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CLARENCE A. "BUD" RYAN
1931—2007

A Biographical Memoir by

R. JAMES COOK

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Biographical Memoir

Clarence A. Lynch

CLARENCE A. ("BUD") RYAN

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BY R. JAMES COOK

CLARENCE A. RYAN, KNOWN AS "BUD" to friends and family alike, was born in Butte, Montana, the second of four children. His mother finished high school but his father never finished grade school, having to go to work at age 12 when Bud's grandfather was killed in an accident in a mine. Bud's family moved to Helena, Montana, when he was 10 years old. There, Bud attended a Catholic high school and then Carroll College, a Jesuit liberal arts college, graduating in 1951 with a B.A. in chemistry. Bud paid for his undergraduate education with part-time jobs and loans and then, when he graduated, took a job with the Montana Highway Department for one year and drove a cab at night to pay off his loans. During that same year, he married Patricia Meunier, "a little gal from Wolf Creek, Montana" that he had met at a barn dance.[1]

It had not occurred to Bud to go to graduate school until he was contacted by a woman that had graduated from Carroll College two years ahead of him and was working on an M.S. degree in chemistry at Montana State University. She called him several times to suggest that he consider graduate school, and then made an appointment for him with the chair of the chemistry department at MSU. Bud kept the appointment, was accepted into graduate school,

and having no graduate assistantship, took jobs in the agricultural experiment station and as a teaching assistant in freshman chemistry to support himself and his growing family. He obtained an M.S. in chemistry in 1956 and Ph.D. also in chemistry in 1959, by which time he and Pat had three children. Bud was one of the first two to obtain a Ph.D. in chemistry from MSU, both in 1959. He was also the first at Washington State University to be elected to the National Academy of Sciences, in 1986.

In an interview[1] Bud explained that "from the time I started high school, I said I'm going to be a chemist." When asked why, he answered that it was sparked partly by a radio program sponsored by DuPont called "Better Things for Better Living Through Chemistry" that described how scientists brought plants back from the jungle as sources of chemicals used for beneficial purposes. He also credited one of his high school teachers, Sister Evangelista, who taught "a great science class."

With his thesis research in cereal chemistry Bud had job offers to work with corn products in Chicago and a brewery in Milwaukee. Coincidently, a new assistant professor in the chemistry department at MSU told him of an opportunity to do postdoctoral research in marine biochemistry at Oregon State University. Tsoo King at OSU had a grant from the Office of Naval Research to study respiratory metabolism of the paramyosin smooth muscle of mollusks. It would be his only sojourn into research on animals. He commented in an interview[1] that "I really didn't like killing animals or working with mice" and added that "it was just a natural thing for me [to work with plants]. I'm really lucky because that doesn't happen to a lot of kids, but I just followed what was interesting to me." In fact, he developed an allergy to mice and could not even be in the same room with them.

Bud then joined A. K. Balls as a postdoc at the U.S. Department of Agriculture's Western Regional Research Laboratory from 1961 to 1964. Balls was interested in the digestive enzymes of animals; specifically, he was working to find the active sites that conferred ability of chymotrypsin and trypsin to digest proteins. Following a report of a substance in potato peel that inhibited choline esterase, and earlier evidence that a potent inhibitor of choline esterase also inhibited trypsin and chymotrypsin, Bud, then 33 years old, purchased three Russet Burbank potato tubers from a neighborhood grocer that he then used to demonstrate the complete inhibition of chymotrypsin by a substance in the tuber cortical tissues. With his discovery that "potato is jammed full of a chymotrypsin inhibitor," Bud asked Balls if he could try to purify it. Balls response was, "Have at it—it's not what I hired you for, but go ahead."[1]

Like all postdocs entering the final year of their appointment, Bud began to look for a more permanent position, preferably at a university where he could continue to study the proteinase (protease) inhibitor. When Balls said that he would not continue work on the protease inhibitor, Ryan submitted a proposal to the National Institutes for Health for a grant that would allow him to continue the work. He was awarded a Career Development Award that paid his salary for five years and allowed him to choose a university and mentor. Coincidentally, his former mentor at Oregon State University was responsible for selecting speakers for a regional meeting of the American Chemical Society to be held in Pullman, and invited Bud as one of the speakers. Balls provided the funding and suggested that Bud look up Romey Legault, formerly a section chief at the USDA Western Regional Research Laboratory in Albany, California, and now head of the Department of Agricultural Chemistry at Washington State University, Pullman. When Bud told

Legault that he had an NIH Career Development Award, Legault offered him a lab in the Department of Agricultural Chemistry. WSU has a long history of strengths in the plant sciences, but Bud was the university's only plant biochemist in 1964.

