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THE CENTER FOR PLANT AND MICROBIAL COMPLEX CARBOHYDRATES AT THE UNIVERSITY OF GEORGIA COMPLEX CARBOHYDRATE RESEARCH CENTER

Five-Year Report
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The Center for Plant and Microbial Complex Carbohydrates at the University of Georgia Complex Carbohydrate Research Center

1987-92 Five-Year Report and 1993-97 Renewal Application

OVERVIEW

1. **The CCRC Philosophy.**

The Complex Carbohydrate Research Center (CCRC) is the home of ten independent but complementary interdisciplinary research groups led by nine regular faculty and one adjunct faculty. The research of these groups represents a broad spectrum of interests, and they are involved in about 90 collaborations with their CCRC and UGA colleagues and with scientists at other institutions and companies in the U.S., Canada, Europe, Israel, and Japan.

The hallmark of the CCRC is the collaborative, interactive environment encouraged by its directors, faculty and long-term staff. Newcomers to the CCRC or short-term members soon learn that everyone benefits from this process. The team-oriented approach in carbohydrate science translates into the day-to-day generous giving of one's time and expertise to the work of others, whether it be in sharing specialized instrumentation, participating in the design of experiments and interpretation of data, providing service to scientists outside the CCRC, or joining collaborative projects. The CCRC is founded on the principle that the cross-fertilization of ideas and know-how leads to the synergistic advancement of science.

2. **The Role in the CCRC of the DOE-Funded Plant and Microbial Carbohydrate Center Grant (the "Grant").**

The Grant is the most important component of the CCRC's support. It was the first multi-investigator "center" grant awarded to the CCRC and remains the largest of the CCRC's grants. Awarding of the Grant convinced the University of Georgia (UGA) administration to construct a building for the CCRC, offer attractive start-up packages for CCRC faculty, provide matching funds for the CCRC's equipment purchases, and assist financially in the construction of an addition to the CCRC building when the building was only one year old. The Grant makes it possible for the CCRC to afford the staff that keeps the CCRC's extensive sophisticated equipment operating more efficiently and better than if the equipment were maintained by manufacturers' service contracts (which, if purchased, would utilize nearly all the funds in the Grant). The Grant supplements the low fees for analytical services for non-proprietary research that the CCRC provides to any scientist who requests assistance. The Grant has provided partial funding for many of the collaborative research projects that have helped to make the CCRC recognized throughout the world. The Grant has made it possible to invest in an innovative, high-risk, potentially high payoff computer-based analytical project that has generated exceptionally important results (see below). And, if this renewal application is approved, the UGA administration has agreed to support a plan to construct a second addition to the CCRC that would increase the building's space by about 40% and allow the CCRC to grow from its approved level of 10 tenure-track faculty to its long-held goal of 12 to 15 tenure-track faculty.

The partial support of the CCRC's infrastructure provided by the Grant made it easier for the animal carbohydrate scientists of the CCRC to win two multi-investigator grants of their own

-- an NIH Resource Center for Biomedical Carbohydrate Science and an NIH program project grant to characterize the structures of the carbohydrate portions of the glycoproteins of the AIDS virus and its physiological receptor. Those grants have led to success in applications for individual investigator grants. The Grant also allowed the CCRC to begin the development of a first-rate computer center for carbohydrate science, an effort that has since received substantial support from the Digital Equipment Corporation and UGA.

3. Plant and Animal Sciences in the CCRC.

The more rapid progress in the field of animal carbohydrate science as compared to plant and microbial carbohydrate science reflects the far greater resources available to support biomedical science. This has meant that, when the CCRC recruits faculty who specialize in analytical carbohydrate science (NMR spectroscopy, mass spectrometry, computational analysis), the best candidates study animal rather than plant or microbial carbohydrates. Nevertheless, and despite the imbalance that exists in most biochemistry, molecular biology, cell biology and developmental biology departments, because of the Grant, the balance of support and positions in the CCRC is about evenly divided between animal and plant science (see below). Indeed, the breadth of the CCRC faculty and the ability of the plant and microbial scientists to collaborate with and learn techniques from the animal carbohydrate scientists (and vice versa) greatly strengthens the plant and microbial science supported by the Grant. Moreover, plant carbohydrate scientists have played an integral role in the CCRC's development of the analytical technology that benefits all carbohydrate science, including chemical and NMR methods for sequencing complex carbohydrates, development of the Complex Carbohydrate Structural Database (CCSD) and its operating program CarbBank, development of the CARBOMASS computer program for assigning structures to mass spectral ions and mass spectral ions to carbohydrate structures, development of the use of artificial neural network technology for the analysis of spectra (see below), and the demonstration that oligosaccharides are regulatory molecules with hormone-like properties.

4. Challenges Facing the CCRC.

The CCRC, and thus the Plant and Microbial Carbohydrate Center, is not without problems. One of the problems stems in part from the very success of the CCRC as a premier center for training of carbohydrate scientists: we are having a difficult time keeping our key faculty and staff. Indeed, 1992 will see the departure of one of our senior faculty members. Richard Cummings has been attracted by financial largess and an endowed chair to the University of Oklahoma Medical School. Although we plan continued interactions with Richard after he moves to Oklahoma, nevertheless, we have been made acutely aware of our vulnerability, and this vulnerability has been accentuated by the hiring by industry of four of the CCRC's carbohydrate chemists by offering salaries that more than double those that the CCRC can provide. This exodus has benefits for industry and our country, but it presents a continual challenge to the CCRC to maintain the high quality of its endeavors.

Another problem the CCRC faces is shared by most science departments: the CCRC would benefit from applications for graduate study from a greater number of United States citizens. Towards that goal, the CCRC is planning to develop, within the UGA Biochemistry Department, a Ph.D. track in carbohydrate science and then apply for a training grant to support the training of a greater number of graduate students and postdoctoral research fellows in this field.

Another challenge is represented by the physical separation of the CCRC's members. Lack of space in the CCRC building necessitates 25% of the CCRC's members being located in the new Life Sciences Building (one mile from the CCRC building). The faculty and staff of the CCRC work hard to overcome the potentially harmful effects of this separation.

5. <u>CCRC Faculty</u>.

Four of the eight regular CCRC faculty (excluding Cummings) are in the plant sciences (Albersheim, Darvill, Hahn and Mohnen), and four are in the animal sciences (Meyer, Moremen, Pierce and Van Halbeek, although Meyer and Van Halbeek can also be considered analytical carbohydrate scientists). All of the faculty (except Moremen and Pierce who joined the CCRC in 1991) have made significant contributions to the Plant and Microbial Carbohydrate Center (see below). Moremen and Pierce are already acting as technical advisors to several projects in the Plant and Microbial Carbohydrate Center, which assures that they, too, will impact the research of the Center in the near future. The above tenure-track faculty are rostered 80% in the CCRC and 20% in an academic department. Eriksson, a fifth plant scientist, is a full member of the CCRC but is fully rostered in the Biochemistry Department.

The CCRC has three non-tenure-track faculty: David Smith, Research Biochemist in the Biochemistry Department (Research Biochemist is equivalent to a non-tenure track full Professor); Russell Carlson, Technical Director of the Plant and Microbial Carbohydrate Center, who is an Associate Research Biochemist in the Biochemistry Department (equivalent to Associate Professor); and Assistant Research Biochemist Marcia Kieliszewski. Carlson and Kieliszewski have independent plant science research programs; each holds an independent competitive research grant. Smith has an independent research program in animal carbohydrate science. The CCRC is recruiting a specialist in mass spectrometry; two individuals have been approved for appointment by the CCRC and Biochemistry Department. An offer of a tenure-track assistant professorship has been presented to the top candidate; he has informally accepted the offer. The CCRC has one remaining assured tenure-track faculty position to fill and has between three and five additional tenure-track faculty positions tentatively allotted. However, space has become the limiting factor to growth (see below).

6. <u>CCRC Staff</u>.

The CCRC is blessed with a group of highly professional, exceptionally competent, and unusually loyal staff led by Administrative Manager Rosemary Nuri, Business Manager Lydia Snyder, Building Manager Carl Bergmann, and Chemical Instrumentation Manager Dennis Warrenfeltz. These individuals, their staffs, and the senior research staff are a dedicated and hardworking group. They are the individuals who make the CCRC a productive unit; their importance to the success of the Plant and Microbial Carbohydrate Center should not be underestimated.

7. <u>The Future</u>.

The CCRC wishes to continue to grow in numbers of faculty, staff, and students, and in terms of sophisticated equipment and facilities. Funds for a super computer have been applied for. The top mass spectrometrist candidate is talking about the acquisition of a four magnetic sector instrument, the nuclear magnetic resonance spectroscopists are planning for a 750-MHz spectrometer (when it becomes available) as well as solid sample analytical capabilities. We are all talking about adding one or two chemical synthesis groups and of expanding our abilities in plant and animal sciences. Most of these goals cannot be realized without additional space. A formal proposal has been made to UGA President Charles Knapp to build an addition to the CCRC building that would add about 40% to the existing space. The request has been approved in principle, and we are optimistic that it will be funded. Renewal of this Grant would be a major positive influence on whether the administration decides to fund the addition.

8. **Industrial Funds for Basic Research.**

UGA provides the CCRC with nine-month salaries of the tenure-track faculty, matching funds for equipment, the building, and maintenance of the building and its grounds. UGA does not provide operational funds or salaries for any of the staff. Since the CCRC is totally dependent on competitive grant funds for its operations, it would be desirable to diversify its funding base. Industrial funds are the most obvious alternative. However, even though the CCRC has been enormously helpful to a wide variety of corporations (see Appendix 1), the CCRC has received very little financial support for its basic research from these corporations.

The CCRC faculty and staff, given their other responsibilities, do not have the time and energy to pursue, negotiate, and formalize research and development alliances with a variety of industries. The CCRC, therefore, established relationships with two entrepreneurs who are both interested in founding corporations to interface the basic research of the CCRC with the applied goals of industry. One corporation, ANN Technology Inc., has been formed to take advantage of the CCRC discovery of the use of Artificial Neural Networks in identifying molecular structures (see below) and managing analytical data relating to molecules; a patent to protect the proprietary aspects of this discovery has been applied for. ANN Technology negotiated with the University of Georgia Research Foundation (UGARF) for the right to develop the CCRC discovery in exchange for support of the CCRC's basic computer science research and for royalties to UGARF on ANN Technology products. ANN Technology is in the process of raising funds to support this venture.

Digital Equipment Corporation (DEC) was so optimistic about the value of the CCRC's artificial neural network technology to increased sales of DEC computer workstations that they provided the CCRC with computers and computer programs worth $600,000 plus the salary of a postdoctoral research associate to develop further the scientific basis of the discovery. DEC has transferred to ANN Technology their rights to commercialize the CCRC neural network technology, and DEC has indicated a willingness to provide additional support for this project.

The second entrepreneur will form a corporation to develop into marketable products the basic research discoveries of the CCRC (outside the computer science area). Like ANN Technology, this interface corporation will have its own research, development, and production facilities. Although this corporation has yet to be formed, we are optimistic that it will be and that an arrangement with the CCRC and UGARF similar to the arrangements negotiated with ANN Technology will result.

COLLABORATIVE RESEARCH HIGHLIGHTS

This application contains a series of appendices that document the extent and breadth of the Plant and Microbial Carbohydrate Center's contributions to collaborative research, training, and service. Several collaborative research projects that have received postdoctoral research associate support from the Grant are highlighted below, as these projects are particularly illustrative of the wide-ranging collaborations that have evolved as a result of this Grant and the quality of the science that the Grant enables. Each of the projects receives support from other grants. In each of the four sections below, we include the scientific background of the projects, our accomplishments to date, and our current and proposed activities.

1. Oligocalacturonide Oligosaccharins.

[**Collaborators:** Cervone and De Lorenzo, University of Rome; Guern, Mathieu, A. Kondorosi and E. Kondorosi, CNRS, Gif sur Yvette, France; Benhamu, Laval University, Quebec; Fluhr, Weizmann Institute, Rehovot, Israel. **CCRC participants:** Faculty members Albersheim, Darvill, Hahn and Mohnen; postdoctoral research associates Bergmann and Eidsness; graduate students Alba, Gelineo, Ito, Marfà, Ridley, Spiro, and Wu; staff biologist Eberhard and staff chemist O'Neill. Note: Alba, Ito, Wu, and Eberhard have not received funds from the Grant.]

Homogalacturonan, the pectic polysaccharide composed of partially methylesterified 4-linked α-D-galactosyluronic acid residues, is thought to be an important structural component of the primary cell walls of higher plants. The structural role of homogalacturonan may be enhanced by its ability to form cross-linked gels in the presence of calcium ions [1,2]. CCRC personnel and their collaborators have been centrally involved in discovering that deesterification and partial depolymerization of homogalacturonan gives rise to α-1,4-oligo-D-galacturonides that exhibit a variety of regulatory effects in plants including elicitation of defense responses, regulation of growth and development, and induction of rapid responses at the cell surface [3-5]. (Note: Methods developed for the purification of oligogalacturonides are described under graduate student Mark Spiro's project in Appendix 2.)

