *et al.*²⁵, and shown to be required for the growth and division in culture of a wide variety of plant cells not derived from an organized meristem. (In such

structures, apical regions synthesize their own auxin, making exogenous additions superfluous.) Groups under both F. C. Steward at Cornell and F. K. Skoog at Wisconsin sought the identity of additional growth factors from natural sources such as coconut milk, which had been found by Johannes van Overbeek *et al.*²⁶ to favour the growth of immature *Datura* embryos that would otherwise not grow in culture. Investigating other natural sources of growth promoters such as yeast extract and herring sperm, the Wisconsin group isolated from the latter an unnatural substance named kinetin²⁷, an artifact formed during the autoclaving of isolated DNA. In the presence of auxin, kinetin promoted the division of cells of tobacco pith. This finding led to a search for naturally-occurring analogues of kinetin, resulting ultimately in the isolation by Letham and others²⁸ of several 'cytokinins', in coconut milk and elsewhere. The molar ratios of auxin and cytokinin in tissue cultures of tobacco pith and other plant materials determines whether the products of cell division remain as undifferentiated callus or whether they differentiate into root- or bud-primordia²⁹. Cytokinins also turn out to promote protein synthesis in excised leaves, and thus to mimic the effect of roots attached to otherwise isolated leaves.

Gibberellin

Japanese investigators had long focused on a growth factor produced when the fungus *Gibberella fujikuroi* invaded rice to produce the elongated stems characteristic of the *bakana-e* disease³⁰. Eventually, gibberellic acid (GA) was isolated from higher plants as well as fungal media³¹, shown to be physiologically active, and to be one of a large number of metabolically interrelated 'gibberellins' whose biosynthetic pathway is now reasonably well understood³². The roles of gibberellins in the control of germination³³ and dwarfism³⁴ were later discovered and investigated, and their role in flowering and other physiological phenomena is now under active investigation.

Absciscic acid

Absciscic acid (ABA) was named and discovered in the context of leaf abscission by Addicott and coworkers³⁵ at Davis, California. At virtually the same moment in history, the same compound was isolated by Wareing and colleagues³⁶ at Aberystwyth, Wales, who were studying bud dormancy. In the hindsight of history, ABA turns out to be much more closely associated with seed and bud dormancy than with leaf abscission, and its suggested designation as 'dormin' or dormic acid seems more appropriate. Most recently, ABA has been shown to be involved in response to water stress, and to function in the gain and loss of solutes during changes in stomatal guard cell turgor³⁷.

Ethylene

It had long been appreciated that unsaturated hydrocarbon gases used as fuels in the heating of greenhouses could cause dramatic changes in growth and morphology, exemplified by leaf abscission and by the 'triple response' of etiolated pea seedlings, i.e. shortening and fattening of epicotyls, ageotropic behaviour and formation of the apical hook³⁸. Later, the role of ethylene in hastening the ripening processes of fruits was found to be associated with its natural occurrence in plants and its rapid rise in titer following a sudden 'climacteric' rise in respiratory release of carbon dioxide³⁹. Eventually, its formation from methionine through the intermediacy of ACC (1-aminocyclopropane 1-carboxylic acid) was described⁴⁰. The mechanism of ethylene action in a variety of physiological responses, from tropisms to leaf fall, is currently under investigation.

In the last decade, considerable attention has been paid to several other substances with presumed hormonal characteristics. Jasmonic acid, synthesized from linolenic acid in the membrane by a lipoxygenase-initiated chain of reactions, activates genes controlling the synthesis of proteinase inhibitors involved in defence reactions⁴¹. A peptide named systemin is said to play a similar role⁴², possibly triggering the biosynthesis of jasmonic acid. Salicylic acid also occurs naturally in plant tissues and plays a role in the high respiratory rate, leading to the warming of the fleshy spathe of skunk-cabbage and related plants⁴³. It may also be related to resistance to plant pathogens and to various stress reactions⁴⁴. Polyamines such as putrescine and spermidine are ubiquitous in plants, and are considered by some to control, or at least to be importantly involved in such processes as mitosis, response to stress and antagonism of ethylene-induced fruit ripening⁴⁵. Diminution of polyamine titer through inhibitor-induced blockage of polyamine biosynthesis leads to inhibition of the above processes. Polyamines are discussed further below.

