Derek Bewley

The Beginning

An early, subliminal influence on my scientific interests was my maternal grandfather, who upon retirement grew tomatoes and bred chrysanthemums and dahlias. He gave me his brass microscope from the 1860's, and a fascinating set of old slides. Years later, I accepted an offer from the University of London (Queen Elizabeth College), with the intention of majoring in Zoology and Physiology. But during my first year the plant lectures were far more interesting, so I opted for a degree in Botany and Biochemistry, the latter being a rapidly expanding topic, with molecular biology still under its umbrella. I particularly remember a guest lecture series by Sydney Brenner (later a Nobel laureate), for each week he would excitedly enter the classroom and announce the latest codon to be cracked. Lectures in plant physiology by Michael Black peaked my interest, with his enthusiasm and dry humor, and this led to.......

The Research

Upon graduating, Mike obtained a PhD scholarship for me to study lettuce seed germination under his supervision. At that time, Joe Varner had shown that GA_3 induces a marked increase in a single protein, α-amylase, in the aleurone layer of Himalaya barley. So the hypothesis to be tested was that when GA breaks dormancy of lettuce seeds there will be a prominent protein

synthesized that is associated with germination. So, armed with Ornstein and Davis gels, newly invented Sephadex columns, and MAK columns, I sought the 'germination protein' and its message. Needless to say no such protein emerged, but with positive information on the physiology of the seed I was able to qualify for a PhD. I learned much while working with Mike, but one lesson that has guided me since is to put my research in the context of the 'Big Picture'.

Seed research conducted in the USA was abundant in the literature, and the opportunity to expand my horizons, both scientifically and geographically, led me there for a postdoctoral fellowship. Particularly interesting was the groundbreaking research of Abe Marcus, who showed that dry wheat embryos commence polysome reformation and protein synthesis very soon after imbibition, and he had developed an *in vitro* wheat germ system to study this. Hence, I was pleased to be accepted into his lab at the Fox Chase Institute for Cancer Research in Philadelphia. My wife Christine and I moved there in 1968 with a suitcase apiece and $100 each in our pockets. My project was to isolate a soluble protein synthesis initiation factor required in the formation of the ribosome-messenger RNA complex, a prelude to translation. Each morning the *in vitro* system had to be prepared from wheat germ, and then used almost immediately for the planned experiment. While the initiation reaction was known in *E. coli*, we gained the first clues how it operates in eukaryotes. A highlight in my second year in the lab was attending a FASEB meeting in Atlantic City, along with some 18,000 other registrants, held in grand hotel ballrooms. My talk, strictly limited to seven minutes including questions, was my second at any meeting and in front of an audience of more than 2,000, including five Nobel prize winners. It was nerve-wracking, especially when one of them asked me a question.

As the end of my two years as a post-doc was approaching, I had to seriously consider what came next. I enjoyed the challenges of research, but I also wanted to incorporate teaching; so I looked to academia for a future. In the UK, positions in universities had dried up and the divisive war in Vietnam made the US an unattractive alternative, so I looked north to Canada.

continued on next page

Derek Bewley *continued*

The University of Calgary advertised for a 'Metabolic Plant Physiologist'; although unsure what that meant, I fortunately fit the description, so we moved there in 1970. Calgary was a smallish frontier city at the time of our arrival, but the first oil boom changed that, money poured into the universities, and a new biological sciences building with spacious and well-equipped laboratories was soon completed.

While wishing to continue research on seeds, I also wanted to develop a niche that would attract both attention and funding. I was intrigued by a recent paper by Ted Hsaio in *Plant Physiology*, showing that relatively mild water stress in maize seedlings can cause an irreversible reduction of protein synthesis. What, I wondered, occurs in vegetative tissues tolerant of much more severe water stress, i.e. desiccation? I found no reports of desiccation-tolerant angiosperms native to the northern hemisphere, so what to use? I mentioned this to David Fountain, my first graduate student, and he recounted when on an undergraduate field trip back home in New Zealand, his instructor had poured beer over a dried moss clump, causing it to green up again within minutes. I had admiration for a plant that responds so positively to the noble brew and thus, with the help of our herbarium, picked for study a suitable desiccation-tolerant moss, *Tortula ruralis* (now *Syntrichia ruralis*). Its ability to dry within an hour, and become reactivated metabolically within minutes of rehydration was ideal for research, especially when I had to fit it in between a demanding teaching schedule. Over the next few years, I welcomed several graduate students and postdocs into the laboratory, and we made considerable progress in understanding how *Tortula* varied in its response to rapid and slow desiccation. Using a structure-function approach we determined, for example, how polysomes dissemble and re-assemble, the fate of different RNAs in the dry state and their re-synthesis upon rehydration, energy metabolism during and following stress, lipid peroxidation, and changes in cellular and membrane integrity. I was pleased to be asked by the *Annual Review of Plant Physiology* to write an article on 'Physiological Aspects of Desiccation Tolerance', which was published in 1979. I learned much about desiccation tolerance in algae to angiosperms, providing me with the opportunity to postulate the requirements for tolerance, which I briefly summarize as: i) limit damage during desiccation, ii) retain physiological integrity in the dry state, and iii) repair damage quickly upon rehydration. Satisfyingly, even today these presumptions are still mostly correct. It was a pleasure to be invited by Mel Oliver and co-authors to give closure to my work on desiccation by participating in a review published in ARPB in 2020, 41 years after my first one!