Elicitation of plant defense responses by oligogalacturonides. Many microbial phytopathogens secrete *endo*polygalacturonases (EPGs) and pectate lyases that release oligogalacturonide fragments from plant cell walls [6]. Depending on the plant, the released oligogalacturonides elicit various defense responses including the accumulation of phytoalexins [6-10], the induction of *endo*-β-1,3-glucanases that degrade fungal cell walls, the induction of chitinases and lysozymes that degrade fungal and bacterial cell walls [11,12], and the increased deposition of lignin, which can serve as a physical barrier to fungal penetration [13,14]. β-Glucan, chitin, and chitosan fragments, generated by the *endo*-β-1,3-glucanases and chitinases, are all elicitors of phytoalexins. Moreover, oligogalacturonide and oligo-β-glucoside elicitors act synergistically, that is, the concentrations required to elicit phytoalexins when both elicitors are present are less than the concentration required for each to elicit phytoalexins individually [11,15].

Oligogalacturonides are also involved when plants, in response to insect infections, produce proteins that inhibit insect-secreted proteases; this effect of oligogalacturonides is localized in the cells surrounding the point of attack [16-18]. Oligogalacturonides can also induce the production of extracellular isoperoxidases [14] and H_2O_2 in suspension-cultured cells [19]; it has been suggested that oligogalacturonide-induced active oxygen metabolism plays a role in early interactions of pathogenic bacteria and plants [20]. The oxidative burst induced in plant cells by oligogalacturonides is strikingly similar to the production of activated oxygen species by animal cells [19,20]. In animal cells, activated oxygen species cause lipid peroxidation that can result in membrane damage and localized tissue inflammation [21].

The size range of oligogalacturonides that activate defense responses is usually quite narrow. For example, oligogalacturonides with DPs between 10 and 15 are generally required to elicit plant defense responses. However, oligogalacturonides with DPs between 2 and 20 induce proteinase inhibitor production in tomato. Although the reason for the frequently observed size dependence of the response to oligogalacturonides is not known, this requirement suggests that oligogalacturonides need ten or more contiguous α-1,4-linked galactosyluronic acid residues in order to assume a biologically active solution conformation.

All dicotyledonous plants examined possess a polygalacturonase-inhibiting protein (PGIP) with broad specificity against fungal EPGs [22,23]. In the presence of excess PGIP, the rate at which fungal EPGs digest polygalacturonic acid (PGA) *in vitro* is reduced ~99.7%, resulting in the accumulation of biologically active oligogalacturonides with DPs > 10 [24]. The larger oligogalacturonides have half-lives of hours rather than minutes because of the reduced EPG activity. It thus appears that many plants have evolved a mechanism to regulate the activity of pathogen-produced enzymes and thereby increase the production of elicitor-active oligogalacturonides.

Hypersensitive resistance (HR) to microbial infection is characterized by plant cell necrosis at the sites of attempted infection. Application of EPGs to plant tissues that possess PGIP results, in at least some cases, in necrosis of the plant cells exposed to EPG. It is particularly interesting that fungal EPGs, in the presence of excess PGIP, are no longer able to macerate plant tissue [25]. The possibility that the oligogalacturonide products of EPG play a role in localized cell necrosis has been considered [26,27], but the evidence remains inconclusive [25]. We have not been able to obtain evidence that bioactive oligogalacturonides cause necrosis. Another possible and potentially important explanation for the necrosis-inducing effect of EPG is that the PGIP-EPG complex is itself a functioning receptor-ligand complex that signals the plant to undergo an HR response. EPG that is bound to PGIP retains 0.3% of its catalytic activity. Therefore, the active site of EPG must not be the same as those parts of EPG that interact with PGIP. Thus, we should be able to use mutagenesis to separate the catalytic and PGIP-binding activities of EPG.

We are in the process of testing the hypothesis that the PGIP-EPG complex functions as a receptor-ligand complex by attempting to generate *Fusarium solani* mutants that produce EPGs that retain their catalytic activity but fail to bind to (are inhibited by) PGIP, and to produce other *F. solani* mutants that produce EPGs that retain their ability to bind to PGIP but are no longer able to hydrolyze polygalacturonic acid. Our efforts to isolate such mutants are made easier by the fact that *F. solani* has only a single EPG gene (unpublished results of our collaborators at the University of Rome). We are attempting to express the cloned *F. solani* EPG gene in yeast and then use site-specific and/or insertion mutagenesis technologies to generate the desired mutants. We are also attempting to screen for the mutants in *F. solani* colonies derived from spores subjected to chemical mutagenesis. The two types of EPG-modified mutants should allow us to determine whether it is PGIP recognition of EPG or of the oligogalacturonide products of EPG that causes the HR-like response when plant tissues are exposed to EPG. The answer to this question will improve our understanding of the molecular basis of plant-microbe interactions.

The PGIP from true beans (*Phaseolus vulgaris*) [28] and the EPG from its fungal pathogen *F. solani* (manuscript in preparation) have been cloned and sequenced by postdoctoral research associate Carl Bergmann in collaboration with Felice Cervone and Giulia De Lorenzo and their coworkers at the University of Rome. Using antibodies and DNA probes specific for the PGIP and its mRNA, respectively, we (Bergmann and graduate student Yuki Ito) and our collaborators (Cervone and De Lorenzo, and Nicole Benhamu of Laval University) have demonstrated that substantial constitutive levels of PGIP are elevated some ten-fold in those plant cells neighboring the hyphae of an infecting fungus. We have shown that the levels of PGIP are also elevated by wounding. Efforts are under way to use transgenic technology to determine if the expression of higher constitutive levels of PGIP endows the plants with a greater ability to resist fungal infection.

Induction of plant growth and development by oligogalacturonides. (Note: The work described in the following two paragraphs was not directly supported by the Grant, but the results are pertinent to the work described in the remainder of this section, which did receive direct support from the Grant.) The ability of oligogalacturonides to regulate plant morphogenesis is another biological activity of this plant cell wall-derived oligosaccharin. Morphogenesis is the development of form and specialized organs such as leaves, roots, and flowers. Most undifferentiated plant cells are totipotent, that is, each cell has the potential to develop into a complete plant. The ability of plant cells to differentiate and develop into specialized organs is controlled by a number of factors including hormones, light, and temperature [29,30].

A tobacco thin cell-layer (TCL) explant bioassay [31,32] has been used to study the effects of phytohormones and plant cell wall-derived fragments on plant morphogenesis [32,33]. TCL explants are thin strips of floral stem tissue containing five to ten cell layers that, when incubated for 24 days on culture medium containing the phytohormones auxin and cytokinin, form flowers, vegetative shoots, or roots [32]. The particular organ type that forms depends on the concentrations of auxin and cytokinin in the culture medium. Oligogalacturonides with DPs 10 to 15 can induce flower formation and inhibit root formation in tobacco TCLs ([32,33] and unpublished results of the authors). Oligogalacturonides with DPs between 12 and 14 are the most active (50% active at 0.5 µg/ml; [32]). Oligogalacturonides of the same size range can also inhibit the formation of auxin-induced roots in tobacco leaf disks (collaboration with Cervone *et al.*).

Oligogalacturonides induce rapid responses at the plasma membrane and cell surface. Oligogalacturonides induce a variety of rapid responses at the plant cell surface. For example, suspension-cultured soybean cells produce H_2O_2 within 5 min of exposure to oligogalacturonide mixtures [19], and tomato leaf mesophyll cells [34] undergo depolarization of membrane potential within 5 min of exposure to relatively high concentrations (1 mg/ml) of oligogalacturonide mixtures (DPs 1 to 7 and DPs 10 to 20). We (postdoctoral research associate Alan Koller, graduate student Mark Spiro, and staff chemist Malcolm O'Neill) and our collaborators (Yves Mathieu and Jean Guern of the CNRS at Gif sur Yvette, France) have demonstrated that lower concentrations (10 µg/ml) of size-specific oligogalacturonides (DPs 12 to 15) induce, within 5 min, a transient stimulation of K^+ efflux, Ca^{2+} influx, cytoplasm acidification, and depolarization of the plasma membrane in suspension-cultured tobacco cells [35]. Size-heterogeneous mixtures of oligomannuronides and oligoguluronides are ~400-fold less effective in inducing K^+ efflux. Furthermore, treatment of tobacco cells with an oligoguluronide preparation did not result in a decrease in the apparent Ca^{2+} concentration of the incubation medium. These observations provide evidence that oligogalacturonides at low concentrations can induce rapid and specific responses at the plant cell surface.

Size-specific oligogalacturonides (DPs 14 and 15) have been shown to enhance the *in vitro* phosphorylation of a 34 kd protein associated with purified plasma membranes isolated from potato and tomato leaves [36]. The phosphorylation of the 34 kd protein does not appear to be directly related to the induction of proteinase inhibitor accumulation in tomato, as oligogalacturonides with DPs < 14 induce the production of these inhibitors but do not enhance *in vitro* phosphorylation of the 34 kd protein [36]. Oligoguluronide preparations, although less effective than oligogalacturonides, induce proteinase inhibitor activity and *in vitro* phosphorylation of the 34 kd protein in tomato [36]. However, the DP and purity of the active oligoguluronides used in the bioassay were not reported.

The bioactive oligogalacturonides and auxin have the opposite effects on a number of physiological responses (see, for example, [32,33,37-39]). Several types of evidence suggest that the oligogalacturonides are not directly interacting with or affecting the rate of uptake or half-life of auxin. On the other hand, indirect evidence suggests that the differential effects of the oligogalacturonides and auxin may occur at the level of the plasma membrane. We have established a collaboration between Jean Guern and Yves Mathieu and their coworkers of the CNRS, and Michael Hahn, his graduate student Rob Alba, Malcolm O'Neill, and the PIs of the CCRC to identify, in suspension-cultured tobacco (CNRS) and soybean (CCRC) cells, membrane proteins whose degree of phosphorylation is "oppositely" affected by oligogalacturonides and auxin. We hypothesize that differential phosphorylation of such a protein or proteins is an early step in the signal transduction pathway by which these hormones regulate gene expression.

Divalent cations, most notably calcium, are known to play a role in the growth and development of plants [40,41]. Calcium or other divalent cations are also required for at least some of the observed bioactivities of oligogalacturonides [35]. For example, depletion of calcium in the growth medium of tobacco cells all but abolishes oligogalacturonide-stimulated K^+ efflux. Mg^{2+} and Mn^{2+} are effective, but somewhat less so than Ca^{2+}, in restoring the K^+ efflux [35]. Oligogalacturonides, but not oligoguluronides, cause a transient decrease in the Ca^{2+} concentration of the growth medium. The results obtained suggest that bioactive oligogalacturonides cause Ca^{2+} to be taken up by the cells and that Ca^{2+} influx results in the decrease in the Ca^{2+} concentration of the medium.

Oligogalacturonides and oligoguluronides bind Ca^{2+} [42-45]. Oligogalacturonides with a DP > 10 are believed to form intermolecular complexes (dimers) in the presence of Ca^{2+} [42-44], which has led to the suggestion that an interaction between oligogalacturonide-Ca^{2+} complexes and plant cells could initiate localized physiological and morphological responses [5,46]. It is not known how such complexes could interact with the binding site of a receptor, if a receptor is, in fact, directly involved in the action of oligogalacturonides. Furthermore, oligogalacturonides with DPs > 15, which also form intermolecular complexes with calcium [42,44], are generally not bioactive, while oligogalacturonides with DPs 2 to 9, which do not form intermolecular Ca^{2+} complexes [44], are occasionally bioactive. Oligoguluronides (DPs 8 to 28), but not oligomannuronides, form intermolecular complexes with Ca^{2+} [42,44]. However, oligoguluronides, like oligomannuronides, do not appear to elicit rapid membrane changes in plants [35]. Thus, it remains to be determined whether the rapid membrane response requirement for divalent cations is caused either by the effect of the cations on oligogalacturonide structure or by the requirement for the cations for some other step in the signal path.

