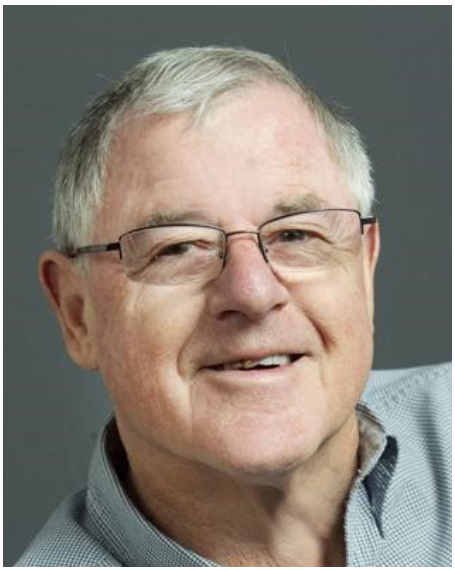
ASPB Pioneer Member

Russell Jones

Russell Jones was born in Wales and received his BSc (1962) and PhD (1965) degrees from the University of Wales, Aberystwyth. He was faced with three career choices following his PhD: to work at the UK sugar company, Tate and Lyle, in the West Indies; to do a post-doc at the Waite Institute of the University of Adelaide with Leslie Paleg; or move to East Lansing, Michigan to the newly established Plant Research Laboratory (PRL). He chose the PRL, and it was indeed a very fortunate choice. The PRL was an inspirational place. A cohort of researchers from the US and overseas, including Phil Filner, Hans Kende, Joe Varner, and Jan Zeevaart, who were particularly influential, had gathered there to do ground-breaking research.

Jones was interested in agriculture and plants from an early age. All family members on his mother's side were farmers, and he spent many of his formative years on family farms. A great deal of his time was spent at his grandfather's farm, less than a mile from his home. Among the crops growing on this farm was malting barley, and each Fall a representative from the malting company that bought the grain came by to test it. The test determined how much a-amylase was present in the grain, as excessive amounts of this enzyme rendered the crop useless for malting malting barley was and continues to be expensive. The seed testers inspired Jones to think



about a career working with seeds. Little did he realize that barley grains and a-amylase would play a key role in his future.

Jones's PhD research focused on determining the site of gibberellin synthesis in plants. He showed that apical meristems of both the root and shoot had the capacity to produce GA. This research required the use of bioassays to measure GA content, and one of the assays he used was a new one developed by Les Paleg in Australia. It involved measuring the reducing sugar produced by half grains of barley that were producing a-amylase in response to GA.

Jones's postdoc at the PRL involved continuing studies of GA synthesis in plants, working with Anton Lang, then director of the laboratory, and with Hans Kende. He also worked closely with Joe Varner, who was using barley half-grains and aleurone layers to study the biochemistry of GA and ABA action. Varner convinced Jones to continue the use of barley half-grains to assay GA, and, instead of measuring reducing sugar production resulting of the action of a-amylase, measuring the enzyme itself, an obvious improvement in the assay which made it more sensitive and much easier to measure. This work with Varner and several of his postdocs, most notably Maarten Chrispeels and Jake Jacobsen, colored Jones's research for the next 20 years.

Anton Lang called Jones into his office one day in early 1996 to tell him that UC Berkeley was searching for an Assistant Professor in the Botany Department. He urged him to apply but also cautioned that Berkeley had a "reputation". Jones was soon to learn what that reputation was. He flew to San Francisco for an interview and was met at the airport by Len Machlis, the chair of Botany. Len would become a great mentor, and he was to have a big influence on Jones's career. Jones was offered the Berkeley job, and he moved there in the summer of 1966.

Anton Lang's caution that Berkeley had a "reputation" soon revealed itself. First, Jones's predecessor in the Berkeley position had been denied tenure, an unfathomable decision, and this clearly had bothered Lang—it also bothered Jones when he learned about it. Second, the campus was still living in the turmoil of the Free Speech Movement (FSM), where students at Berkeley were asking for a greater role in their education. Initially, the FSM was opposed by the Regents

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of the University but supported by the faculty. Soon, student activism included demonstrations against the Vietnam War, the US military's adventures in Cambodia, and in protest over firing of the then University of California's President, Clark Kerr, by Governor Ronald Reagan. The turmoil was to last into the late 1960s.

Against this background of turmoil and change at Berkeley, the work of education and research continued. With the encouragement of Varner, Jones decided to focus his attention on the barley aleurone layer as an experimental system and to examine in more detail the action of GA. One route that he chose was to use microscopy to study aleurone cells that were responding to GA. This approach was stimulated in part by the work of George Palade, who was using the electron microscope to study pancreatic cells that also synthesized and secreted a-amylase.

