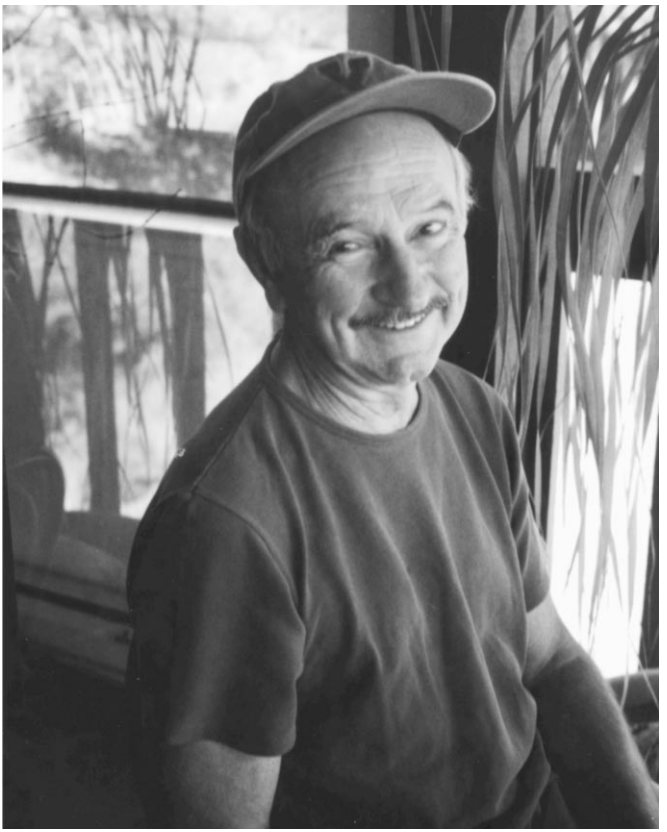

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THEMES IN PLANT DEVELOPMENT

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INTRODUCTION

Recently I was reading an alumni magazine in which the editor had asked a number of retired or soon-to-be retired professors how they got into their disciplines. I was surprised that for over half of them it had been a seemingly trivial or incidental event such as "I was planning to be a physics major, but I picked up my roommate's introductory biology text and became fascinated by the subject" that had precipitated the initial decision.

In reflecting on my own life, I am also surprised at the number of times when seemingly chance events, such as being in the right place at the right time, had major consequences for my career as a biologist. I think that the realization that there is no one true path to be followed has also influenced how science has been done in our lab by myself and by the people who came to study with me. Instead of defining a biological problem and following it to its end, with everyone contributing a part, my approach has been to find a question that is interesting to me, and to investigate it as long as it remains interesting, then to move on to a new question.

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So my research has involved organisms as diverse as fungi, algae, lycopsids, ferns, monocots, and dicots, and has examined questions as varied as gametophyte-sporophyte relations; the initiation of leaves, roots, and flowers; embryonic polarity; somatic embryogenesis; hormonal regulation of storage proteins and dormancy; auxin metabolism; and some others.

Most of the people who worked in our lab chose their own projects, and the only restriction was that it should be something on which I could give them encouragement and some advice. This was what I learned from Claude Wardlaw at Manchester during my PhD studies. Unfortunately, it seems to be a way of doing science that is dying out as the pressure of large lab groups, competitive grants, and professors who prefer to be called PIs increases.

PLACES

I was born and grew up in a semirural suburb of Auckland, New Zealand, where we had access to pastures, salt marshes, mud flats, beaches, and native bush. It was easy in this environment to develop an interest in plants.

In the 1940s, there was no career advising in New Zealand schools, and I probably would not have attended university had not a teacher suggested this to my parents. At Auckland University College, my undergraduate degree was a BSc in botany. This required all science and no humanities or social science courses but gave me an excellent education in what are now called the classical fields. While I was there, VJ Chapman came from England to be head of the department, and his enthusiasm inspired a generation of students, me included, to study marine and shoreline biology. After completing an MSc degree in 1949 on the ecology of a shoreline plant, I was fortunate enough to obtain a three-year scholarship to Manchester University in England.