There is evidence that at least some plant cells become rapidly desensitized to oligogalacturonides. A correlation has been reported between prolonged H^+ influx, K^+ efflux, and the bacteria-induced HR in tobacco cells [27,47]. The bacteria-induced HR is inhibited by treating tobacco cells with pectate lyase (PL) and PL-digested PGA, a treatment that also results in a transient rather than a prolonged H^+/K^+ response [27]. These transient ion fluxes are not detected after a second addition of PL or PL-digested PGA. Pre-treatment of tobacco leaf disks with PL-digested PGA prior to inoculation with *Pseudomonas syringae* reduces but does not block the prolonged H^+ influx (and presumably the K^+ efflux) induced by HR-causing bacteria. However, the HR is inhibited by addition of PL-digested PGA [27]. Tobacco cells treated with bioactive oligogalacturonides to induce K^+ efflux also do not respond to a second treatment with the same fragments [27,35]. Furthermore, oligogalacturonide-induced ethylene biosynthesis in cultured pear cells results in a time-dependent decrease in the ability of the cells to produce ethylene in response to a second elicitor treatment [48]. All of these desensitization effects could be explained by internalization of plasma membrane-localized oligogalacturonide receptors [49].

We have begun to study the question of the possible internalization of the bioactive oligogalacturonides in an extension of our collaboration with the Guern team. Radiolabeled, size-specific oligogalacturonides have been generated by sodium borotritiide reduction.

Roughly 20% of oligogalacturonides with a DP = 13, when mixed with tobacco cells, bind rapidly and strongly to the walls of those cells. We have also found that the tobacco cells secrete a polygalacturonase into their own cell walls that more effectively cleaves bioactive oligogalacturonides (DPs = 13) than bio-inactive oligogalacturonides (DP = 8 and DP = 18). We have obtained no evidence that the bioactive oligogalacturonides are taken into the cells, although we cannot rule out that < 2% of the oligogalacturonides are internalized. In an effort to determine whether oligogalacturonide receptors are present in plant cells, graduate student Mark Spiro and the PIs are attempting to use a ^{125}I-labeled tyramine derivative of tridecagalacturonide to identify binding proteins (see Appendix 2). Graduate student Brent Ridley, working with Debra Mohnen and the PIs, is attempting to synthesize a biotin derivative of tridecagalacturonide in order to use its high affinity for avidin for selecting a binding protein gene cloned in a system that expresses the encoded proteins extracellularly (see Appendix 2).

Graduate student Ivana Gelineo is using the ability of the bioactive oligogalacturonides to evaluate the hypothesis of our collaborator Robert Fluhr (Weizmann Institute, Rehovot, Israel) that the general perturbation of a cell's membranes causes abnormal protein folding and abnormal *N*-linked carbohydrate side chain synthesis, resulting in increased total levels of a BiP-like chaperone that binds to incorrectly folded proteins while depleting the level of the unbound chaperone. In this hypothesis, it is the reduced concentration of unbound chaperone that triggers resistance responses in plant cells, including the HR.

Fluhr has obtained evidence of increased accumulation of a BiP-like chaperone in cells undergoing a defense response. Fluhr tested his hypothesis that abnormal glycoprotein side chains lead to activation of defense responses by treating cells with several chemicals known to alter carbohydrate side chain synthesis; each of these chemicals increased the total chaperone levels, and each activated known defense responses (unpublished results).

To further test the hypothesis that elicitors alter glycoprotein side chain synthesis, Fluhr has introduced into tobacco plants a chloramphenicol acetyl transferase (CAT) gene containing a prepeptide signal sequence that causes the CAT protein to be *N*-glycosylated and secreted into the cell wall. Graduate student Gelineo and the PIs are trying to determine if treating the transgenic tobacco cells with agents that disturb the cell's membranes or that cause an HR response (examples are oligogalacturonides, *endo*-β-1,4-xylanase, and EPG) also alter the structure of the *N*-linked oligosaccharide attached to the CAT protein. In other words, we are attempting to use carbohydrate structure analysis to assess the effect of these bioactive molecules on the activities of the endoplasmic reticulum- and Golgi-located *N*-linked carbohydrate synthesis and processing enzymes. Such studies of the effects of oligogalacturonide-induced membrane responses will increase our understanding of the first steps in the oligogalacturonide-induced signal transduction pathways.

Are bioactive oligogalacturonides present *in vivo*? Detecting free bioactive oligogalacturonides in plants would provide additional evidence that these fragments play a role *in vivo*. Preliminary evidence has been obtained that bioactive oligogalacturonides are present in the growth medium of suspension-cultured sycamore cells (unpublished results of graduate students Victoria Marfà and Mark Spiro and the PIs). We propose to confirm these observations and to ascertain whether bioactive oligogalacturonides exist in the walls or cells of intact plants. The possibility that bioactive oligogalacturonides exist in plant tissues is strengthened by the demonstration that a number of different pectic-degrading enzymes present in plant tissues have the potential to generate oligogalacturonides by deesterification of pectic methyl esters and cleavage of homogalacturonides. Postdoctoral research associate Marly Eidsness, working with the PIs, is attempting to clone a polygalacturonase that she demonstrated is present in the flower stems of tobacco, which is the source of the tobacco TCL explants that oligogalacturonides induce to flower. Graduate student Yeong-Tong Wu, with assistance from Russell Pressey (of the USDA's Richard Russell Research Center in Athens) and postdoctoral research associate Eidsness, is attempting to clone the pectin methylesterase he has demonstrated to be present in the same tissue.

Finally, Jean Guern and his colleagues have found that bioactive oligogalacturonides have dramatic effects on legume root hair plasma membranes (personal communication). This suggests that *Rhizobium* species, when they infect roots, may secrete an EPG that generates bioactive oligogalacturonides. Others have tried to demonstrate the ability of *Rhizobium* species to secrete this enzyme without success, but there is new evidence of a gene encoding an EPG in *Agrobacterium tumafaciens* [50,51], a member of the *Rhizobiaceae* that is very closely related to *Rhizobium meliloti*. Marly Eidsness and the PIs, in collaboration with Adam Kondorosi and his colleagues (of the CNRS-ISV in Gif sur Yvette, France) are attempting to identify the *R. meliloti* EPG gene and, if it is present, to express the gene and then to generate *R. meliloti* isolates that lack the functioning gene. Once we have generated an EPG$^-$ *R. meliloti* mutant, Kondorosi will determine whether it retains its normal ability to infect alfalfa root hairs.

The studies of oligogalacturonides and their biological effects is perhaps the best example of how the Grant enables the plant and microbial carbohydrate scientists of the CCRC to form, with scientists located in a variety of institutions, in-depth collaborations aimed at solving fundamental problems involving oligosaccharins.

2. <u>Development of a Computer-Based Method for Determining the Structure of Complex Carbohydrates</u>.

[Internal CCRC/UGA collaboration between the groups of faculty members Albersheim, Darvill, Meyer and Van Halbeek, including senior staff scientists Radomski, Sellers, Thomsen and York, and graduate student Hansen (UGA Artificial Intelligence Program). Note: Radomski has not received funds from the Grant.]

Roughly 80% of asparagine-linked oligosaccharides that are being structurally characterized have had their structures delineated at an earlier date, but analysts are unaware of this fact, as they have no way to know the identity of the oligosaccharides they are characterizing until they delineate the complete structures of the oligosaccharides and then compare those structures to the structures listed in the Complex Carbohydrate Structural Database. To delineate the structure of a complex carbohydrate can take anywhere from a week to six months of a skilled carbohydrate chemist's time and extensive use of expensive instruments. We reasoned that one of the most important contributions the CCRC could make to carbohydrate science would be to develop a method to reduce or eliminate these duplicate efforts and their associated expense. We further reasoned that such a project could best be undertaken by a center like the CCRC with its broad range of expertise, sophisticated instrumentation, and collaborative, interactive environment. Moreover, since Dr. Robert Rabson (the DOE administrator of the Grant) encouraged the PIs to invest some funds in risky, potentially high pay-off research, we decided to make a limited investment in a project that, if successful, would eliminate much of the duplication of structural studies. The results so far portend far greater rewards than we had imagined, for the discoveries emanating from this project show promise to increase the efficiency of a variety of analytical endeavors carried out in non-academic and academic laboratories.

We decided to try to develop a method to identify complex carbohydrates from their one-dimensional (1-D) ^{1}H-NMR spectra, as these spectra require only about 10 nmols of sample. The initial vision, formulated in 1985, was to use pattern recognition techniques to "recognize" molecular spectra. We knew that pattern recognition had been developed to a rather sophisticated level by the needs of the military, and Dr. Charles Bender, an expert in this field, had just joined UGA as head of UGA's Advanced Computational Methods Center. Bender encouraged us to begin these studies and, in 1986, provided some of the initial funding for the endeavor. We hired postdoctoral research associate Jan Thomsen to initiate the project. It was Thomsen who decided to use an artificial neural network program (rather than the more classical pattern recognition program with which Bender was expert) in an attempt to recognize spectra of carbohydrates. Jan spent about three-fourths of his postdoctoral year (1987) writing the CCRC's first artificial neural network program. It was at about this time that Bernd Meyer joined the CCRC faculty with his extensive computer experience gained while writing the force field-based GESA program that predicts the favored solution conformations of oligosaccharides. Meyer and Thomsen formed a close collaboration; it was Meyer who convinced Thomsen to apply his neural network program to the identification of the 1-D ^{1}H-NMR spectra of simple alditols (reduced monosaccharides); the effort was a success: the network could be trained to recognize the different alditols [52].

We hired information systems manager Jeff Sellers to replace Thomsen when he returned to Denmark. Meyer took over leadership of the project and attracted graduate student Torben Hansen (UGA Artificial Intelligence Program) to work with them, in collaboration with William York and the PIs. The ability of software-emulated neural networks to distinguish the mass spectra of partially methylated alditol acetates (PMAAs) (that had been prepared for studies of plant cell wall xyloglucans) was demonstrated next [53]. Indeed, the neural network could even be trained to distinguish the mass spectra of the PMAAs of stereoisomers, something even the most accomplished chemists could not do. This was perhaps the first of many times that we found neural networks can recognize spectra far better than we had expected [53].

We next demonstrated that neural networks could be trained to distinguish the 1-D ^{1}H-NMR spectra of a closely related and structurally redundant set of xyloglucan oligosaccharides containing up to 20 glycosyl residues. These results, which were published in *Science* [54], have increased interest in this technology. These studies included experiments to establish that neural networks can deal with the normal imprecisions of ^{1}H-NMR spectra, including those resulting from differences in chemical shifts due to concentration or temperature variations, different signal-to-noise (S/N) ratios, variable absolute signal intensities, and different line widths. Another very important feature of neural networks, which became apparent from these studies, is their ability to identify the spectra of those molecules in the database that are most closely related to the molecule from which the unknown ^{1}H-NMR spectrum being presented to the neural network was obtained, when the spectrum of the unknown molecule had not been included in the neural net training set.

Our experiences with neural networks were convincing us that they could be effective for structural analysis under normal laboratory conditions. We had established that an artificial neural network can distinguish between the ^{1}H-NMR spectra of oligosaccharides that differ by only one glycosyl residue out of 20. Since the architecture of the neural network used had not been optimized and since we had not yet met any significant obstacles, we realized that we would be able to find ways to train artificial neural networks to recognize the ^{1}H-NMR spectra of most complex carbohydrates. These very productive early results led us to begin a major effort to develop the use of neural networks to identify the ^{1}H-NMR spectra of the asparagine-linked (*N*-linked) oligosaccharide side chains of glycoproteins.

***N*-linked oligosaccharides.** Meyer's postdoctoral research associate Jan Radomski obtained 107 spectra of *N*-linked oligosaccharide side chains of glycoproteins from Van Halbeek's collection of ~500 such spectra. Ninety-five of the spectra came from different molecules, while 12 spectra were duplicates generated from *N*-linked oligosaccharides attached to different positions of either the same or different proteins. The neural network was asked to identify the chemical structure of each carbohydrate chain when its spectrum was presented to the input layer of the neural network. To test whether the neural network could do this, the set of 107 spectra was divided into two sets of about equal size. The neural network was trained with about 53 unique spectra in about 10 hours of CPU time on a DECsystem 5500. The trained neural network was able to recognize each of the 12 spectra for which duplicates had been included in the training set. The other spectra of the test set were not known to the neural network, but the neural network could identify close structural relatives. For example, when a spectrum of a tetraantennary glycopeptide with three fucosyl residues at the non-reducing termini was presented, the neural network identified, as the closest relative, the same tetraantennary structure with a different point of substitution of a fucosyl residue and a different peptide. In another instance, the presentation of the spectrum of a *mixture* of two biantennary *N*-linked oligosaccharides (one containing a 2,3-linked sialic acid and one with a 2,6-linked sialic acid) gave, as the closest relative, the spectrum of the same biantennary structure with the combined sialylation pattern of the two compounds but lacking one of the two glucosamine residues in the core region. Although the ^{1}H-NMR spectra used for this work were of varying quality, they were perfectly usable by the neural network. Therefore, we believe that, with sufficient computer power, this approach can be used to train a neural network to identify all 1-D ^{1}H-NMR spectra of *N*-linked oligosaccharide side chains.