My return to lettuce seeds was stimulated by the arrival in Calgary of Peter Halmer as a post-doctoral fellow with expertise on cell walls. I told him of a puzzle that had concerned me since my PhD years: in a publication in 1963, Ikuma and Thimann suggested that germination of lettuce seeds requires weakening of the cell walls of the embryo-enclosing endosperm. This, they suggested, is accomplished by cellulase synthesized in the radicle, and then released into the endosperm. If so, then why does the enzyme not degrade the cell walls of the radicle also? Could it be that the cell wall composition of the two tissues is different? Peter's GC analysis of the cell walls showed that while those of the radicle are rich in cellulose, the main component of endosperm walls is the hemicellulose, galactomannan. This was the start of an extensive project that initially showed weakening of the endosperm walls by endo-β-mannanase is post-germinative. Synthesis of the enzyme in the endosperm initially requires signaling from the radicle, through the cotyledons to the endosperm, its site of synthesis, and is influenced by GA and ABA. The oligomannans released by the endo-β-mannanase are converted to mannose by β-mannosidase already in the cell walls of the cotyledons, and the galactose can be released by α-galactosidase synthesized in both the endosperm and cotyledons.

In 1985, I moved to the University of Guelph to assume the Chair of a newly independent Botany Department. I had to invest considerable time in making it a stronger unit, but with my colleagues we greatly

continued on next page

Derek Bewley *continued*

enhanced its reputation, in particular developing a solid research and graduate program while maintaining its strength in teaching. An advantage to me in Guelph was having access to a larger number of plant researchers, both in my own department and in the agricultural college.

Research on endo-β-mannanase intensified in Guelph, including on tomato seeds. Three impediments to studying the enzyme were overcome: 1) development of a fast gel enzyme assay by Bruce Downie, 2) production of a specific antibody by Hiro Nonogaki in Japan, and 3) the isolation of a cDNA clone for tomato seed endo-β-mannanase, which I achieved under the mentorship of Rachel Burton during a sabbatical at the Waite Research Institute in Adelaide. The roles of the enzyme in germination and mobilization of endosperm cell walls were studied also in seeds of white spruce, fenugreek, rice and coffee. Extensive studies on, and cloning of, β-mannosidase and α-galactosidase completed the picture of how galactomannan degradation is achieved in tomato seeds, and in 2004 the gene encoding endo-β-mannanase was cloned from the seed on which my research began, lettuce.

While on a brief research break in Henk Hilhort's lab in Wageningen University in The Netherlands, I found that endo-β-mannanase in tomato fruit is present as an insoluble enzyme, and using fluorescent secondary antibodies this later led to its detection only in the skin (epidermis) and a few adjacent outer cell layers of the pericarp. To summarize several years of work, a single fruit endo-β-mannanase gene was identified, the enzyme purified, crystallized, and its structure elucidated using X-ray crystallography. Some cultivars of tomato synthesize a permanently inactive fruit enzyme due to a two-base deletion, resulting in a frame-shift and the absence of a single leucine (L-398) close to the C-terminal end, yet the enzyme still locates correctly. Computer simulation showed how this mutation changes the structure of the enzyme, and hence its active site.

A series of questions melded my interests in desiccation and seed development and germination; most of this research was conducted on seeds of castor bean, *Phaseolus* bean, maize and alfalfa, and some of the results are summarized briefly. When during development are seeds capable of surviving desiccation? This occurs surprisingly early, in some species close to mid-development. If a developing seed is dried prematurely, upon rehydration does it still complete its development, or does it switch to being a germinating seed? The answer is the latter; the seed's metabolism and gene expression patterns change from anabolic to catabolic upon rehydration. When a seed, such as that of tomato, develops in a wet environment in the fruit, what prevents it from germinating? The osmotic potential of the sheath and locular tissue surrounding the fruit, and ABA within the seed are responsible. Will a tomato seed germinate if taken from the fruit during development and prevented from drying? Yes it will, at a similar rate to that of a dried seed, and the metabolism of the seeds is very similar. When an imbibed seed is re-dried during germination, e.g. in peas after 16 hours, upon rehydration will it revert to being a dry seed again and repeat its metabolism, or will it continue from the time when it was dried? It does not revert to the dry state, but rather restarts from 2-3h before the time of water loss.