Bud's discovery of protease inhibitors in potato would lead to a succession of fundamental breakthroughs in plant biology research, including discovery of a major defense mechanism in plants against insect pests, the first peptide hormone known in plants, the jasmonic acid signaling system in plants, and a mechanism for amplification of the plant immune response to pathogens, all while remaining at Washington State University in Pullman. Reflecting on Bud's widely recognized low-key personality, his accomplishments in plant biology research, and his decision to remain in Pullman, Maartin Chrispeels[2] commented that "Bud was a gentleman in the real meaning of that word. He loved the simple life in his small town. No city slicker he was. His research slowly matured over a 50-year period. An amazing guy." Peter Quail[2] commented that "he was a gentleman and a scholar whose pioneering work will have a lasting impact on the field of plant biology. He was renowned for his quiet, low-key presentations, during which he was frequently announcing truly paradigm shifting new research data."

ENDOGENOUS PEPTIDE SIGNALING MEDIATES PLANT DEFENSE AGAINST INSECTS

Clarence A ("Bud") Ryan, Charlotte Y. Martin Professor of Biochemistry and fellow of the Institute of Biological Chemistry, Washington State University, led the team that discovered and named systemin, an 18-amino-acid peptide in tomato and the first peptide hormone known in plants (1991). With this discovery Bud joined the ranks of Banting and Best (1922) who discovered the first polypeptide

hormone known in animals, the 48-amino-acid insulin in dog, and Kurjan and Herskowitz (1982) who discovered the first polypeptide hormone known in microorganisms, the 13-amino-acid α-factor in yeast. While one of the seminal discoveries in plant biology in the 20th century, it followed Bud's election to the National Academy of Sciences in 1986 by five years. And typical of his leadership style of elevating the members of his team to the highest pedestal, he made his senior and highly dedicated lab technician, Greg Pearce, an experienced protein chemist taught in the Ryan lab, as first author of the landmark paper.

Bud (1998) describes his discovery of systemin and its precursor prosystemin as the result of some 30 years of incremental advances that started in 1962 with the discovery of the inhibitor of the digestive enzyme chymotrypsin in the cortical tissue of potato tubers (1962); that production of the protease inhibitor was induced by wounding, including when an insect takes a bite from a leaf ; and that this wound response/production of protease inhibitor represents a major defense response of plants to herbivorous insects. After "an immense amount of frustration and confusion" (1998), systemin turned out to be the elusive protease inhibitor inducing factor (PIIF). Currently, it is thought (Greg Pearce, personal communication) that the precursor protein prosystemin, released upon wounding, is processed to form systemin that travels in nanomolar amounts to neighboring cells where it binds to a transmembrane receptor and leads to production of jasmonic acid (see below). Jasmonic acid, in turn, does the long-distance signaling expressed as systemic production of the protease inhibitors responsible for starvation of the leaf-chewing insects by inhibition of their digestive enzymes. Formation of more prosystemin is also induced by jasmonic acid, thereby providing an amplification loop for the systemic defense response.