The ability of neural networks to identify the spectra of the molecules most closely related to the spectra of molecules not included in the database is very useful, but it is not, by itself, sufficient to identify the structures of new molecules. However, the structure of molecules not included in the database could often be deduced from this "closest relative" information if the investigator also knew the building block composition of the polysaccharide (i.e., the identities of the component glycosyl residues together with their points of attachment to neighboring glycosyl residues). An example of a building block would be a 3- and 6-linked mannosyl residue that is attached though an α-1,4-linkage to another glycosyl residue.

Realizing that knowledge of closely related structures together with the building block composition of oligosaccharides might allow us to use neural networks to determine the structures of oligosaccharides that had not been characterized previously, Meyer and Radomski investigated whether neural networks could be trained to recognize the building blocks of the oligosaccharides from their 1-D ^{1}H-NMR spectra. A neural network was successfully trained that had 57 target neurons corresponding to 57 different building blocks. In preliminary results, the trained neural network can correctly identify ~90% of the test substructures in the oligosaccharides presented to it. Those building blocks that were missed resulted, as far as could be ascertained, from the failure to include in the training set a sufficient number of examples of oligosaccharide spectra containing those building blocks. In other words, the network needs more examples to be fully trained. We anticipate that the network can be trained to recognize all building blocks when a larger, more diverse training set is available. No other rapid analytical procedure can simultaneously provide the substructures (building blocks) and anomeric configurations of oligosaccharides.

Radomski and Meyer have recently discovered a new attribute of neural networks of great importance. They have found that artificial neural networks can recognize the spectra of samples so dilute that the scientist, looking at the 1-D ^{1}H-NMR spectrum, cannot see the spectrum over the background noise. Thus, neural networks can identify the oligosaccharide side chains of glycoproteins with ^{1}H-NMR spectra obtained with from 0.01 to 1 μg of carbohydrate sample; the exact limit of this procedure has not yet been ascertained. This method may require for structural analysis as little as 100 times less sample than the Kobata method. (The laborious but highly effective and widely used Kobata method, based on sequential glycosidase digestion and gel-permeation chromatographies of Radiolabeled oligosaccharides, is the most sensitive method now available for sequencing the oligosaccharide side chains of glycoproteins. Akira Kobata is so impressed by the neural network results that he has offered to be the PI of a collaborative Human Frontiers Science Program grant proposal that will combine his laboratory's ability to separate oligosaccharides by serial lectin chromatography with the CCRC's application of combined 1-D ^{1}H-NMR and neural network analysis.)

The research into the use of neural networks to recognize 1-D ^{1}H-NMR and mass spectra is continuing with funds from this grant as well as other grants including a DOE grant to Darvill (one of the Grant's PIs) for studies of plant cell wall structures (DE-FG09-85ER13426). The major research goals of our impending studies include: (i) optimization of a neural network structure so that it will be applicable to a large number of ^{1}H-NMR spectra of complex molecules; (ii) determination of the factors limiting the use of neural networks in recognizing ^{1}H-NMR spectra; (iii) optimization of neural networks for substructure elucidation and characterization of unknown structures; and (iv) using artificial neural networks to extract new, useful parameters from spectra, including deciphering the 3-dimensional structures of molecules from their 1-D and 2-D NMR spectra.

We conclude that computer-based neural networks can form the core of a pattern recognition system for complex spectra of all types obtained from all types of molecules. Unlike traditional rule-based expert systems, neural networks discriminate between spectra without requiring the researcher to "hard-code" a set of rules. Teaching new spectra to the network simply involves adding the new spectra to the learning set and repeating the learning process. Furthermore, neural network-based database search routines are likely to replace those search methods currently used, as neural network pattern-matching is more sensitive, more versatile, faster, and more tolerant of experimental variables that occur during data acquisition.

Our work is limited now by computer time and manpower. We have great need for a massively parallel computer with the ability to allow us to optimize the neural network architecture for spectral recognition and to train large data sets. However, even our present results have enabled UGARF to apply for a patent on the use of artificial neural networks to recognize spectra and an entrepreneurial company (ANN Technology) to be organized that will develop neural network-based products for the pharmaceutical, chemical, agrochemical, food processing, and biotechnology industries, and that are likely to be used in all laboratories that analyze the structures of molecules.

3. Complex Carbohydrates have Important Functions in Rhizobium-Legume Symbioses.

[**Collaborators**: Brewin, John Innes Research Institute, Norwich, England; Hollingsworth, Michigan State University; Kondorosi and Putnoky, CNRS, Gif sur Yvette, France and Hungarian Academy of Sciences, Szeged; Long, Stanford University; Noel, Marquette University; Spaink and Lugtenberg, University of Leiden; Stacey, University of Tennessee; postdoctoral research associate Kannenberg (with Brewin) and graduate student Atkinson (with Long). **CCRC participants**: Faculty members Albersheim, Darvill, and Carlson; postdoctoral research associates Bhat, Glushka, and Puvanesarajah; senior staff member York; graduate student Reuhs; and technician Bhagyalakshmi.]

An excellent example of the CCRC's contributions to plant carbohydrate science and molecular biology is provided by its many collaborative projects in the area of *Rhizobium*-legume interactions. The establishment of a nitrogen-fixing symbiosis between a *Rhizobium* and its legume host involves a complex "infection" process. This process requires an exchange of signal molecules between the bacterium and the cells of its eukaryote host that results in coordinated regulation of gene expression in both the *Rhizobium* and the legume. The result of the infection process is a root nodule containing bacteroids, that are modified nitrogen-fixing rhizobia.

The infection process leading to nitrogen-fixing nodules is host-symbiont-specific. For example, *Rhizobium leguminosarum* biovar *trifolii* infects clover but not alfalfa, while *Rhizobium meliloti* infects alfalfa but not clover. *Rhizobium* biovar-specific and legume species-specific signal molecules determine host-symbiont-specific recognition and regulate differentiation processes in both the host (e.g., nodule formation) and symbiont (e.g., bacteroid formation). Many of the signal molecules have been identified. Species-specific flavones or isoflavones secreted by the host legume activate symbiont *Rhizobium* genes (*nod* genes) required for nodulation [55-58].

Russell Carlson and postdoctoral research associate V. Puvanesarajah, in collaboration with Gary Stacey's team at the University of Tennessee, have contributed to the flavonoid story in their studies of *Bradyrhizobium japonicum*, the symbiont of soybean. The *nod* genes are activated by isoflavone molecules secreted by the legume. In addition to the isoflavones, activation of *nod* genes requires the product of *nodD*, one of the *nod* genes.

Some rhizobia contain more than a single *nodD* gene; *B. japonicum* has two *nodD* genes. One of these, *nodD1*, was found to be essential for flavonoid induction of the other *nod* genes (e.g., *nodYABC*). In contrast to other rhizobia, the *nodD1* gene is also inducible. The isoflavones genistein and daidzein are good inducers of all the *B. japonicum nod* genes, including *nodD1*. Carlson and his coworkers discovered that soybean seeds also excrete compounds that are not inducers of the *nodYABCSUIJ* genes, but enhance induction of this operon in the presence of a sub-optimum genistein concentration. These compounds, which were purified and shown to be isoflavone derivatives, are specific inducers of *nodD1* but not of the *nodYABCSUIJ* operon. The active isoflavones were shown to be malonylglucosyl derivatives of genistein (6"-malonylgenistin), daidzein (6"-malonyldaidzin) and glycitein (6"-malonylglycitin) [59].

Rhizobium nod genes consist of those that are common to all *Rhizobium* species (the *nodABCD* genes) as well as those that determine host specificity and are, therefore, unique to a particular *Rhizobium* biovar or species. Some of the common and host-specific *nod* genes encode enzymes (or regulatory proteins) required for the synthesis of lipooligosaccharide oligosaccharins (called nodulation factors or simply nod factors) that specifically induce, in their host legumes, root hair deformation and cortical cell division (and, indeed, nodules in the legume hosts of the fast-growing rhizobia; [60-64]; G. Stacey *et al.*, unpublished results; R. Carlson *et al.*, unpublished results). Root hair deformation and cortical cell division can be induced by nod factor concentrations as low as 10^{-12} M and 10^{-8} M, respectively ([62-64]; G. Stacey *et al.*, unpublished results; R. Carlson *et al.*, unpublished results). We consider the lipooligosaccharide nod factors to be a family of oligosaccharins.

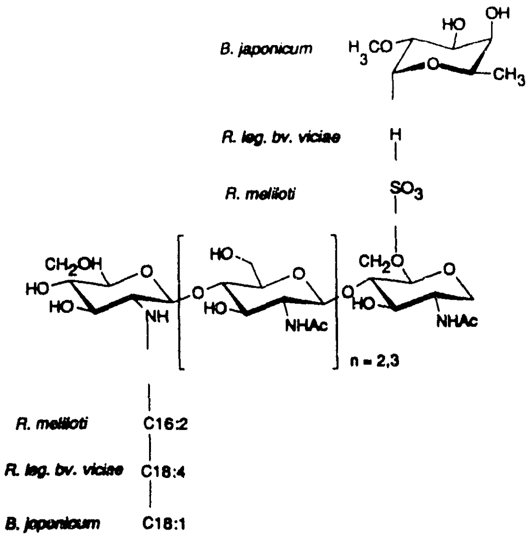


Figure 1: *Rhizobium* nodulation factors.

Structures of the nod factors. The isolation and structural characterization (see Figure 1) of the first nod factors were reported by Lerouge *et al.* in April 1990 [60]. The initial report of the alfalfa-specific nod factors secreted by *R. meliloti* further stimulated the already great interest in elucidating the nod factors secreted by other *Rhizobium* biovars. The CCRC was contacted by three groups to assist in determining the structures of nod factors that they were studying. Even though these groups were, in some ways, competing with one another, they were able to avail themselves of the CCRC's expertise, because we informed each group of our involvement with the other groups and because we did not divulge one group's results to the others.

Senior postdoctoral research associate John Glushka and graduate student and manager of the mass spectrometry facility William York of the CCRC assisted Herman Spaink and Ben Lugtenberg and their coworkers of the University of Leiden by identifying the structures of the nod factors secreted by *R. leguminosarum* biovar *viciae*, the symbiont of pea. The most active of these nod factors has an unusual fatty acyl substituent containing four double bonds (see Figure 1), the analysis of which had contributed to the problems of previous workers trying to elucidate these structures. Glushka and York are co-authors of a full-length article published in *Nature* [63]. This effort was also acknowledged by the following letter:

April 26, 1991

Dear Peter,

Your Center and its people are great. You did what Harvard could not do! Thanks to everybody.

Warm regards,

(signature)

Ben Lugtenberg

John Glushka assisted Adam Kondorosi and his group (CNRS in Gif sur Yvette, France) in identifying the structures of previously unidentified *R. meliloti* nod factors (see Figure 1) with host specificities that differ from those described by Lerouge and Dénarié and coworkers [60]. Again, the Kondorosi team spent nearly a year being frustrated by the inability of their analytical collaborators to identify the nod factors before they asked the CCRC for assistance. Glushka is co-author of a *PNAS* article describing these results [64].

Russell Carlson, Technical Director for the Grant, has a collaboration with Gary Stacey and his coworkers at the University of Tennessee to purify and structurally characterize the nod factors secreted by *B. japonicum*, the symbiont of soybean. This work has led to nod factors with novel structures (see Figure 1). A manuscript describing this work has been submitted for publication, and another manuscript is in preparation.

The structural features of the Rhizobium nod factors that determine host specificity. Knowledge of the structures of a variety of nod factors has provided a great deal of information about the structural attributes of the factors that determine host specificity. The structural features of the *R. meliloti* factors that endow them with specificity for their host legume are the sulfate at C-6 of the reducing GlcNAc residue and, in the case of the most active factor, the C16:2$\Delta^{2,9}$ *N*-fatty acyl residue (as opposed to a different *N*-fatty acyl residue) on the non-reducing terminal GlcN residue [60-62,64]. Removal of the sulfate group or reduction of the double bonds of the unsaturated fatty acyl component completely eliminates the host-specific biological activity of these factors [62]. Mutations in the *R. meliloti* host specificity genes *nodP* and *nodQ*, which code proteins that have ATP sulfurylase activity [65], result in *R. meliloti* factors that lack the sulfate group [60,65]. Relatively high concentrations of the nod factor lacking sulfate can induce root hair deformation and nodule formation in pea, but not in alfalfa, the normal host of *R. meliloti* [60,62]. Thus, the presence of sulfate is crucial for the host-specific biological activity of the *R. meliloti* factors.

Other structural features of the *R. meliloti* nod factors have more subtle effects. These were detected because *R. meliloti* also synthesizes smaller amounts of nod factors that contain five rather than four GlcN residues, as well as nod factors with five and four GlcN residues that have an *N*-fatty acyl substituent with three double bonds (C16:3$\Delta^{2,4,9}$) [64]. All of these nod factors are bioactive, but only at higher concentrations than are required for factors with the C16:2 *N*-fatty acyl substituent [64].