The Botany department at Berkeley attracted the very best graduate students, and Jones was fortunate to have a stream of truly outstanding students who came to Botany because of the department's reputation. These students are too numerous to list. Lincoln Taiz, Jones's first PhD student, finished his thesis in 1972 and went on to become a faculty member at UC Santa Cruz.

Taiz was the first to isolate protoplasts from aleurone cells, and the utility of protoplasts as research models led Jones to ask Ted Cocking at Nottingham University, one of the authorities at the time working with protoplasts, whether he would host him on sabbatical. Jones applied for and received a Guggenheim Fellowship to spend a year in Cocking's lab. The sabbatical was not particularly rewarding and part of the blame rests with Jones's lack of planning and his failure to understand what Ted Cocking's lab could contribute. This lesson was learned for planning future sabbaticals.

In the meantime, the Jones lab was using electron microscopy to monitor changes in the cell biology of aleurone cells and found that dramatic proliferation of the endoplasmic reticulum accompanied the synthesis and secretion of a-amylase. To further study this topic, Jill Deikman joined the lab as a graduate student in 1980, with the goal of cloning the amylase gene as a first step in learning more about the synthesis and secretion of the enzyme. One of the questions the lab hoped to answer from an understanding of the sequence of the amylase gene concerned the role of calcium (Ca) in its synthesis. It was known that a-amylases were calcium-containing metalloproteins and that the synthesis of a-amylase by barley aleurone required both GA and Ca. Having cloned the gene, Jill was able to show that a-amylase mRNA only accumulated in aleurone cells when GA and Ca were present, showing a key role for Ca in regulating the expression of the amylase gene.

Jones spent his second sabbatical in 1980 with Jake Jacobsen at the Division of Plant Industry of CSIRO in Canberra, Australia. This was a successful and productive leave, as Jake and Jones knew each other very well and had sketched out a plan of research. They used pulse labeling to follow the synthesis of a-amylase and focused on the number of amylase isoforms and their posttranslational modification. There turned out to be very few; for example, the protein is not glycosylated nor covalently modified.

Jones's lab branched out to investigate the effects of GA on plant cell elongation growth. Part of this was the legacy of his experience in Anton Lang's lab at the PRL, and the work of Bob Cleland at the University of Washington, who was studying the fundamentals of extension growth in response to auxin. Cleland showed that auxin stimulated acidification of the cell wall, and that the acidic environment caused the wall to yield and allow cells to expand. Two graduate students, Wendy Silk and David Stuart, were very much involved in the work on GA and growth. Wendy developed the excised lettuce hypocotyl seedling assay, which responds strongly to GA, as her experimental system. Wendy and Dave showed that expansion of the lettuce hypocotyl in response to GA resulted solely from cell expansion due to cell wall loosening. In contrast to auxin, GA did not bring this about by acidification of the wall.

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The role of Ca in a-amylase synthesis prompted the Jones lab to ask about Ca homeostasis in aleurone cells. It was widely accepted that eukaryotic cells maintained cellular Ca in the range of 1-10 mM, but aleurone cells required 10 mM Ca as well as GA in the external medium for sustained amylase synthesis. The lab embarked on the very difficult task of measuring cytosolic Ca in aleurone cells using protoplasts as the model. Two talented postdocs, Douglas Bush and Simon Gilroy, worked on this challenging project. They used fluorescent dyes to make real-time measurements of Ca in protoplasts and showed that these cells were like other eukaryotic cells in maintaining cytosolic Ca in the low mM range when not synthesizing a-amylase; but when treated with GA and Ca, the steady state level of Ca rose to around 100 mM.

Because of the central role of Ca in the response of aleurone cells to GA, one of Jones's post docs, Rob Schuurink, set about finding Ca receptors, the most obvious being calmodulin (Cam). Rob showed that Cam levels in aleurone cells were regulated by both ABA and GA, with GA upregulating the amount of CAM transcripts and protein and ABA depressing it. Rob also discovered a novel Cam binding transporter at the plasma membrane; members of this class of transporter have now been shown to be the long sought-after cyclic nucleotide plasma membrane-localized Ca channel.