Early in the course of this research (1968, 1970) Bud discovered that protease inhibitor was formed in leaves of both potato and tomato, a close relative of potato. He then chose tomato as the plant of choice for the studies that led to discovery of systemin. He made the connection between wound-induced expression of protease inhibitor and plant defense against insect pests with the observation that some tomato plants accumulated the protease inhibitors in leaves while still in the greenhouse and before wounding. Noticing that these plants were usually near an open window and hence possibly the first to be visited by insects, he collected Colorado potato beetles from a friend's garden one summer evening, and placed these on several tomato and potato plants. The result was the accumulation of large quantities of protease inhibitor in both plant species within two days of placing the beetles on the plants but not in plants kept free of the beetles (1972). In sharp contrast to the relatively simple steps and small amount of biological material required to find the protease inhibitors and come up with the first clue of their role in the wound response and plant defense, isolation and purification of the elusive systemin involved growing thousands of tomato plants each week in a greenhouse, with the final preparation of 800 ng of pure material used for sequencing having come from nearly 30 kg of fresh tomato leaves.[1]

It seemed obvious that systemin would turn out to be but one of many peptide hormones functional in plants, considering that peptide hormones serve so many different roles in animals. Bud then led the discovery of two hydroxyproline-rich defense-signaling glycopeptide hormones in tobacco (2001), followed by three in each of tomato (2003) and petunia (2007). Like potato, these three plant species are all members of the Solanaceae family. And like the cleavage of the 18-amino-acid systemin from the larger prosystemin

precursor protein coded by a single gene, these HypSys signal peptides are cleaved from a precursor protein coded by a single gene. Bud also collaborated in work with sweet potato that identified six different peptide hormones derived from a single precursor protein coded by a single gene and shown to activate a defense gene (2008). Dozens of peptide hormones have now been identified in plants and shown to regulate reproduction, development, and other functions in addition to defense.

JASMONIC ACID SIGNALING: A NEW FRONTIER IN PLANT BIOLOGY RESEARCH

Prior to the discovery of the systemin signaling pathway, the Ryan lab had pursued several leads in search of the protease inhibitor inducing factor (PIIF) in potato and tomato plants. Following a report of elucidation of the biosynthesis of jasmonic acid (JA) from linolenic acid (Vick and Zimmerman, 1983), and recalling an earlier unpublished experiment done by Mary Kay Walker-Simmons when working as a postdoc in his lab showing that linolenic acid induced protease inhibitor synthesis,[3] tomato plants were sprayed with a sample of JA in the form of methyl jasmonate found on the WSU campus. The induction of protease inhibitor synthesis within the first 24 hours was spectacular. Edward ("Ted") Farmer, a postdoc with Ryan, quickly showed that systemin regulates the defensive genes through the octadecanoid pathway—the pathway for biosynthesis of jasmonic acid from α-linolenic acid. Ten years later graduate student Justin Scheer obtained evidence that a cell receptor for systemin is a member of the leucine-rich repeat (LRR) kinase family of receptors. With the signal peptide systemin as the first step, evidence of an LRR kinase receptor for systemin, the JA-signaling pathway for expression of genes for production of protease inhibitors, and finally the interference with the digestive enzymes of

herbivorous insects upon ingestion of the inhibitors by the leaf-chewing insects, this system remains the best understood example of a systemic defense response in plants to either insect pests or plant pathogens.

Jasmonic acid was of relatively unknown function when first shown by Vick and Zimmerman (1983) to form through the octadecanoid pathway from linolenic acid. The discovery of its regulatory role in the expression of genes for production of protease inhibitor in tomatoes opened an entirely new avenue of research into the role of this plant hormone in the defense response of plants to insect pests and pathogens.

The induction of the signaling effect by spraying tomato plants with the volatile methyl jasmonate led to the hypothesis that the defense response could be turned on in neighboring plants—a kind of plant-to-plant communication. Like the experiments with potato tubers fresh from a local market and Colorado potato beetles from a colleague's garden, a test conducted with sagebrush (*Artemisia tridentata*) found abundantly in the semiarid lands just west of Pullman showed that methyl jasmonate emitted from these plants induced production of protease inhibitor in tomato. Not only does the defense response extend systemically from a leaf under attack to other leaves on the same plant not yet under attack, the proteinase-inhibitor-mediated defense response can also be turned on in neighboring plants not yet under attack.