The host-specific structural features of the *R. leguminosarum* bv. *viciae* nod factors reside in the nature of the *N*-fatty acyl substituent and in the presence of an *O*-acetyl group at C-6 of the non-reducing terminal GlcNAcyl residue. The fatty acyl substituent of the major nod factor responsible for host-specific biological activity is highly unsaturated C18:4$\Delta^{2,4,6,11}$ [63]. The *R. leguminosarum* host specificity genes *nodL*, *nodE* and *nodF* are responsible for *O*-acetylation and the nature of the *N*-fatty acyl group [63]. Both the *O*-acetyl group at C-6 of the non-reducing terminal GlcNAcyl residue and the C18:4 fatty acyl substituent appear to be essential for host-specific biological activity. Tetrasaccharide versions of *R. leguminosarum* bv. *viciae* factors are also synthesized in smaller amounts [63].

B. japonicum strains are nitrogen-fixing symbionts of a broader host range than *R. meliloti* or the *R. leguminosarum* biovars. *B. japonicum* strains also have a larger number of *nod* genes involved in determining host specificity than do *R. meliloti* or the *R. leguminosarum* biovars [66-70]. The structures of *B. japonicum* nod factors are characterized by the required presence for biological activity of a terminal 2-*O*-methylfucosyl residue glycosidically linked to C-6 of the reducing GlcNAc residue (see Figure 1). A *B. Japonicum nodZ* mutant that lacks the ability to nodulate soybean also lacks the 2-*O*-methylfucosyl residue.

The *nodABC* genes also encode enzymes required for the production of nod factors, but the products of these genes are required for the synthesis of nod factors of all the *Rhizobium* biovars. This suggests that the *nodABC* genes are involved in the synthesis of the oligoglucosamine backbone of the nod factors. Sharon Long of Stanford University and her graduate student Morrey Atkinson have synthesized *in vitro* putative nod factors whose *in vitro* synthesis requires the products of the *nodABC* genes. The amount of product they can synthesize is very limited. We (the PIs) have agreed to collaborate with Long by assisting in the purification and characterization of these *in vitro*-synthesized products.

The structure and function of *Rhizobium* lipopolysaccharides. The collaboration of Carlson and Stacey has shown that a Tn5-insertion mutant of *B. japonicum* that failed to nodulate soybean has a lipopolysaccharide lacking its *O*-antigen polysaccharide [71]. Further analysis of the lipopolysaccharide from the mutant divulged the structure of the core region of the lipopolysaccharide and established the synthetic step that was defective in the mutant [72].

-Complete lipopolysaccharides are required for symbiosis. Carlson and postdoctoral research associate U. Ramadas Bhat have collaborated with Dale Noel of Marquette University on the structural characterization of the lipopolysaccharides from *R. leguminosarum* bv. *phaseoli*, the symbiont of bean. Noel was the first to discover that certain *Rhizobium* mutants defective in nodule development had defective lipopolysaccharide structures. The collaborators established that the altered polysaccharides lacked the *O*-antigen polysaccharides. Structural analysis of several of these mutants established that the lipopolysaccharides had defective core oligosaccharides [73]. These investigators together with those in other laboratories have established that, in every *Rhizobium*-legume symbiosis studied for this trait, a complete lipopolysaccharide is required for symbiosis.

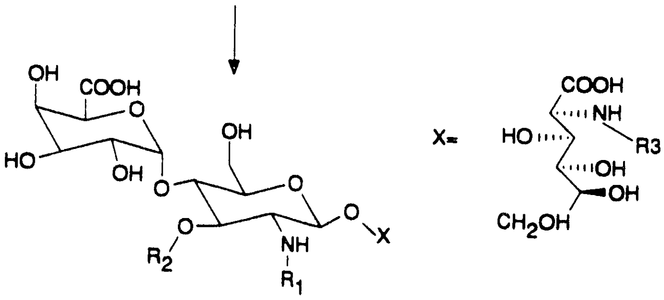
-The structures of the lipopolysaccharides of bacteroids are not the same as the structures of the lipopolysaccharides of the parent bacterium. Noel, using lipopolysaccharide-specific monoclonal antibodies, found that a structural epitope of the lipopolysaccharide changes in the nodule at the time the bacteria differentiate into bacteroids. He also showed that at least one of these changes depends on the presence in the *Rhizobium* of the symbiotic plasmid and on conditions that induce the expression of the *nod* genes. These epitope changes can be induced, at least in part, by growing the rhizobia at pH 5 (rather than pH 7). Bhat and Carlson found that the major change in the lipopolysaccharide resulting from growth at pH 5 was the disappearance in the *O*-antigen polysaccharide of 2,3,4-tri-*O*-methylfucose and the appearance of 2,3-di-*O*-methylfucose [74]. Further characterization of the symbiotically defective mutants with altered lipopolysaccharides is in progress. This research will be expedited while Dale Noel spends a six-month sabbatical at the CCRC starting in June 1992.

-Lipopolysaccharides have different functions in determinant and indeterminant nodules. Nick Brewin of the John Innes Research Institute (Norwich, England) was the first to use monoclonal antibodies to demonstrate that *Rhizobium* lipopolysaccharides undergo structural changes during symbiotic infection. His observations are similar to those of Noel, and, in fact, the two laboratories are now collaborating on studies of the biology of the *R. leguminosarum* bv. *phaseoli*-bean symbiosis. Brewin has also been studying the *R. leguminosarum* bv. *viciae*-pea symbiosis. The bean nodules are morphologically determinant (have a defined size), while the pea nodules are indeterminant. The *Rhizobium* lipopolysaccharides appear to serve different functions in these systems, as the lipopolysaccharide is required for infection thread development in the determinant nodules (bean), while the lipopolysaccharide is required for release of the bacteria in the root cortical cells and does not appear to be required for development of the infection threads in the indeterminant nodules (pea). Understanding the lipopolysaccharide structural requirements for these phenomena is the goal of research that will be carried out this summer when Brewin's postdoctoral research associate, Elmar Kannenberg joins Carlson and Noel at the CCRC beginning in June 1992. Carlson's postdoctoral research associate Tong-Bin Chen (hired with NIH grant funds) will also participate in this project.

-A Kdo-rich polysaccharide can, in certain rhizobia, be substituted for the extracellular polysaccharide as an essential component of the symbiosis. Carlson and his graduate student Brad Reuhs are collaborating with Adam Kondorosi and Peter Putnoky of the CNRS (Gif sur Yvette, France) and the Hungarian Academy of Sciences (Szeged) in studies of *R. meliloti* strains that synthesize a novel polysaccharide which can be substituted for the essential symbiosis functions of the acidic polysaccharide normally secreted by this symbiont of alfalfa. Most *R. meliloti* mutants that cannot synthesize and secrete an acidic polysaccharide (usually referred to as an extracellular polysaccharide or EPS) also cannot form a nitrogen-fixing symbiosis. However, Kondorosi (and, independently, Ethan Signer of MIT) obtained mutants of *R. meliloti* that did not synthesize or secrete the acidic polysaccharide but, nevertheless, still formed effective nitrogen-fixing nodules in alfalfa.

Reuhs and Carlson looked for other polysaccharides synthesized by the single mutant that does not secrete the acidic polysaccharide but still forms an effective symbiosis, and they also looked for polysaccharides synthesized by a double mutant that neither secretes an acidic polysaccharide nor forms an effective symbiosis. Kondorosi generated the double mutant from the single mutant that does not secrete the acidic polysaccharide but is still symbiotic. Reuhs and Carlson found that the single mutant synthesizes a hitherto unobserved, high molecular weight 3-deoxy-D-*manno*-2-octulosonic acid (Kdo)-rich polysaccharide, and they found that this Kdo-rich polysaccharide was missing in the double mutant. Genetic analysis by Kondorosi and his collaborators found that the gene responsible for the double mutant is located in the *fix*23 region of the *R. meliloti* chromosome. DNA homology studies showed that this and related genes in the *R. meliloti fix*23 region are similar to genes involved in the synthesis of *E. coli* K-antigens; many K-antigens consist of Kdo-rich polysaccharides. A paper describing the genetics and initial chemical characterization of the Kdo-rich polysaccharide synthesized by *R. meliloti* has been submitted to the *EMBO Journal*. Reuhs and Carlson have purified the polysaccharide and are generating and purifying its repeating unit. Structural characterization is planned. A sample of the purified polysaccharide has been sent to Kondorosi to see if it can restore the symbiotic capacity of the double mutant. The collaborators' unpublished results demonstrate that other *R. meliloti fix*23 mutants show alterations in their Kdo-rich polysaccharide. Carlson is interested in determining the structural basis for these altered genotypes.

-Lipid A studies. Carlson's own research, which is supported by the Grant as well as by funds from the NIH, centers on structural studies of the lipid portion of *Rhizobium* lipopolysaccharides. The lipid portion of all previously characterized lipopolysaccharides, which is called lipid A, is a disaccharide highly substituted with fatty acyl groups. The lipid As of enterobacteria are classically referred to as endotoxins because of their profound toxic effects in animals. Carlson and his postdoctoral research associate Bhat have found that the lipid A of *Rhizobium* is even more structurally complex and very different from the lipid A of the *Enterobacteriaceae*. The lipid A of *R. leguminosarum* bv. *phaseoli* (see Figure 2) contains a galactosyluronic acid residue α-1,4-linked to a glucosaminyl residue that, itself, is apparently β-linked to a 2-deoxy-2-aminogluconic acid. This appears to be the first lipid A based on a trisaccharide that has been characterized. The core region of the lipopolysaccharide is attached, via the ketosidic linkage of a Kdo residue, to *O*-6 of the glucosaminyl residue of the lipid A (see Figure 2).



R_1 = 3-OH-C14:O, -C16:O or -C18:O

R_2 = 3-OH-C14:O or 27-OH-C28:O

R_3 = 27-OH-C28:O

Figure 2: *R. leguminosarum* biovar *phaseoli* lipid A

Compositional analysis of the lipid As from rhizobia, in collaboration with Rawle Hollingsworth of Michigan State University, led to the discovery of the presence of the never-before-isolated 27-hydroxyoctacosanoic acid [75] and a demonstration of its presence in the lipid As of various *Rhizobium*, *Bradyrhizobium* and *Agrobacterium* species [76]. The lipid As from all of these species contained this characteristic fatty acid, but analyses of the sugars of the lipid As revealed three classes: those containing only glucosamine (as in *R. meliloti* and *R. fredii*), those containing galacturonic acid and glucosamine (as in *R. leguminosarum* bvs. *phaseoli*, *trifolii*, and *viciae*), and those containing 2,3-diamino-2,3-dideoxyglucose either alone or in combination with glucosamine [as in *B. japonicum* and a *Bradyrhizobium* sp. (*Lupinus*); [76]].

The development by Carlson of procedures to characterize amide-linked hydroxy fatty acids (paper submitted to *Glycobiology*) led to the demonstration that several different hydroxy fatty acids are *N*-linked to the lipid A glucosaminyl residue including 3-OH-C14:0, 3-OH-C16:0, and 3-OH-C18:0, while 3-OH-C14:0 and the 27-OH-C28:0 hydroxyoctacosanoic acid are found ester-linked to the glucosaminyl residue. The hydroxyoctacosanoic acid is ester- and probably also *N*-linked to the 2-deoxy-2-aminogluconic acid residue of lipid A.

-The degree of esterification and points of substitution by O-acetyl and O-(3-hydroxybutanoyl) groups in the acidic polysaccharides secreted by R. leguminosarum biovars viciae, trifolii, and phaseoli are not related to host range. This statement was the conclusion of a paper published by Michael McNeil and the PIs in 1986 [77]. Philip-Hollingsworth *et al.* published two papers in 1989 contradicting those findings [78,79]. We were confident of our results, so we repeated and extended our findings by demonstrating that the above statement was true even when the *Rhizobium* strains were grown in the presence of *nod* gene-inducing flavonoids. Our findings were confirmed by the joint efforts of groups in Holland and Australia. Two papers, confirming and extending the results of McNeil *et al.* and refuting the principal conclusions of Philip-Hollingsworth *et al.* were published consecutively in 1991 in the *Journal of Biological Chemistry* [80,81].