The 1950s were exciting times to be in Manchester. It had a large botany department. Professor Eric Ashby was doing research on control of leaf shape and aging in *Lemna*. HE Street, with whom I worked for three months after completing my PhD, was using excised tomato root cultures to study metabolism, and Wardlaw's work on morphogenesis was attracting international attention. In addition, Alan Turing, after picking up a pine cone on a Sunday afternoon walk and being intrigued by the spirals of scales that he observed, began developing his diffusion-reaction theory of morphogenesis. During this time he consulted frequently with Wardlaw, who helped to translate his mathematical models into terms that would be understandable to biologists.

At Manchester I discovered the joys of having access to a good library, and I read a lot. I was especially influenced by Joseph Needham's *Biochemistry and Morphogenesis*, Paul Weiss's *Principals of Development* and D'Arcy

Thompson's *On Growth and Form*. Although these books were principally or entirely devoted to animal questions, they had a major influence on the way I thought about developmental questions in plants.

Taylor Steeves came to Wardlaw's lab for a year on a Sheldon Traveling Scholarship to continue his PhD studies, and he and I began a personal and scientific friendship that has lasted through two editions of *Patterns in Plant Development* and still continues today. After Taylor returned to Harvard, he convinced Ralph Wetmore, his advisor, to invite me to postdoc in his lab, which I did for eight months. Taylor and I then spent four months together working in France with Georges Morel at CNRS, which was located on the palace grounds at Versailles.

Finally, in 1954, it was time to face reality, and I got a position as junior lecturer in the botany department of Victoria University College in Wellington. Although I had intended to remain in New Zealand, I stayed only one year, because the department was unable to support the level of research activity that interested me and there were no external funding sources available. Happily, the situation is very much better in New Zealand universities now.

I applied for and got (without an interview) an assistant professorship at the University of Pittsburgh where I stayed for five years. When Yale offered Taylor Steeves and myself positions in the botany department, it seemed like an ideal opportunity to resume our research collaboration. However, Taylor had only the year previously moved to the University of Saskatchewan and felt that he could not move again. So I went to New Haven in 1960, pleased to be in a coastal environment again. Within two years of my arrival, the botany and zoology departments merged, and I had the opportunity to teach plant development in courses collaboratively with animal developmental biologists. My years at Yale were very happy and productive ones, largely because of the succession of excellent graduate students, postdocs, and sabbatical visitors who worked with me.

In 1989, another of those unanticipated events occurred when I was serving on an NSF/USDA/DOE panel to evaluate biological science centers' grant applications. Interactions with panel members led to my being invited to apply for a position at Berkeley in the newly organized plant biology department. Berkeley presented a great opportunity: a new building, a new lab, and a much larger number of plant colleagues than I had been used to. The lab quickly filled with graduate students and postdocs, and we have had an extended period where people worked together without too many comings and goings. Now that I have decided to retire at age 70, after 43 years of teaching and writing grant proposals, the lab is emptying out as people leave for jobs. I am getting back to doing research with my own hands.

RESEARCH

Above, I commented that my research has involved working on a problem until it was no longer interesting to me and then beginning something new. Often "no longer interesting" meant that there were no methods currently available to advance the problem further. However, in many cases, new approaches did become available later, and I frequently found myself returning to an old problem but using new approaches and new methods. So, although I have used many different organisms in my research, and have investigated many different questions, there are themes that run through these studies of plant development connecting work that was done many years apart. I discuss three of these where the theme is particularly strong.

Meristem Organization

Anyone who has dissected the bud of a vascular plant under a stereomicroscope must surely have been thrilled by the translucent, glistening beauty of the apical meristem and the surrounding leaf primordia. There are subtleties that are lost in the starkness of an SEM image. As well as being thrilled by the appearance of the meristem, one must surely also be awed, thinking, "How does it work and how can I find out?" My interest in meristem organization began in Claude Wardlaw's lab. He was using microsurgical procedures to investigate morphogenesis in fern apical meristems. He agreed that I should work on similar problems, but in angiosperms.