One conspicuous failure in phytohormone research is our continuing inability to define the nature of the flower-inducing stimulus, named florigen, more than three decades ago⁴⁶. The physiological evidence for the existence of such a substance is overwhelming: photo-periodic induction of one region of an appropriate plant leads to flowering in the non-induced region as well; the same effect can be produced by grafting an induced region to an uninduced region. A single leaf is effective as a donor when it is photo-induced and grafted to a defoliated receptor; translocation of the stimulus is apparently through the phloem, and the rate of movement resembles that of sucrose⁴⁷. While we know of substances, including phytohormones like gibberellin and ethylene, whose application can lead to flowering in some uninduced plants, their limited action compels us

to reject them as candidates for florigen. Since both long-day and short-day plants are capable of inducing the other type to flower by grafting, we must assume that the floral stimulus is the same or at least functionally equivalent in both types. To date, no candidate compound fulfills all these requirements, although ethylene and gibberellin are effective in some plants. Thus, despite much recent molecular genetic information about the control of flowering in *Arabidopsis* and other plants⁴⁸, we remain in the dark about the nature of the transmissible floral inducer that triggers the flowering process. The presently dominant theory is that flowering is controlled not by a single substance, but by the interactions of a multiplicity of unconnected stimuli, some endogenous and some environmental. By contrast, a simple theory, based on interactions among three genes, satisfactorily explains the formation of sepals, petals, stamens and carpels⁴⁹.

A discussion of the mechanism of action of plant hormones can be found in a subsequent article in this special section by Johri and Mitra.

Plant organ tissue and cell culture

In the early 1930s, P. R. White⁵⁰ had learned how to cultivate excised root tips on media containing yeast extract; shortly thereafter, F. C. Robbins and J. Bonner separately repeated this finding for tomato and pea roots, and defined the effective components of the yeast extract as thiamin (needed by all roots), pyridoxine (needed by tomato roots) and niacin (needed by pea roots). At about the same time Gautheret and Nobécourt in France had experienced success in achieving the potentially unlimited and largely undifferentiated growth of slices of carrot root. The product here was largely undifferentiated callus containing isolated whorls of tracheids. Two decades later, F. C. Steward would demonstrate that even a single isolated cell of this material could be made to regenerate embryoids and even the entire organism, thus proving that each cell contains the entire functional genome of the plant, and is potentially totipotent. Developments in this field are well summarized and referenced in the monograph edited by Street⁵¹.

After another decade or so, in which many other investigators succeeded in regenerating entire plants from single cells (for details, see ref. 52), the ultimate step in plant regeneration was successfully made. It was shown, especially in the Solanaceae⁵³, that even isolated protoplasts, enzymatically deprived of their cellulosic walls and stabilized by osmotica, could give rise to the entire plant. Such naked protoplasts became useful in a great variety of experiments, ranging from purely physiological to developmental and genetic. It was even found possible to fuse protoplasts of different origins by chemical or electrical means to achieve 'parasexual plant hybridization'⁵⁴.

Investigations on crown gall disease led to the discovery of the crucial roles of *Agrobacterium tumefaciens* and of wounding in the tumefaction process⁵⁵. Grafting demonstrated the transmissibility of the 'tumour-inducing principle' and culture of excised secondary crown gall tumours revealed auxin autotrophy and an absence of recoverable bacteria⁵⁶. Based on these observations and comparisons between virulent and avirulent strains of the bacterium, it was not long until a plasmid was isolated that carried genes for tumour initiation as well as for synthesis of hormones and other substances⁵⁷. This was one of the first documented cases of interspecific gene transfer, and *Agrobacterium* became one of the first effective tools for producing transgenic organisms.

Circadian rhythms

Many creatures change their appearance and morphology in response to light, and we have already described the importance of light-dark cycles on floral initiation. Frequently correlated with these effects on flowering are rapid, reversible change in leaf attitude. For example, some plants have leaves that droop in darkness and are erect in the light, but if maintained in continuous darkness after exposure to a diurnal light-dark cycle, the leaves continue to rise and fall in a rhythmic fashion⁵⁸. Erwin Bünning noted that the effect of a light period on leaf movement, flowering and other phenomena depended not only on its intensity, duration and wavelength, but also on the time of day or night the light is administered. When an artificially lengthened dark period was probed by a light flash given at various times, the response curve described a sine wave, with alternating periods of promotion and inhibition, the peaks occurring approximately 24 h apart⁵⁹. Such phenomena, involving endogenous, self-sustained oscillations were called circadian rhythms; and such rhythms have since been described for animals^{60,61}, plants⁶² and micro-organisms⁶³. The period of such rhythms is independent of temperature (implying the operation of a compensatory mechanism), but is markedly affected by light absorbed by either a phytochrome or a flavoprotein receptor. It is now believed that photoperiod works by 'resetting the clock' that drives the rhythmic behaviour, thus keeping the organism in synchrony with the external environment. Several genes showing rhythmic behaviour in transcription have now been isolated⁶⁴.