4. Other Collaborations.

CCRC personnel are involved in a variety of other collaborations that receive financial assistance from the Grant. We will briefly describe four of these. In 1989, Bernard Fritig and coworkers at the CNRS in Strasbourg collaborated with the PIs in demonstrating that a fungal glucan activates an unknown but potent viral defense in *Nicotianae* [82]. Fritig's group has, without success, tried to identify a defense response-related enzyme or other protein whose activity or concentration is increased by spraying the fungal glucan on tobacco plants. Postdoctoral research associate Kevin Brady, working with the PIs, has looked to see if the concentrations of defense-related mRNAs associated are increased by spraying with the glucan. Of a dozen such mRNAs examined, the concentration of only one, a glycine-rich protein, increases rapidly following exposure to the glucan. Its concentration increases about ten-fold over about two hours. Such glycine-rich proteins have been proposed to be involved in defense responses [83,84]. Brady has cloned the tobacco glycine-rich protein gene (manuscript in preparation), and is using the gene's promoter region to make a construct in which the promoter regulates a β-glucuronidase reporter gene. This construct will be used to make transgenic tobacco plants that will be used to assay for the ability of the fungal glucan to activate expression of the β-glucuronidase. In this way, the smallest active glucan fragment that activates the β-glucuronidase can be generated, purified and structurally characterized. After each major purification step, the glucan fragments will be assayed by Fritig's group for their ability to protect *Nicotianae* against virus infection. The fungal glucan that elicits the defense response contains, within its covalent structure, the well-characterized hepta-β-glucoside elicitor of soybean phytoalexins, but that hepta-β-glucoside does not activate *Nicotianae* against viral infection.

Visiting scientist Noboru Yamazaki, postdoctoral research associate Stephen Fry, and the PIs demonstrated in 1983 [85] that fragments generated from suspension-cultured sycamore cell walls inhibit the ability of the cells to incorporate [^{14}C]leucine into proteins. This was followed by the demonstration by graduate student Steven Doares, postdoctoral research associate Peter Bucheli, and the PIs that enzymes secreted by *Magnaporthe grisea*, the fungus that causes rice blast, generates fragments of plant cell walls that kill or inhibit protein synthesis in suspension-cultured maize cells [86,87]. Later, postdoctoral research associate Serge Kauffmann purified two enzymes from the *M. grisea* culture filtrate that were required to release, from maize cell walls, the fragments that inhibit protein synthesis in maize cells. These enzymes, which were purified to homogeneity, turned out to be an *endo*-β-1,4-D-xylanase and an α-L-arabinofuranosidase (manuscript in preparation). Separately, Doares characterized the wall fragments released by the *M. grisea* culture-fluid enzymes and showed that they were arabinoxylan fragments with ferulic acid esters, and that esters were required for inhibitory activity (manuscript in preparation). These two lines of research have provided evidence that *endo*-β-1,4-D-xylanase is an important virulence factor of the rice blast pathogen and that the *endo*xylanase might participate in eliciting the hypersensitive defense response (rapid cell death) in non-hosts. To test this hypothesis, we decided to clone the two *endo*xylanases of *M. grisea* and then, by replacement genetics, see if one or both of the *endo*xylanases is required for virulence on a susceptible rice host and for elicitation of the HR on non-hosts. Senior postdoctoral research associate Sheng-Cheng Wu has purified to homogeneity and cloned the gene of the 22 kD *endo*xylanase and is planning to clone the gene of the 32 kD *endo*xylanase (the one purified by Kauffmann). Our collaborator, Barbara Valent of DuPont, is using our clone to map the gene of the 22 kD *endo*xylanase and will learn if it is associated with any known virulence or avirulence genes. Valent, who is providing us with the *M. grisea* strains and rice cultivars needed for the replacement genetics, will assist us in all aspects of this project.

The technical directors of the Plant and Microbial Carbohydrate Center (Carlson) and of the Biomedical Carbohydrate Resource Center (Roberta Merkle) have on occasion received collaboration requests that could best be handled by the other technical director. For example, Merkle and her postdoctoral research associate, Izabella Poppe, are obtaining interesting results in a collaboration with a group in Belgium to characterize the oligosaccharide side chains of three S-allele glycoproteins from petunia that are involved in determining self-incompatibility of the pollen. Russell Carlson, on the other hand, has used his expertise in bacterial polysaccharide structure analysis and Herman van Halbeek's and John Glushka's proficiency in NMR analysis to assist Frederick Cassels of the Walter Reed Army Institute of Research

(Washington, DC) by elucidating the structure of a *Streptococcus sangui* H1 polysaccharide involved in dental plague formation. The polysaccharide has a six-glycosyl residue repeating unit with a glycerol phosphate attached through a phosphodiester bond to *O*-6 of the 3-linked α-galactopyranosyl residue of the polysaccharide ([88], and manuscript in preparation).

Collaborations with industry can lead to challenging projects with interesting results. Texaco scientists were interested in two highly viscous polysaccharides with properties that suggested the polysaccharides might be of value in enhanced oil recovery. Peter Robison and Kechia J. Chou of the Texaco Research Center in Beacon, NY, purified the polysaccharides, and Malcolm O'Neill and the PIs determined their structures. The first polysaccharide, from a *Xanthobacter* sp., turned out to have a novel structure related to a series of polysaccharides secreted by *Alcaligenes* and *Pseudomonas* species [89]. The second polysaccharide, which is secreted by *Agrobacterium radiobacter*, was an enormous challenge. We obtained strong evidence that this acidic polysaccharide has a heptadecasaccharide repeating unit, which make it by far the largest repeating unit of a bacterial polysaccharide yet characterized. [The previous record for a bacterial polysaccharide has an undecasaccharide repeating unit; it is synthesized by a *R. leguminosarum* bv. *phaseoli* strain [90], which, like *A. radiobacter*, is a member of the *Rhizobiaceae*.] Our results supporting the 17 glycosyl-residue repeating unit were so extensive that they required 24 pages in *Carbohydrate Research* to report the data [91].

EPILOGUE

The CCRC's carbohydrate science expertise and technology has attracted and enabled most of the collaborations described above as well as the CCRC's many other ongoing collaborations (listed in Appendix 2) that could not be described because of space limitations. The collaborations in plant and microbial carbohydrate science are evidence that the Grant enables the center to be widely recognized for its state-of-the-art technology and expertise and that CCRC personnel have taken advantage of the opportunities presented to them. Our goal for the next five years is to continue and extend the collaborations, the graduate student research projects (Appendix 2), and the service (Appendices 3 and 6) and training (Appendices 2, 3 and 5), and to try to improve on quality in every facet of our endeavors.

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86. Doares, S. H., P. Bucheli, P. Albersheim, and A. G. Darvill. 1989. Host-pathogen interactions XXXIV. A heat-labile activity secreted by a fungal phytopathogen releases fragments of plant cell walls that kill plant cells. *Mol. Plant-Microbe Interac.* 2:346-353.
87. Bucheli, P., S. H. Doares, P. Albersheim, and A. Darvill. 1990. Host-pathogen interactions XXXVI. Partial purification and characterization of heat-labile molecules secreted by the rice blast pathogen that solubilize plant cell wall fragments that kill plant cells. *Physiol. Mol. Plant Pathol.* 36:159-173.
88. Cassels, F. A., H. M. Fales, J. London, R. W. Carlson, and H. van Halbeek. 1990. Structure of a streptococcal adhesion carbohydrate receptor. *J. Biol. Chem.* 265:14127-14135.
89. O'Neill, M. A., A. G. Darvill, P. Albersheim, and K. J. Chou. 1990. Structural analysis of an acidic polysaccharide secreted by *Xanthobacter* sp. (ATCC 53272). *Carbohydr. Res.* 206:289-296.
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91. O'Neill, M. A., P. D. Robison, K. J. Chou, A. G. Darvill, and P. Albersheim. 1992. Evidence that the acidic polysaccharide secreted by *Agrobacterium radiobacter* (ATCC 53271) has a seventeen glycosyl-residue repeating unit. *Carbohydr. Res.* 226:131-154.

The Center for Plant and Microbial Complex Carbohydrates at the University of Georgia Complex Carbohydrate Research Center

1987-92 Five-Year Report and 1993-97 Renewal Application

Appendix 1: CCRC Chronology, Advisers, Visitors & Interactions

Curricula Vitae of PIs Peter Albersheim and Alan Darvill

Chronology of the CCRC, 1985-April 1992

Plant Science Center Board of Advisers

Visitors to the CCRC, 1985-May 1992

Center-Wide Weekly Research Seminar Speakers, 1991-92

CCRC Industrial Interactions

May 1992

CURRICULUM VITAE

PETER ALBERSHEIM

Born:	[redacted]
Married:	Joyce Elizabeth [redacted], 1958
Children:	Renee, [redacted] Jim, [redacted] Stephi, [redacted]

Academic Background:

1956	B.S., Plant Pathology, Cornell University, Ithaca, New York
1959	Ph.D., Biochemistry, California Institute of Technology, Pasadena, California

Academic Positions:

1959	Postdoctoral Fellow, California Institute of Technology, Pasadena, California
1959-60	NSF Postdoctoral Research Fellow, Swiss Federal Institute of Technology, Zurich, Switzerland
1960-61	Instructor of Biology, Harvard University, Cambridge, Massachusetts
1961-64	Assistant Professor of Biology, Harvard University, Cambridge, Massachusetts
1964-67	Associate Professor of Biochemistry, Department of Chemistry, University of Colorado, Boulder, Colorado
1967-85	Professor of Biochemistry, University of Colorado, Boulder, Colorado
1970-85	Professor of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, Colorado
1985-present	Research Professor of Biochemistry, Chemistry, Botany, Plant Pathology and Director, Complex Carbohydrate Research Center, University of Georgia, Athens
1987-present	Co-director DOE/NSF/USDA Center for Plant and Microbial Complex Carbohydrates
1989-present	Director, NIH Resource Center for Biomedical Complex Carbohydrates

Awards:

1973 Charles A. Shull Award for outstanding research in plant physiology by a scientist under 40 years of age. American Society of Plant Physiologists.

1973 Sponsor of three students (W.D. Bauer, K. Keegstra and K. Talmadge) who received the George Olmsted Award from the American Paper Institute.

1977 Ruth and Tracey Storer Life Sciences Lecturer, University of California, Davis.

1978 Visiting Distinguished Professor, University of Hawaii, Honolulu.

1979 Robert L. Stearns Award for outstanding contributions to the progress of the University. University of Colorado, Boulder.

1980 Faculty Research Lectureship, University of Colorado, Boulder.

1983 Elected as a Fellow of the American Association for the Advancement of Science.

1984 Kenneth A. Spencer Award from the American Chemical Society for outstanding achievement in agricultural chemistry.
1986 Philip C. Hamm Memorial Lecture, University of Minnesota, St. Paul, Minnesota.
1989 Walter E. Loomis Memorial Lecture, Iowa State University, Ames, Iowa.

Professional Societies:

American Association for the Advancement of Science
American Chemical Society
American Phytopathological Society
American Society for Microbiology
American Society for Biological Chemists
American Society of Plant Physiologists
Council for Agricultural Science and Technology
International Association for Plant Tissue Culture
International Society for Plant Molecular Biology
Japanese Society of Plant Physiologists
Phytopathological Society of Japan
Plant Molecular Biology Association
Society for Complex Carbohydrates
CODATA (Committee on Data for Science and Technology)

Other:

1986-88 Chairman, International Advisory Committee CNRS Plant Science Institute, Gif-sur-Yvette, France
1986- Director, CarbBank Carbohydrate International Database
1988-90 DOE/NSF/USDA Advisory panel for Plant Science Centers (3/1/88-2/28/90)
1988-89 Member, UGA Biotechnology Committee
1989 Member, CNRS International Evaluation Committee for Plant Science Centers at Gif-sur-Yvette (ISV), Strasbourg (IBMP) and Grenoble, France
1989 Member, UGA Biotechnology Postdoctoral Grant Review Committee
1989-90 Member, UGA Special Professorships Nominating Committee
1989-90 Member, UGA Franklin College Biological Science Promotion and Tenure Committee
1990-92 Member, American Chemical Society Committee
1991 Member, Peer Review Committee of the Institute of Biological Sciences, National Research Council of Canada

Publications (Past 5 years, from a total of 236):

Thomas, J.R., M. McNeil, A.G. Darvill, and P. Albersheim. 1987. The Structure of Plant Cell Walls XIX. Isolation and characterization of wall polysaccharides from suspension-cultured Douglas fir cells. *Plant Physiol. 83*:659-671.
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Ham, K.-S., S. Kauffmann, P. Albersheim, and A.G. Darvill. 1991. Host-Pathogen Interactions XXXIX. A soybean pathogenesis-related protein with β-1,3-glucanase activity releases phytoalexin elicitor-active heat stable fragments from fungal walls. *Mol. Plant-Microbe Interac. 4*:545-552.

Mathieu, Y., A. Kurkdjian, H. Xia, J. Guern, A. Koller, M.D. Spiro, M.A. O'Neill, P. Albersheim, and A. Darvill. 1991. Membrane responses induced by oligogalacturonides in suspension-cultured tobacco cells. *The Plant Journal 1*:333-343.