In those days in England there were no course requirements for the PhD, hence the need to read independently, and graduate students did not prepare a thesis prospectus or have a dissertation committee. Wardlaw simply said to me, "Go away and do something." For the next nine months, I wrestled with what to do and what to do it on. One day Frank Cusick, another Wardlaw graduate student, brought me some sprouted potato tubers that he had found in his vegetable bin. These turned out to be ideal experimental material. I could punch out "eyes" on plugs of tuber tissue that were easy to orient on the stage of a stereomicroscope.

The buds contained only about 10 developing scale leaves with no obscuring trichomes and could be dissected easily and quickly. New leaf primordia were initiated at about 24-hour intervals, so development was very rapid. Within a few months, I had done a series of experiments that involved surgically bisecting the shoot apical meristem, isolating the meristem or parts of it from lateral tissue by four incisions, and puncturing it in terminal and subterminal positions. These experiments pointed to the importance of the slowly dividing, distally located cells in maintaining the integrity of the meristem. However, this was about as

far as I could go with the surgical approach, and when I left the lab I moved on to other questions.

My next venture into meristem organization came in the 1970s when I became interested in how meristem fate is established. The angle meristem of *Selaginella* was ideal for study of this question. In intact plants, these meristems form rhizophores, root-like structures that initiate roots when they contact the soil surface. If the main shoot apex of the plant is removed, the angle meristem produces a leafy shoot. Thus the meristem is formed before its fate as rhizophore or shoot apex is determined. By the time we began these studies, organ sterile culture systems were quite well developed, so we excised angle meristems devoid of surrounding tissue and found that when we cultured them on a basal medium they developed as shoots, but when cultured on an IAA-containing medium they developed as rhizophores. Furthermore, by culturing excised angle meristems first on basal medium then transferring to the IAA medium, we found that meristem fate was determined several days after the meristem began to grow.

The idea of meristem fate reappeared in our research in the 1980s and 1990s when we produced fate maps of shoot apical meristems. In my reading of the animal literature I had been impressed by the fate maps that embryologists had produced, and how these had formed a foundation for experimental analysis of embryonic development. In 1978, Coe & Neuffer published a fate map of the shoot apical meristem of maize. This showed that specific parts of the meristem gave rise to specific parts of the shoot. This idea of meristem cells being partitioned out in a modular fashion was at odds with anatomical descriptions of shoot development, in which cell lineages from the apex appear to "flow" seamlessly into the developing shoot. In order to investigate whether the model was restricted to maize or was more general, we made fate maps of the shoot apical meristem of *Helianthus* and *Arabidopsis*. These are both dicots, but, like maize, flower terminally. These fate maps were strikingly similar to the Coe & Neuffer fate map of maize, suggesting that in the shoot meristem, cells are partitioned out as components of morphological units consisting of node, leaf, internode, and axillary bud. The beauty of this concept is that it tells us that the morphological units that we identify visually in a plant have a developmental reality. Now we need to find out how that reality is achieved.

One of the most fascinating questions concerning meristems is how cells in the different layers integrate their activities to maintain meristem organization, and function together in organ and tissue formation. In most dicotyledons, the shoot apical meristem consists of three cell layers (L1, L2, and L3) that generate discrete lineages in the plant. L1 forms the epidermis, L2 several underlying cell layers, and L3 the core of the plant. Since there is no exchange of cells between

these layers within the meristem, how do the cells of different layers interpret their positions and integrate their functions? Animal developmental biologists were able to analyze similar questions by generating chimeric organisms. In these, embryonic cells of two genetically identifiable organisms were disaggregated and allowed to reassociate as a single embryo in which the cellular contribution of each partner to the new organism could be assessed. Comparable experiments have not been possible in plants because disaggregation and reassociation of tissue cells has not been achieved consistently. However, chimeric plants can be produced from graft regions of two genetically different plants if one of the meristem cell layers in a regenerated apex originates from one of the graft partners and the other layers from the other. By exploiting this approach, we were able to show that many features of meristem function such as meristem size and number of floral organs per whorl are controlled by the L3 layer, the L1 and L2 cell layers behaving as though they respond to signals from the L3 layer. Now the nature of these signals and how they are transferred between layers remain to be discovered.