In support of an apparently lost cause – polyamines

Over two decades ago, Ravindar Kaur-Sawhney and I were investigating the culture of cereal protoplasts, when we made an observation that led us and others to a

long series of investigations on polyamines (summarized in ref. 65). In order to obtain protoplasts from young oat leaves, we had to peel off the epidermis before exposing the leaf to cellulase, which liberated the mesophyll cells as protoplasts into the surrounding medium containing hypertonic mannitol. We noted that leaves pre-exposed to arginine, or to any of the polyamines derived from arginine, greatly stabilized the integrity and green colour of the peeled leaves and the protoplasts derived from them⁶⁶. We thus proposed that polyamines might be involved in the control of leaf senescence. Later investigations by one of my graduate students, Hector Flores, showed that protoplasts contained much higher titers of putrescine than the leaf cells from which they were derived⁶⁷. This was found due to *de novo* synthesis of arginine decarboxylase (ADC), one of the two enzyme systems involved in the formation of putrescine from arginine, the other being ornithine decarboxylase, ODC. We later found that numerous other stresses, such as low pH⁶⁸, potassium limitation⁶⁹ and heavy metals⁷⁰ produced the same effect through the same mechanism, and investigations by others added to the list of stresses that activated putrescine accumulation in stressed cells. By the mid 1980s, high putrescine titer had come to be recognized as a criterion of stress in cereals and some other plants as well. Yet, to our surprise and chagrin, most subsequent conferences and reviews on the physiology of stress in plants have not mentioned polyamines at all! The cause of this omission remains a mystery to me, a mystery which I leave to future workers to resolve.

Polyamines seem also to be involved in the control of cell division and morphogenesis, being required for embryoid formation from cultured carrot cells⁷¹, for flower production in 'thin-layer'-derived cultures of tobacco callus⁷², for the growth⁷³ and prevention of senescence and ethylene production of tomato fruits⁷⁴, for export of the florigenic stimulus from induced leaves of the long-day plant *Sinapis alba*⁷⁵ and for bolting and flowering in *Arabidopsis thaliana*⁷⁶. A summary of the literature on polyamines as related to reproductive activities and stress in plants has recently appeared⁷⁷, and a masterful overall summary of polyamine biochemistry and physiology in all kinds of creatures has been written by Seymour Cohen⁷⁸, a pioneer in this field.

Since polyamines and enzymes regulating their biosynthesis and metabolism are present in all cells, some basic function of these compounds is implied. Their titer in cells is usually in the fractional millimolar range, and they must be applied as multiple millimolar solutions to elicit physiological reactions. This is, of course, at least a thousand-fold higher concentration than the effective range for traditional plant hormones, and may be one reason why the possible physiological regulatory role of these compounds is so seldom considered. At physiological pH, they are strongly cationic,

and can couple with many cellular polyanions such as DNA and RNA, as well as certain proteins and surface components of membranes. In doing so, they change the configuration of the anionic molecule with which they couple, often with important consequences. This includes activation of kinases and phosphatases⁷⁹ and the conversion of active B-configuration DNA chains to the inactive Z-form⁸⁰. Polyamines may also be covalently linked to proteins through the action of transglutaminase⁸¹, again with possible consequences for functional alteration. When presented to plant cells, polyamines tend to accumulate in vacuoles, inhibiting the uptake of K⁺ (ref. 82). *In vitro*, they are powerful inhibitors of proteases⁸³. Because of these properties and demonstrated physiological actions, it is likely that polyamines will turn out to be important in some physiological regulations. If this prediction is correct, many textbooks and reviews will have to be altered.

A look into the future

Whither plant physiology in the next millennium, especially in areas dealing with growth and development? Realizing that prediction is an inexact art, I will be conservative here, while expecting progress in three directions: one toward the *molecular and cellular* analysis of growth and development, a second toward *agricultural and ecological* considerations, and a third toward *ethical concerns*. The molecular-cellular movement will bring us closer to explanations of basic problems in plant physiology, while the second and third will lead us to confront urgent societal problems for which we have relevant information and expertise, and for which equitable and ethical solutions are required.

One obvious trend is *the increasing use of molecular genetic ideas and techniques* to obtain information on mechanisms controlling the attainment of morphogenetic patterns. It has already been brilliantly successful in arriving at a rational and provable series of rules for development of each of the four whorls of flower parts⁸⁴. The imminent completion of the *Arabidopsis* genome project will make possible the identification, cloning and transgenic use of new regulatory genes. Comparative genomics will also make it possible to specify the function of some genes and their protein products. The use of individual cells and protoplasts in transgenic experiments in which regeneration of organs or entire plants must be attained will also obviously increase, providing a parallel boost in research on cell and tissue culture.