Always with the big picture in mind, Bud (1996, 1998) pointed out that this signaling pathway for plant defense against herbivorous insects is analogous to the inflammatory response of macrophages and mast cells in animals to pathogens, whereby a polypeptide activates release of arachidonic acid and this leads to synthesis of prostaglandin. Bud (1998) also suggested that "with plants and animals having both derived from a common ancestor hundreds of millions of years ago, it seemed logical that plants would have also

evolved such a fundamental signaling strategy to regulate various processes."

ENDOGENOUS PEPTIDE SIGNALING AMPLIFIES THE INNATE IMMUNE RESPONSE OF PLANTS TO PATHOGENS

For most of Bud's career his lab focused almost exclusively on the role of the wound response and associated production of protease inhibitors as a mechanisms of defense against herbivorous insects in tomato and potato. After his retirement in 1999 at age 68, he was hired back on a 40 percent appointment ("more like 90%"[1]) and began to look at plant defense systems against plant pathogens. Greg Pearce had become the expert at isolating and purifying peptide hormones by then and stayed on as Bud's technician. His lab group, while never large, went down to one visiting scientist, Yube Yamaguchi from Japan, and his last graduate student, Alisa Huffaker. Remarkably it was during the later part of this period—the last three to four years of his life—that the lab group under his leadership discovered an entirely unknown endogenous peptide signaling system for amplification of the innate immune response of plants to plant pathogens.

While many proteins had been shown over the previous 15 to 20 years to elicit an immune response in plants, all were produced by the would-be pathogens, so-called elicitors or pathogen-associated molecular patterns (PAMPs). The known plant proteins involved in the innate immune response to pathogens were typically transmembrane receptor or recognition molecules and products of the leucine-rich repeat (LRR) kinase family of disease-resistance genes. In a series of three papers published in Proceedings of the National Academy of Sciences (PNAS) in 2006 and 2007, the Ryan lab reported the isolation and characterization of a 23-aa peptide named AtPep1 from *Arabidopsis thaliana* (2006), showed the precursor of AtPep1 to be a 98-aa protein (PROPEP1) produced

from a single gene that, when expressed constitutively in *A. thaliana*, caused expression of the PROPEP1 gene and the well-known plant defense genes PDF1.2 and PR-, and led to enhanced resistance of the plant to the root pathogen *Pythium irregulare*. Based on estimations of amino acid similarities and identities, six different versions (paralogs) of the PROPEP1 gene were shown to occur in the genome of *A. thaliana*, and other versions (orthologs) of the gene were shown to occur in rice, wheat, maize, barley, canola, potato, soybean, *Medicago sp.*, birch, and poplar (2006). A companion paper (Yamaguchi et al., 2006) reported that the transmembrane cell-surface receptor for AtPep1 in *A. thaliana* is a member of the LRR receptor kinase family of recognition ligands, named PERP1, and that induced expression of its own precursor PROPEP1 gene by the AtPep1 signal peptide provides a feedback loop that amplifies the innate immune response putatively turned on by PAMPs.

Initial results showed that the PEPR-1 cell-surface receptor activates the expression of the defense genes PDF1.2 and the precursor PROPEP1 gene through the jasmonate/ethylene signaling pathway. Huffaker and Ryan (2007) then reported that the six paralogs of the precursor PROPEP1 gene in *A. thaliana*, as well as the defense genes PDF1.2 PR-1, were differentially expressed in accordance with whether the plants were treated with methyl jasmonate or methyl salicylate. Another remarkable discovery, these results reveal that the endogenous AtPep1 signal peptide can amplify plant responses to PAMPs through either the jasmonic acid or salicylic acid signaling pathways not just in *A. thaliana* but also as a general response of plants where PROPEP1 orthologous genes occur.

PERSONAL ASIDES

While excelling in academics, Bud was also a star on the Carroll College basketball team and was elected to both the Carroll College Alumni Hall of Fame in 1981 and the Carroll College Basketball Hall of Fame in 1982. He continued to play basketball throughout his four-plus decades at WSU, including in city and county leagues and with faculty, staff, and students at WSU three times a week in what was called "noonball." He commented to a colleague that the most enjoyable recognition received when he was elected to the National Academy of Sciences was when his basketball teammates from Colfax (small country town 15 miles north of Pullman) took him to lunch. An enthusiastic supporter of Washington State University athletics generally and of Men's Basketball more specifically, he served a five-year term as chair of the WSU Athletic Council and faculty representative to the PAC-10 Conference and National Collegiate Athletic Association.