The lab continued to focus on the elusive role of the Golgi apparatus in secretion of hydrolases from aleurone cells. Neither electron microscopy nor organelle isolation methods showed a prominent Golgi, leading a graduate student, Diane Melroy, to investigate the involvement of Golgi using the drug, monensin, which blocked a-amylase production by aleurone cells. EM showed cisternae of the Golgi to be swollen in monensin-treated cells. This work led Jones to spend a sabbatical with David Robinson, a world class plant cell biologist, at the University of Göttingen. This sabbatical was supported by a generous grant from the Alexander von Humboldt Foundation. David was an exceptionally supportive host. One of our goals was to use protoplasts to look at exocytosis in the aleurone, but technical difficulties dogged this work. To make functional protoplasts, mannitol was used as an osmoticum. However, unbeknownst to us, mannitol in Germany is isolated from sugar beet, and we discovered from the stomatal guard cell researchers, Rainer Hedrich and Julian Schroder in Klaus Raschke's lab in Gottingen, that the mannitol available in Germany was rich in ABA. Adding German mannitol to the medium of aleurone protoplasts ensured that they did not respond to GA. This stymied our research during the sabbatical year. But the sabbatical in Gottingen had the added benefit that Hedrich and Schroder introduced Jones to patch clamping and Douglas Bush came to Gottingen to master the technique. This led to publication of an article on the flux of potassium from aleurone protoplasts measured using the patch-clamp technique.

A grad student, Paul Bethke, became interested in the functioning of protein storage vacuoles (PSV) and investigated the proteases they contain. Paul developed methods to isolate PSV in quantity to study their complement of proteolytic enzymes. Paul's work showed aspartic and cysteine proteases were specifically localized to PSV and that these enzymes increased on treatment of cells with GA. The presence of proteases in PSV prompted Paul to ask how these amino acids crossed the tonoplast membrane. He embarked on an ambitious series of experiments using patch clamping of individual PSV to study the transport properties of the tonoplast. He identified the slow vacuolar channel and showed that the opening and closing of this channel was controlled by reversible phosphorylation.

The Jones lab became expert at using both protoplasts and fluorescent dyes to measure various aspects of aleurone cell function. Sarah Swanson showed that pH-sensitive dyes could be loaded into aleurone cells and made to accumulate in PSV. Work from the lab had shown that PSV were organelles that produced the amino acids that were converted to secretory proteins by the elaborate ER in the cytoplasm. Sarah was able to

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show that acidification of the PSV in response to GA and Ca was a requirement for the synthesis and secretion of a-amylase and other proteins.

One key aspect of non-embryonic seed tissues, such as endosperm, is that they are dispensable. In cereal grains, the starchy endosperm is dead at grain maturity and is digested by enzymes produced by the aleurone. The aleurone also dies after its role as a producer of digestive enzymes ends. Several graduate students and post docs in the lab became involved in examining cell death in aleurone tissue and showed that it was brought about by reactive oxygen species. ROS are kept under tight control in GA-treated cells by the presence of enzymes such as catalase, ascorbate peroxidase and superoxide dismutase. The transcription of ROS genes is sharply downregulated after GA treatment, but ABA stimulates their expression. Aleurone protoplasts could be kept for up to six months in culture in the presence of ABA, whereas in the presence of GA all would die within 72 hours of culture.

Like barley, Arabidopsis seeds possess an aleurone layer, also known as endosperm, and the lab used Arabidopsis as a model for dormancy studies. This work was initiated in 2004, when Jones spent a second sabbatical at CSIRO Canberra with Jake Jacobsen and Frank Gubler. Jones's CSIRO colleagues were interested in studying the roles of ABA and GA in seed dormancy and determined that Arabidopsis was a far better model for this work than barley grains. Frank introduced Jones to the C-24 cultivar of Arabidopsis that was very strongly dormant, but where 100% germination could be achieved with GA treatment. Because Arabidopsis provided a route to molecular genetic studies of dormancy, the Jones lab began to use the C-24 cultivar exclusively. By this time (2003) Paul Bethke had earned his PhD and continued in the lab as a postdoc. His work on programmed cell death in barley aleurone showed that nitric oxide was as potent an inducer of cell death as GA. What's more, he showed that a copious amount of NO was produced in aleurone tissue by the reduction of nitrate at low pH in the cell wall. This important finding helped explain a very old observation that simple compounds such as nitrite and nitrate could break seed dormancy in a wide range of species.

Work with Arabidopsis established the endosperm/aleurone imposed dormancy by restraining growth of the embryo. Work with GA, NO, ABA and the *Spy-1* mutant showed that the endosperm was essential for dormancy and that germination ensued when endosperm cell walls were weakened by GA, NO and in *Spy-1*. ABA and NO scavenging compounds all inhibited germination.

The Bottom Line

A successful scientific career comes about largely because of mentors, colleagues, and academic and research institutions. At Aberystwyth, Jones benefited from the influence of two mentors, Philip Wareing and Dai Phillips. At the PRL, he drew on its concentration of talented researchers, especially Joe Varner. The resources of the University of California, Berkeley are immense. The institution attracts the very best graduate students and postdocs, and its faculty are the grateful beneficiaries. Finally, sabbatical leaves can be powerful learning experiences by providing exposure to new ideas and approaches.