Most recently, we have taken a new direction in the study of plant meristems, and this is to examine the molecular and cellular events involved in the initiation of a meristem. This work was begun before the discovery of PCR, so it was necessary to use an experimental system that would provide large amounts of developmentally synchronous material for extraction of mRNA. Lateral root initiation turned out to be excellent for this work. Lateral roots can be induced synchronously along the whole length of seedling radish roots that have been exposed to IAA. From these, subtracted cDNA libraries were made that were enriched in genes expressed at specific times in meristem initiation and development, and we identified many such genes. Continuation of this work in *Arabidopsis* focused on the cellular origin of the meristem, and in vitro culture experiments revealed that there was formation of an initial primordium within which a subset of cells became organized to function as the root apical meristem.

Leaf Development

While I was carrying out my graduate studies on the apical meristem of potato, I noticed that if one of the surgical incisions was located between the shoot apical meristem and the presumptive site of the next leaf to be initiated, that leaf frequently would develop as a radially symmetrical and not a dorsiventral organ. This result was strikingly similar to results that Wardlaw had obtained on the fern apex, but in his experiments the radial organ developed as a new shoot apex. Evolutionary morphologists had earlier suggested that the leaves of vascular plants are modifications of branch systems, and these experimental results seemed to indicate that the shift from radial to dorsiventral symmetry

resulted from an influence of the shoot apical meristem on the developing lateral organ. However, at that time, molecular or genetic approaches to examine this question were not available.

My next venture into leaf development was begun in collaboration with Taylor Steeves, who had been working on leaf development in the fern *Osmunda cinnamomea*. We found that leaf primordia excised from the bud and placed on quite simple culture media would continue to develop and mature as small replicas of normal leaves even to the extent of initiating sporangia. Fern leaves were ideal for study of the effects of nutrition on leaf shape because, in contrast to dicot leaves that typically have limited or no apical growth, fern leaves develop from an apical meristem that functions for a long time, forming a succession of leaflets along the axis. Later, we showed that by modifying the sucrose level of the medium we could reproduce all the leaf forms from juvenile to adult in excised leaves, thus providing support for Goebel's idea that the simple juvenile leaf form of ferns results from carbohydrate starvation.

Above, I referred to the absence of apical growth in typical dicot leaves. This was not generally realized at the time, and the model that had been developed from anatomical studies invoked apical and subapical initial cells that generated the leaf axis, and marginal and submarginal initial cells that generated the leaf blade. Even at the time, it could have been seen that this model was incorrect because leaves of chimeric plants in which L1 is genetically green, L2 is albino, and L3 is green have white margins and green centers, indicating that all three layers of the meristem contribute to formation of the leaf. But at that time, chimeric plants were thought to be anomalies, and it was not until we carried out a clonal analysis of leaf development in tobacco that experimental proof of the absence of extensive apical and marginal activity in this leaf was provided. Clonal analysis also showed that each leaf is initiated from many founder cells and not from the very few initial cells that earlier anatomical studies had suggested.

A final proof that all three cell layers from the meristem contribute to leaf shape came from studies of tomato/nightshade chimeras in which leaves were large, compound, and tomato-like if L3 was from tomato, and were small, simple, and nightshade-like if L3 was from nightshade, regardless of the genotype of L1 and L2.