The Department of Agricultural Chemistry, where Bud with his NIH Career Development Award was given a laboratory in 1964, resided administratively in the College of Agriculture but was located physically with the chemistry and biochemistry departments in the College of Sciences and Arts. Accordingly, Ryan was given a tenure track appointment as assistant agricultural chemist in the College of Agriculture and assistant professor of biochemistry in the College of Sciences and Arts starting with his arrival at WSU in 1964. He was promoted to associate agricultural chemist and associate professor of biochemistry in 1968 while still salaried by his NIH grant, and agricultural chemist and professor of biochemistry in 1972 after transferring seamlessly to salary provided by WSU. He served as chair of the Department of Agricultural Chemistry from 1977 to 1980.

The Department of Agricultural Chemistry was formed primarily for the purpose of obtaining data required for registration of minor-use pesticides (i.e., pesticides for use on minor crops with market size too small to attract investments by the chemical companies that owned and manufactured the products). Through multiple discussions Bud convinced Legault, and Legault convinced the dean of agriculture that the mission of the Department of Agricultural Chemistry would become increasingly less relevant, that the faculty doing applied research in the department should be replaced as they retired with faculty hired to do basic research in plant biochemistry, and that the department should be physically as well as administratively in the College of Agriculture. In 1980 Bud along with five plant biochemists hired to replace applied faculty were named fellows of the now famous Institute of Biological Chemistry (IBC) and moved to new space in the College of Agriculture and Home Economics, while retaining their academic appointments in the Department of Biochemistry, College of Sciences and Arts. Pappachan Kolattukudy was the first director of the IBC. Bud served as acting director of the IBC in 1989-1990. He was appointed Charlotte Y. Martin Distinguished Professor in 1991.

Bud maintained membership in a wide range of professional scientific societies and was a regular participant if not an invited symposium or plenary speaker at the annual meetings of these societies. He was also a regular invited participant in national and international meetings such as NATO, Gordon and Keystone workshops, conferences, and symposia and averaged six to seven invited addresses, named lectures, or symposium talks somewhere in the world each year for the last 10 years before his retirement in 1999, and served on several scientific advisory boards until his death. In 1990 he organized an international conference at WSU, Biotechnology: Practice and Promise, with speakers connected

by satellite communications from around the world. This was surely among the first satellite conferences conducted in the sciences. In 1994 he organized an Academy colloquium on Self Defense in Plants, and in 1996 he was an invited speaker at the Academy's annual meeting symposium on Frontiers in Plant Biology.

Bud did only one sabbatical leave during his 43 years at WSU, which was six months in 1981 in the Department of Biochemistry at the University of Washington, Seattle, followed by six months in 1982 with the Center for Biochemical and Biophysical Sciences in Medicine, Harvard Medical School, Boston. In May 1997 he returned to the Harvard Medical School as the Bert and Natalie Foundation Visiting Professor. He served in several leadership roles at WSU, including president of the WSU chapter of Phi Kappa Phi and chair of the faculty Association of Research Professors, both in 1985 and 1986, but tended to avoid appointments or election to committees and offices outside of WSU unless it was to chair or organize a symposium. Ted Farmer reminded me that Bud had a message taped to his telephone that read, "Just say no, unless you really want to."

With all the honors and acclaim Bud remained among the most humble and nicest men I have known. Anthony Cashmore shared that "in addition to his excellent science, his distinguishing feature was that I never heard anyone say anything bad about him" and Winslow Briggs noted that "one only heard great things about him, whether about his science or his remarkable humanity."[2] His senior technician, Greg Pearce, shared with me that every 10 years Bud would have an engraved plaque made for him with words to the effect "In Recognition of Outstanding Work," and he shared a portion of each cash prize with Greg.