Space does not allow me to write about a host of other research projects on seed development and germination, including the influence of ABA; seasonal variations in storage polymers in perennial roots; desiccation-tolerant ferns and *Ranunculus* roots; the collet region and radicle emergence; phytin synthesis; somatic embryogenesis; long-term secondary dormancy; mitochondria development; SO_2 pollution and moss survival; etc. They were equally stimulating, and were started by asking a simple question, leading to more and more questions as the complexity of the answers increased. Nor do I not have space to name the large number of graduate students, postdoctoral fellows, technicians, visiting researchers, and co-researchers elsewhere with whom I was very fortunate to work, and credit goes to them for the successes of our research programs. My role was to provide the lab as a forum in which everyone could pursue their curiosity

continued on next page

Derek Bewley *continued*

and to invest their energies as they wished: success has come to the many who took up the challenges.

In 2005, I opted for early retirement. After a long and brave fight with cancer, my wife was no longer winning the battle, so I wanted to spend more time with her, without distraction. Sadly, the end came faster than we anticipated. I took some extra time off, and because I still had grant money and access to my lab I returned for another few years. But the fire was no longer there, so I decided to close the lab door and walk away for the last time.

The Books

These started in 1973 with a chance encounter with a representative from Springer-Verlag during the first joint meeting of the Canadian and American Societies of Plant Physiology in Calgary. He asked if I knew of any subject in need of a book: I suggested that one on seeds was overdue and introduced him to Mike Black as the ideal author. After some hesitation, Mike agreed and asked me to help with the writing. Originally there was to be one book, 'Physiology and Biochemistry of Seeds', covering most aspects of seed structure and function, with Mike as the first author. However, when I was on sabbatical in London in 1976 I was able to finish most of my writing, while Mike's commitments meant that he had still much to do. Springer agreed that we could split the book into two volumes, the first one with the subtitle 'Development, Germination and Growth', as Bewley and Black, and the second subtitled 'Viability, Dormancy and Environmental Control', as Black and Bewley. The first volume went to print in 1978, and the second, mostly written by Mike, in 1982. But Springer broke our agreement and insisted that both volumes have the same order of authors.

While we were pleased with the response to two-volume work, we realized there was also a need for a shorter and more affordable version that covered the key aspects of seed physiology. The intended audience was students and teachers in institutions of higher learning, whose courses included plant biology, e.g. plant physiology, agriculture and horticulture. Hence a contract was signed with Plenum to publish 'Seeds. Physiology of Development and Germination', the first edition in 1985 and the second in 1994; both received critical acclaim.

Mike suggested a book to cover seed technology and aspects of seed biology that underpin this. We attracted a number of experts to write chapters, and the publication appeared in 2000 as 'Seed Technology and its Biological Basis' edited by Black and Bewley. Mike's second project was a much greater challenge, to put together a single comprehensive tome with authoritative articles on all aspects of seeds. Thus was born 'The Encyclopedia of Seeds. Science, Technology and Uses' edited by Black, Bewley and Halmer, with Peter's experience in the seed industry providing vital links to the commercial side of seed biology. A total of 112 authors provided articles for the Encyclopedia and when printed by CABI in 2006 it was 828 pages long.

After retirement, I was pleased to be invited to participate in seed courses in Brazil and UC Davis. The spring course in Davis on the fundamentals of seed biology was organized by Kent Bradford, with Hiro Nonogaki, Henk Hilhorst and myself as co-lecturers. We discussed an update of the 'Seeds' book, and Springer agreed to be our publisher; Mike had another project underway, but allowed us to include some sections he wrote for the previous editions. Published also as an e-book and e-chapters on 2013, we were pleased with the quality of the third edition, my 'swan song' in a career in seed biology.

For several years I taught in a plant physiology course at Shezhen University. As a finale in 2017, I recorded the course for online presentation, and Beixin Mo and I completed an accompanying introductory level bilingual book on Plant Physiology. She wrote and translated the Chinese, and I edited the English, and on each page information in English was printed in the left column and the Chinese translation on the right, with bilingual tables and figures. Thus, I ended my teaching career in a unique way.