Embryogeny

After my early studies on meristems had been frustrated by their small size and the absence of biochemical methods to carry the work further, we decided in 1970 that embryo development would be an interesting way to investigate developmental programs. However, because the earliest stages of a plant embryo are as small as or smaller than its meristems, we decided to focus on later events

when the embryo is larger, and to choose plants in which the embryo becomes very large. For this reason, we selected *Phaseolus vulgaris*, the kidney bean, and *P. coccineus*, the scarlet runner bean. These were ideal for our studies. *P. vulgaris*, in particular, is a spontaneously self-pollinating, day-neutral, determinate plant in which the stage of embryo development can be accurately determined from morphological features of the pod and seed. An interesting question in plant embryogeny is the developmental arrest of the embryo and formation of the seed. These phenomena, which were crucial in the evolution of gymnosperms and angiosperms by providing dispersal and survival mechanisms, are in marked contrast to the situation in ferns where embryogeny and postembryonic development occur without interruption. So, how did this new event, developmental arrest, intervene between two previously continuous stages? Our investigations led to the conclusion that the synthesis of abscisic acid, possibly in response to the changing osmotic environment of the embryo, brought about growth cessation and arrest. This conclusion was supported by work on the viviparous *vp1* mutant of maize in which arrest of the embryo does not occur and the embryo germinates precociously on the cob because it fails to respond to the presence of ABA in the seed.

However, the role of ABA was even more profound than this, because in excised embryos we showed that application of exogenous ABA resulted in the activation of synthesis of seed-specific storage proteins and the suppression of expression of genes required for chloroplast function. Thus, ABA appeared to play a pivotal role in the shift from embryo growth and development to arrest and seed maturation.

A way to study developmental events in very early stages of the embryo became available when it became possible to generate a large number of embryo lethal mutants in *Arabidopsis*. Continuation of this line of work has resulted in molecular and biochemical analysis of embryogeny in many labs.

Flower Development

At about the same time that we began work on meristem fate determination in *Selaginella*, we also became interested in how meristem fate is changed in the transition from vegetative to reproductive function so that the lateral organs are now the various kinds of floral organs and not leaves, and the meristem is determinate, not indeterminate, in its growth. Our first approach to this question was to combine surgical and in vitro culture methods. We bisected and excised tobacco floral meristems at different stages of development and grew them in culture where they completed organogenesis. The new organs were floral and were formed in the appropriate whorls so we concluded that floral meristem fate was fixed irreversibly in tobacco and that the factors that determined organ identity were located within the meristem.

Much later, we returned to examine the question of floral meristem determinacy. This was after the ABC model of floral organ identity had been established and when progress was being made in defining the functions of the floral homeotic genes by means of mutant analysis. We were able to show that the AP1 and AP2 genes are required for determinate development of the floral meristem and that they also suppress the formation of axillary buds in first whorl floral organs.

CONCLUSIONS

What are some of the changes I have seen during my academic career? First is the huge increase in the number of scientists who are working in the fields that can broadly be defined as developmental plant biology. In my early years, it was rare to find another person working on the same organism or the same question that I was. Now it is usual to find several labs working on the same genes in the same plant. This has had many consequences. The level of competition has increased, but so has the rate of progress. Two of the great advantages of this population explosion are that it has provided opportunities for more women to become scientists, and it has virtually overwhelmed the "old boy network."

Next is the increased frequency of scientific meetings. I used to go to two meetings a year, the Growth Society, the forerunner of the Society for Developmental Biology, and the AIBS meetings. These were held on a university campus and were cheap to attend. They were small, and it was possible to know essentially all the people attending because they returned year after year. Now there are Gordon and Keystone conferences and FASEB meetings as well as many others that compete for our time and attention. These are usually held in expensive resorts. Most of these meetings are large or their subject matter changes year-to-year so it gets harder to know even a good fraction of the attendees.

Small science versus big science. By small science I mean a project on which one or two people in a lab are working. By big science I do not mean the genome project, valuable as it undoubtedly is, but "the lab project" where the PI has obtained one or more federal grants, and essentially everyone in the lab works on a part of it. It seems to me that the latter way of doing science is a disservice to graduate students because their focus is necessarily narrowed. Because of this, granting agencies have repeatedly urged interdisciplinary collaborations between labs that may be physically distant. But, with small science, which I like to think our lab practiced, at any one time the lab might contain a cell biologist, a biochemist, a molecular biologist, and a geneticist each working on their own projects, but also able to contribute to other projects. In this way, our lab seemed to have many of the aspects of an interdisciplinary approach.

ACKNOWLEDGMENTS

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