I started my career at Washington State University in 1965, one year after Bud. Unbeknown to either of us, we lived only

a few blocks apart in Albany, California, from 1961 to 1964, during his time with the USDA Western Regional Research Laboratory and my time in graduate school in plant pathology at the University of California, Berkeley. I first met Bud in the late 1960s when we and our wives became part of a six-couple monthly bridge group that continued for some 20 years. Like me he enjoyed the social gatherings but only tolerated bridge. In addition to his passion for basketball, Bud was an avid golfer and loved to fish, going back to his days growing up in Montana. Not being inclined myself to these activities, we became best of friends through the monthly bridge gatherings plus times in his office or mine visiting mainly about science with the occasional sojourn into campus politics. Bud was the one to call me near sunrise (Pacific time) on an April morning in 1993 to inform me that I had just been elected to the National Academy of Sciences.

Bud and Pat Ryan had two daughters, Jamie and Janice, and two sons, Steven and Joseph Patrick. Jamie, Steven, and Janice were born in that order while Bud was in graduate school at Montana State University and Joe Pat was born shortly after the Ryan family moved to Pullman. Tragically Steven died in a scuba diving accident on Easter Sunday morning in 1976, and one week after the memorial service for Steve, Joe Pat at the age of 10 was killed in a car accident while accompanying a friend and his family. In their honor Bud and Pat set up the Steve and Joe Pat Memorial Fund at Carroll College in Helena, Montana. Bud is survived by his wife, Patricia, his two daughters, Jamie and Janice, and two granddaughters, Kymberly and Haleigh Thrall, daughters of Janice and Terry Thrall, who were very special to him.

In addition to his election to the National Academy of Sciences in 1986, he was recognized with the Steven Hale Prize in 1992 from the American Society of Plant Physiologists (now the American Society of Plant Biologists), the Kenneth

Spencer Award in 1993 from the American Chemical Society, The Silverstein-Simione Award in 1997 from the International Society of Chemical Ecology, and was named an honorary fellow of the American Academy of Microbiology in 2002. In 2005 he was awarded an honorary doctorate in science from Washington State University, only the third honorary doctorate awarded by WSU in the previous 50 years.

Bud died suddenly from a massive brain aneurysm. Dating back to 1957 when he published his first journal article, he published 289 peer-reviewed journal papers, book chapters and reviews, including 37 papers in PNAS. Starting in 1989 he was identified every year by the Institute of Scientific Information (ISI) as an original member of "Highly Cited Researchers," which includes less than 0.5 percent of all researchers in the world. ISI identified his 1973 article in the *Annual Review of Plant Physiology* as a Citation Classic in the agricultural and biological literature, his 1992 PNAS paper reporting the isolation and identification of systemin as "the most cited article in world literature in ecology/environmental science for the period 1990-1992, and a 1992 paper with Ted Farmer in *The Plant Cell* as the most cited Hot Paper in the agricultural sciences. The American Society of Plant Biologists listed one of his articles in *Plant Physiology* and two articles in The *Plant Cell* in the top 50 cited articles in the histories of the journals. He published 42 articles, including 13 of his 37 papers in *PNAS* after retirement. Through the efforts of his technician, Greg Pearce, and collaborators his publications have continued to appear in print after his death (2008). Roger Beachy probably summed Bud up best with the comment that "Bud…made contributions so great that it will be difficult to account for his impact for years to come."[2]

I AM INDEBTED TO GREGORY PEARCE, Bud Ryan's senior laboratory technician of more than 30 years, for assembling a file of information and publications used to write this memoir. I also thank Ted Farmer for suggestions and reflections added to this memoir and Anthony Cashmore for collecting reflections from colleagues in the Academy, some of which are quoted in this memoir

NOTES

1. Clarence "Bud" Ryan oral history. Interview by J. Kerssen, Mar. 18, 2004. Museum of History and Industry, Washington State University, Pullman. http://www.seattlehistory.org/col_transcript_tech.cfm.
2. Comments collected by Anthony Cashmore from colleagues of Ryan in Section 25 of the National Academy of Sciences and shared at his memorial service.
3. T. Farmer, G. Howe, G. Pearce, and A. Schaller. 2008. Bud Ryan obituary. *ASPBNews* 35:37-38.

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