Koller, A.L., M. O'Neill, A.G. Darvill, and P. Albersheim. 1992. A comparison of the polysaccharides extracted from dried and non-dried walls of suspension-cultured sycamore cells. *Phytochem. 30*:3903-3908.

Albersheim, P. 1991. Commentary on: "Laminin carbohydrates are implicated in cell signaling," Chandrasekaran *et al. Chemtracts. 2*:390-392.

De Lorenzo, G., F. Cervone, M.G. Hahn, A. Darvill, and P. Albersheim. 1992. Plant cell wall pH prevents tissue maceration and enhances the stability of phytoalexin elicitor-active oligogalacturonides in the presence of a bacterial endopectate lyase. *Physiol. Mol. Plant Pathol. 39*:335-344.

Albersheim, P., C. Augur, J.-J. Cheong, S. Eberhard, M.G. Hahn, V. Marfà, D. Mohnen, M.A. O'Neill, M.D. Spiro, W.S. York, and A. Darvill. 1992. Oligosaccharins -- oligosaccharide regulatory molecules. *Accts. of Chem. Res. 25*:77-83.

Hisamatsu, M., W.S. York, A.G. Darvill, and P. Albersheim. 1992. Structure of Plant Cell Walls. XXXIV. Characterization of seven xyloglucan oligosaccharides containing from seventeen to twenty glycosyl residues. *Carbohydr. Res. 227*:183-194.

Albersheim, P. 1992. Commentary on: "Molecular cloning of a putative receptor protein kinase gene encoded at the self-incompatibility locus of *Brassica oleracea*," Stein *et al. Chemtracts 3*:50-52.

O'Neill, M.A., P.D. Robison, K.J. Chou, A.G. Darvill, and P. Albersheim. 1992. Evidence that the acidic polysaccharide secreted by *Agrobacterium radiobacter* (ATCC 53271) has a seventeen glycosyl-residue repeating unit. *Carbohydr. Res. 226*:131-154.

Hahn, M.G., A. Darvill, P. Albersheim, C. Bergmann, J.-J. Cheong, A. Koller, V.-M. Ló. 1992. Preparation and characterization of oligosaccharide elicitors of phytoalexin accumulation. In: *Molecular Plant Pathology: A Practical Approach* (D.J. Bowles, Ed.), IRL Press. In press.

Augur, C., L. Yu, K. Sakai, T. Ogawa, P. Sinaÿ, A.G. Darvill, and P. Albersheim. 1992. Further studies of the ability of xyloglucan oligosaccharides to inhibit auxin-stimulated growth. *Plant Physiology.* In press.

Toubart, P., L. Daroda, A. Desiderio, G. Salvi, F. Cervone, G. De Lorenzo, C. Bergmann, A.G. Darvill, and P. Albersheim. 1992. Cloning and characterization of the gene encoding the *endo*polygalacturonase-inhibiting protein (PGIP) of *Phaseolus vulgaris* L. *The Plant Journal*. In press.

Darvill, A., C. Augur, C. Bergmann, R.W. Carlson, J.-J. Cheong, S. Eberhard, M.G. Hahn, V.-M. Ló, V. Marfà, B. Meyer, D. Mohnen, M.A. O'Neill, M.D. Spiro, H. van Halbeek, W.S. York, and P. Albersheim. 1992. Oligosaccharins - oligosaccharides that regulate growth, development and defense reponses in plants. *Glycobiology*. In press.

CURRICULUM VITAE

ALAN GEOFFREY DARVILL

Born: [redacted]

Married: Janet Elizabeth [redacted] 1975

Children: Sarah Jayne [redacted]

Academic Background:

1970-73	B.S., Plant Biology, Wolverhampton Polytechnic
1973-76	Ph.D., Plant Physiology, University College of Wales, Aberystwyth, Wales
1975-76	President, Biological Society of Aberystwyth University

Academic Positions:

1976-78	Postdoctoral Research Associate, University of Colorado, Boulder, CO, USA
1978-83	Senior Research Associate, University of Colorado, Boulder, CO, USA
1983-84	Assistant Professor, Attendant Rank, Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO, USA
1984-85	Associate Professor, Attendant Rank, Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO, USA
1985-87	Associate Professor, Departments of Biochemistry and Botany, and Associate Director, Complex Carbohydrate Research Center, University of Georgia, Athens, GA, USA
1987-88	Associate Professor, Departments of Biochemistry and Botany, and Director, Complex Carbohydrate Research Center, University of Georgia, Athens, GA, USA
1987-present	Co-director, DOE/NSF/USDA Center for Plant and Microbial Complex Carbohydrates
1988-present	Professor, Departments of Biochemistry and Botany, and Director, Complex Carbohydrate Research Center, University of Georgia, Athens, GA, USA

Professional Societies:

International Plant Growth Substance Association
American Society of Plant Physiologists
Plant Growth Regulator Society of America
Japanese Society of Plant Physiologists
International Society for Plant Molecular Biology
Society for Complex Carbohydrates
Member, American Chemical Society
Member, American Association for the Advancement of Science

Editorial Board:

Plant Physiology
Glycobiology
The Plant Journal for Cell and Molecular Biology

Publications (Past 5 years, from a total of 117):

Darvill, A.G., K.R. Davis, S.H. Doares, D.J. Gollin, R.A. O'Neill, P.R. Toubart, W.S. York, and P. Albersheim. 1987. Oligosaccharins - molecules that can regulate plant morphogenesis and defense against disease. In: *Molecular Mechanisms in the Regulation of Cell Behavior*, Alan R. Liss, Inc., pp. 283-284.

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Appendix 1: CCRC Chronology, Advisers, Visitors and Interactions

CHRONOLOGY OF THE CCRC, 1985-APRIL 1992

[Current positions of staff given in parentheses]

<u>1985</u>

September	Dr. Peter Albersheim (Research Professor and Co-Director) and Dr. Alan Darvill (Professor and Co-Director) move with 16 of their group from the University of Colorado (Boulder) to found the Complex Carbohydrate Research Center at the University of Georgia. The group occupies approximately 10,000 sq.ft. of laboratory and office space rented by the University of Georgia from the USDA in the Richard Russell Research Center in Athens.
September	Dr. Richard Cummings (Professor of Biochemistry), who was already a UGA Biochemistry Department faculty member, joins CCRC faculty.

<u>1986</u>

February	Dr. Herman van Halbeek (Associate Professor of Biochemistry) joins CCRC faculty
March	Lydia Snyder (Business Manager) joins CCRC
April	-Rosemary Nuri (Administrative Manager) joins CCRC -250-MHz nuclear magnetic resonance spectrometer delivered to CCRC (NSF and CCRC start-up funds)
May	Dennis Warrenfeltz (Chemical Instrumentation Manager) joins CCRC
July	500-MHz nuclear magnetic resonance spectrometer delivered to CCRC (NSF and CCRC start-up funds)
August	DOE-funded workshop attended by 36 leading carbohydrate scientists and database design and implementation specialists select the CCRC to develop the Complex Carbohydrate Structural Database and its management program CarbBank, to implement and direct the database and as the operational site for the project
October	Dr. Carl Bergmann (CCRC Building Manager and Postdoctoral Research Associate) joins CCRC

<u>1987</u>

Summer	Construction begins on $5 million, 40,000 sq.ft. CCRC building on Riverbend Road
September	CCRC wins five-year grant funded by the DOE (as part of the USDA/DOE/NSF Plant Science Centers program) for the Center for Plant and Microbial Complex Carbohydrates

<u>1988</u>

March	Dr. Bernd Meyer (Associate Professor of Biochemistry) joins CCRC faculty

May	Dr. Russell Carlson (Associate Research Biochemist and Technical Director - Plant and Microbial Carbohydrates) joins CCRC
September	-Dr. Michael Hahn (Assistant Professor of Botany) joins CCRC faculty (July 1986-September 1988 Assistant Research Scientist at the CCRC) -CCRC wins five-year NIH program project grant to characterize the carbohydrate portions of the glycoproteins on the surface of the AIDS virus
November	Dr. Karl-Erik Eriksson (Professor of Biochemistry and Eminent Scholar of Biotechnology) joins UGA and CCRC faculty

1989

June	CCRC moves into new building on Riverbend Road
July	Digital Equipment Corporation awards contract for the development of a laboratory research information system
September	CCRC wins five-year grant from NIH for a Resource Center for Biomedical Complex Carbohydrates
October	-Dr. Roberta Merkle (Technical Director - Biomedical Carbohydrates) joins CCRC (November 1987-September 1989 Postdoctoral Research Associate with Dr. Richard Cummings) -First distribution of CCSD/CarbBank to subscribers

1990

May	Formal dedication of CCRC building and open house
September	Dr. Debra Mohnen (Assistant Professor of Biochemistry) joins CCRC faculty
August	Construction begins on 5,000 sq.ft. extension to the CCRC building to house NMR and computer labs
December	Construction completed on CCRC building extension

1991

January	600-MHz nuclear magnetic resonance spectrometer delivered to the CCRC (NIH and UGA funds)
April	Dr. Michael Pierce (Associate Professor of Biochemistry) joins CCRC faculty
May	Dr. Kelley Moremen (Assistant Professor of Biochemistry) joins CCRC faculty
December	-SCIEX triple quadrupole electrospray mass spectrometer delivered to CCRC (NIH and UGA funds) -Dr. Marcia Kieliszewski (Assistant Research Biochemist) joins CCRC

1992

January	CCRC opens CCSD/CarbBank office in Bellingham, WA

Appendix 1: CCRC Chronology, Advisers, Visitors & Interactions

Plant Science Center Board of Advisers

The Plant Science Center Board of Advisers met in June, 1988, September, 1989, and April, 1991. The advisers and their addresses are as follows:

Dr. Gerald O. Aspinall
Department of Chemistry
York University
4700 Keele Street
North York, Ontario M3J 1P3
Canada

Dr. Alan D. Elbein
Department of Biochemistry &
Molecular Biology
Slot 516
4301 West Markham
Little Rock, AR 72205-7199

Dr. Jack Preiss
Department of Biochemistry
Michigan State University
E. Lansing, MI 48824

Dr. James Prestegard
Department of Chemistry
Yale University
New Haven, CT 06520

Appendix 1. CCRC Chronology, Advisers, Visitors, and Interactions

Visitors to the CCRC, 1985 - May 1992

December 1985	Ole Hindsgaul	University of Alberta, Canada
February 1986	Tom Jacobs	Stanford University
	Michael Hahn	The Salk Institute
	Rita Colwell	University of Maryland
March 1986	Subramaniam Sabesan	DuPont, Wilmington, DE
	David Smith	VPI and State University
	James C. Paulson	UCLA Medical School
	Joseph Ecker	Stanford University
	K.C. Nicolau	University of Pennsylvania
	Bernd Meyer	University of Oldenburg, Germany
May 1986	Debbie Delmer	ARCO Plant Cell Research Institute, CA
	Nancy LeClair Stults	Johns Hopkins University
June 1986	Akira Kobata	University of Tokyo
July 1986	Kierem Fleming	United Kingdom
	Kiem Tran Thanh Van	CNRS, Gif sur Yvette, France
	Robert Rabson	Department of Energy, Washington, DC
	Menaro Fukuda	La Jolla Cancer Research Foundation
	Tsune Kosuge	University of California, Davis
	Peter Quail	University of Wisconsin
	Vernon Reinhold	Harvard Medical School
	L.O. Sillerud	Los Alamos National Laboratory
August 1986	Klaus Bock	Technical University of Denmark
	Osamu Kodama	Ibaraki University, Japan
	Robert Selvendran	AFRC Institute of Food Research, UK
	Jan Williams	Medical Research Council, UK
September 1986	Peter Doerner	University of Oldenburg, Germany
	Bernd Meyer	University of Oldenburg, Germany
	LuAnn Aquino	University of Illinois, College of Medicine
	Andrea Vasell	University of Zurich
	Sam Nunn	U.S. Senate, Washington, DC
October 1986	James Lau	Hewlett-Packard, NJ
	Allan Bradbury	General Foods
	Jason Salsbury	Georgia Institute of Technology
	Ronald Highsmith	Allied Signal
	Archie Davis	C&S Bank, Atlanta, GA
	Fred Greer	C&S Bank, Atlanta, GA
	Chuck Williams	C&S Bank, Atlanta, GA
	Dunbar Harrison	C&S Bank, Athens, GA
	Bill Loftin	C&S Bank, Athens, GA
January 1987	Adrienne Clark	University of Melbourne, Australia
	Ed Clark	Duke University, Durham, NC
	V. Puvanesarajah	University of Tennessee, Knoxville
	Burt Fraser-Reid	Duke University, Durhamn, NC
	Bernard Durand	University of Orléans, France