While *the search for new regulatory factors in plants* has probably almost run its course, recent discoveries about jasmonic acid and salicylic acid strongly indicate that some new ones still remain to be discovered. One class with probable significance will be the peptide

hormones, like systemin⁴². In general, investigations into the reasons for specific obligate relations between parasites or saprophytes and their host plants should give new leads.

More attention needs to be paid to the physiology of *roots and the rhizosphere*. For example, while the importance of mycorrhizae is partially understood, their possibilities for further improving plant growth have not been well explored.

As the world's human population grows beyond 6 billion toward still uncharted heights, *agricultural productivity* must obviously also increase if these extra people are to be fed, since there is virtually no new land to bring into production. In the past 50 years, waves of improvement in productivity have come from conventional genetics, fertilizers, irrigation, plant protection and the recent 'green revolution'. Yet now, we see the necessity for a new wave, since we are facing a new 'ceiling' probably imposed by existing genomes. Will the new increase come from transgenic plants, from improved agronomic technology, from improved understanding of plant-plant interaction, or some other direction?

Along with improved productivity, we must strive for better *nutritional quality*, an endeavour in which gene transfer techniques will almost certainly prove important. With the introduction of transgenes into agricultural crops, we must concern ourselves with problems voiced, especially in Europe, about their possible harmful ecological side-effects. Will such genes escape to the wild, creating 'superweeds'? Will introduced genes be allergenic or toxic to human recipients or to the ecosystem? Should transgenically modified crops be so labelled for the consumers who are concerned? These problems, relating to the health of individuals and entire ecosystems, must be attacked and solved if genetically-modified foods are to become important. Other environmental problems, like those resulting from the massive use of nitrogenous fertilizers and the increasing salinization of irrigated soils, must also be addressed.

One ethically distressing result of plant physiological research in the last century was the massive use of *defoliants as weapons of war* in south-east Asia from about 1967 to 1971 (ref. 85). About 45 million litres of a formulated mixture containing herbicidal levels of 2,4-D and 2,4,5-T, designated in military parlance as Agent Orange, were sprayed by United States airplanes onto about 1.7 million hectares of mainly upland forests and mangroves and some crop lands in Vietnam. The object was to reveal enemy combatants hidden by the otherwise dense vegetation, but among the unwanted side-effects were a series of ecologically damaging events, including a loss of minerals from the ecosystem by increased nutrient dumping, soil erosion and gulleying, replacement of valuable species like teak by useless and noxious weeds like scrub bamboo, and the destruction

of some food crops by deliberate or accidental drifting of the aerial spray. In addition, it was later discovered that Agent Orange contained potentially toxic levels of dioxins, amongst the most noxious materials ever synthesized⁸⁶. Vietnam veterans, whose service led to their being sprayed, received many millions of dollars in compensation from the manufacturers of Agent Orange after a lengthy court battle⁸⁷, but no recompense has yet been offered to Vietnamese mothers who may have produced teratogenic babies or suffered from other toxic effects.

Since the use of these chemical weapons, the largest such use in military history, may have violated important international treaties⁸⁸, and in any event led to numerous public protests at home and abroad, their use in future warfare appears unlikely. Their use has led to a proposal that *ecocide* be designated as a war crime to parallel the crime of genocide, justly condemned at the Nuremberg trials following the Nazi era. It would seem appropriate for plant physiological associations to register their opposition to any such antisocial use of materials originally designed to enhance agricultural productivity and practice.

The growing importance of *industrial research laboratories* has brought new dimensions into plant physiology, including new jobs, new money for academic research, and new practices, such as the use of patents and the concomitant restriction in the flow of information. Decisions about what research to pursue and how much to publish are now frequently made not by scientists, but by corporate officials relatively untrained in science and perhaps more interested in increased profitability than in increased knowledge. Our scientific organizations must take note of this trend and propose methods to deal with it.

Finally, as evidence for *global climatic change* becomes more and more convincing, plant physiologists need to concern themselves with its possible agricultural consequences. Should we develop crops with higher temperature optima for growth and productivity? Or simply look for new crops to fit the new climatic situation? Should the emphasis be on C3 or C4 plants?

Clearly, plant biology faces many important problems and opportunities ahead. We need to continue to train able scientists to discharge these responsibilities in an efficient, ethical and timely manner.

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