February 1987	Klaus Bock	Technical University of Denmark
	Martin Peter	University of Bonn, Germany
	Jasna Peter-Katalinic	University of Bonn, Germany
March 1987	Russell Carlson	Eastern Illinois University, Charleston, IL
	H. Kalembasa	Eastern Illinois University
	S.J. Kalembasa	Poland
	Gerald Aspinall	York University, Toronto, Canada
	Thomas Peters	National Research Council, Canada
April 1987	Chris Lamb	The Salk Institute
	Karl-Erik Eriksson	Swedish Pulp and Paper Institute, Stockholm
	Marguerite Rinando	CERMAV, Grenoble, France
	Ian Sussex	Yale University
	Sacco de Vries	Agricultural University, Wageningen, The Netherlands
June 1987	Robert Forrest	Scottish Crop Research Institute
	Jean Negrutiu	Institute of Molecular Biology, Brussels, Belgium
	Tomoya Ogawa	RIKEN Institute, Saitama, Japan
September 1987	Kei Matsuzaki	Shinshu University, Japan
	Yasuyuki Koie	Emeryville, CA
	Alfred M. Mayer	Hebrew University, Jerusalem, Israel
October 1987	Kiem Tran Thanh Van	CNRS, Gif sur Yvette, France
	Russell Carlson	Eastern Illinois University, Charleston, IL
November 1987	Bo Nilssen	BioCarb, Lund, Sweden
	Hans van Etten	Cornell University, NY
	Michael Spellman	Genentech, Inc., San Francisco, CA
	Serge Perez	INRA, Nantes, France
	Anne Imberty	CERMAV, CNRS, Grenoble, France
	Donald P. Miller	Clemson University, Clemson, SC
	Steven Levery	Biomembrane Institute, Seattle, WA
December 1987	John R. Yates	Department of Chemistry, University of Virginia
	Robert Cherniak	Georgia State University
	Carlos A. Stortz	Department of Chemistry, Georgia State University
January 1988	Bruce Wasserman	Department of Food Science, Rutgers University, New Brunswick, NJ
February 1988	Ned Friedman	Department of Botany, The University of Georgia
	Keith Davis	Massachusetts General Hospital, Boston
	Arnd Sturm	University of California, San Diego
March 1988	Jack Paxton	University of Illinois, Urbana, IL
	Brent Ridley	Okemos, MI
	Arthur Galston	Department of Biology, Yale University
	Mark Spiro	Amherst, MA
April 1988	Fred Meins	FMI, Basel, Switzerland
	Kevin Brady	Indiana University, Bloomington, IN
	Børre Robertson	University of Tromsø, Norway
	Jan Raö	University of Tromsø, Norway
	Ray Lemieux	University of Alberta, Canada

June 1988	Jack Priess	Department of Biochemistry, Michigan State University
	Alan Elbein	Department of Biochemistry, University of Texas
	Gerald Aspinall	York University, Canada
	James Prestegard	Department of Chemistry, Yale University
	Amnon Erez	Volcani Center, Bet-Dagan, Israel
	Ronald Damiani	Norristown, PA
July 1988	Derek Horton	Department of Chemistry, Ohio State University
	Byron Bossenbrock	Chemical Abstracts, Columbus, OH
	Joseph Mockus	Chemical Abstracts, Columbus, OH
September 1988	Avi Nachmias	Gilat Experiment Station, Negev, Israel
	Haruki Yamada	Japan
	Stan van Boeckel	Orgarion Int., Oss, The Netherlands
	V. Poszgay	NRCC, Ottawa, Canada
October 1988	Peter M. Gresshoff	Plant Molecular Genetics, University of Tennessee
November 1988	Carl Schnaitman	Department of Microbiology, University of Virginia
	James Campbell	Department of Biology, Deakin University, Australia
December 1988	Peter Roone	Texas
January 1989	Bruce Stone	LaTrobe University, Australia
	Jeffery Sellers	Perkin-Elmer, Atlanta, GA
March 1989	Michael Janus	Harvard Medical School
	Akira Hasegawa	Applied Bioorganic Chemistry, Gifu University, Japan
April 1989	Hans Paulsen	University of Hamburg, Germany
	Thomas Peters	University of Frankfurt, Germany
May 1989	Gerhard Franz	University of Regensburg, Germany
June 1989	Victor J. Morris	AFRC Institute of Food Research, Norwich, England
July 1989	Sabine Koepper	University of California, San Clemente
	Barbara Langer	Philadelphia, PA
August 1989	Laurie Melton	University of Otago, New Zealand
October 1989	Louise Brisson	Concordia University
	Dale Noel	Marquette University, WI
November 1989	Naoto Shibuya	National Food Research Institute, Tsukuba, Ibaraki, Japan
	Michael McNeil	Colorado State University, Ft. Collins
	Dianne Bowles	University of Leeds, UK
	Daotian Fu	Iowa State University, Ames, IA
December 1989	Barry McCleary	MegaZyme, Australia
	Zhenbiao Yang	VPI & State University, Blacksburg, VA
March 1990	Carel Wuffelmans	Leiden, The Netherlands

April 1990	Michael Pierce	University of Miami School of Medicine
	Robert Spiro	Harvard Medical School, Boston, MA
	Mary Jane Spiro	Boston, MA
	C.A. Ryan	Washington State University, Pullman, WA
May 1990	Hannelie Witsenboer	Genetics, Free University, Amsterdam, The Netherlands
	Michael Walter	Institute für Pflanzenphysiologie, Germany
	Kelley Moremen	MIT, Cambridge, MA
	Michel Bergh	Genzyme, Belmont, MA
	Bruce Stone	La Trobe University, Australia
	Phillip Harris	La Trobe University, Australia
	Demitrius Paris	Georgia Institute of Technology
	Lowell Greenbaum	Medical College of Georgia
	Debbie Bell	Georgia Institute of Technology
	Clarie Airington	Georgia State University
	Haskin Pounds	Board of Regents, University System of Georgia, Atlanta, GA
	Charlene R. Black	Georgia Southern
	Dennis Piszkiewicz	Baxter Healthcare Corporation, Duarte, CA
	Hans Marquardt	Oncogen, Seattle, WA
	Ellen Berg	Stanford University Medical School, Palo Alto, CA
	Therese Simmons	Baylor College of Medicine, Houston, TX
	David Zarley	Lederle-Praxis Biologicals, Pearl River, NY
	Michael Dumas	Miles, Inc., Berkeley, CA
	Sam Dasgupta	Medical University of South Carolina
	Edward Yeh	Harvard Medical School, Boston, MA
	Ibrahim Barsoum	Vanderbilt University School of Medicine, Nashville, TN
	Jamie De Stefano	University of Cincinnati Medical School
	Sen-itiroh Hakomori	University of Washington, Seattle, WA
	J.H. Prestegard	Yale University
	David Bundle	National Research Council, Canada
	Gerald W. Hart	Johns Hopkins University
	A.L. Burlingame	University of California, San Francisco
	Ajit Varki	University of California, San Diego
	Akira Kobata	University of Tokyo
	Leena Rasemen	University of Helsinki
	Hamdi Zahran	Egypt
	Betty Yan	Eli Lilly & Co., Indianapolis, IN
	Rajan Nair	University of Michigan Medical Center
	Nate Wardrip	University of California, Davis
	Paul Aeed	The Upjohn Company, Kalamazoo, MI
	Brian O'Connell	University of Rochester Medical School
June 1990	Pat Schuller	University of Colorado
	Rawle Hollingsworth	Michigan State University
July 1990	Marie Devins	Forsyth County High School
	Mason Hill	Gainesville, GA
	Dawn Jen	Gainesville, GA
	Lynn Verdery	Baldwin, GA
	Nancy Crittendon	Hartwell, GA
	Miriam Neal	Gainesville, GA
	Debbie Sommer	Blairsville, GA
	Melissa Kelley	Blairsville, GA
	Jenni Gregory	Blairsville, GA
	Teresa Thomason	Blairsville, GA
	Jackie Ruds	Clarkesville, GA
	Vickie Washington	Clarkesville, GA
	Gwen Fry	Clarkesville, GA
	Beth Crawford	Clarkesville, GA

	Jane Shetaker	Clarkesville, GA
	Mary Butehilder	Clarkesville, GA
	Joi Campbell	Clarkesville, GA
	Ella Baldwin	Toccoa, GA
	Sandra Payne	Toccoa, GA
	Jane LeMaster	Toccoa, GA
	Wanda Carpenter	Toccoa, GA
	Amelia Cochran	Toccoa, GA
	Beverly Patterson	Toccoa, GA
	Judy Carter	Toccoa, GA
	Deborah Winsor	Toccoa, GA
	Sandra Thurman	Royston, GA
	Rachel Schneider	USDA Forest Service
	Eddie Bennett	Pioneer RESA, Cleveland, GA
August 1990	Steven Levery	Biomembrane Inst., University of Washington, Pullman, WA
	Andrew Mort	Dept. of Biochemistry, Oklahoma State University
	Joe Varner	Dept. of Biology, Washington University, St. Louis, MO
	Anne Datko	USDA, Beltsville, MD
	Machi Dilworth	National Science Foundation, Washington, DC
	Robert Rabson	Department of Energy, Washington, DC
	Herman Spaink	Leiden University, Leiden, The Netherlands
September 1990	Hakon Leffler	Dept. of Psychiatry, University of California, San Francisco, CA
October 1990	Ben Lugtenberg	Leiden University, Leiden, The Netherlands
	Tom Stevenson	University of Montana, Missoula, MT
	Jon M. Case	Centocor, Malvern, PA
	Rod McEver	Centocor, Malvern, PA
	Nicole Benhamou	Laval University, Quebec, Canada
	James Gray	Australian National University
	W. Goodenough	Washington University, St. Louis, MO
November 1990	Herman Spaink	Leiden University, Leiden, The Netherlands
	Chris Somerville	MSU/DOE Plant Research Laboratory, East Lansing, MI
	John Glushka	The Rockefeller University, New York, NY
	Bernard Brau	Rhone Merieux, France
	Remy Bayle	Rhone Merieux, France
	D. Mark Keister	Rhone Merieux, Athens, GA
	R.L. Highland	Rhone Merieux, Athens, GA
December 1990	Jaap J. Boon	FOM Institute for Atomic and Molecular Physics, Amsterdam, The Netherlands
	Don Scott	Alko Biotechnology, Schaumburg, IL
	Brian Larkins	University of Arizona, Tucson, AZ
January 1991	Widmar Tanner	Universität Regensburg, Germany
	Ronald J. Alain	NALCO Chemical Company, Naperville, IL
	Robert L. Wetegrove	NALCO Chemical Company, Naperville, IL
	Robert Linhardt	University of Iowa, Ames, IA
	T.E. Conners	Institute of Paper Science and Technology, Atlanta, Georgia
	R.J. Dinus	Institute of Paper Science and Technology, Atlanta, Georgia

	D.A.I. Goring	University of Toronto, Canada
	R. Malcolm Brown	University of Texas, Austin, TX
	Robert Blanchette	University of Minnesota, Minneapolis/St. Paul, MN
February 1991	Joe Modelevsky	Digital, Atlanta, GA
	Jonathan Arnold	Digital, Atlanta, GA
	James L. Van Etten	Plant Pathology, University of Nebraska, Lincoln, NE
	Ann Matthyssee	University of North Carolina, Chapel Hill
	Jinhua An	Oklahoma State University
	Bob Barker	Cornell University, Ithaca, NY
	Michael Gold	Oregon Graduate Institute, Beaverton, OR
	Jonathan Seals	Cambridge Biotech, Worcester, MA
	Desh Pal Verma	Ohio State University, Columbus, OH
	Sasha Djelineo	University of Belgrade, Yugoslavia
April 1991	Ellen Kerr Warren	Kimberly-Clark, Roswell, GA
	Kenneth R. Smith	Kimberly-Clark, Roswell, GA
	Elliott Meyerowitz	Caltech, Pasadena, CA
	Gary Stacey	Dept. of Microbiology, University of Tennessee, Knoxville, TN
	Herman Spaink	Leiden University, Leiden, The Netherlands
	Gregory Payne	Chapman and Hall, New York, NY
	Tomoya Ogawa	RIKEN Institute, Japan
	Bernard Fritig	IBMP-CNRS, Strasbourg, France
	Felice Cervone	University of Rome, Italy
	Hans Forsburg	Swedish Academy of Engineers, Stockholm, Sweden
	Jean Guern	CNRS, Gif Sur Yvette, France
	Toshio Shimizu	Aloka Co. Ltd., Japan
	Shumji Nakamura	Aloka Co. Ltd., Japan
	Osamu Hasegawa	Nisshinbo Ind., Japan
	Fernando Davu	Oxford Glycosystems, NY
	Jack Preiss	Michigan State University, East Lansing
	James Prestegard	Yale University, New Haven, CT
	Alan Elbein	University of Arkansas, Little Rock, AR
	Gerald O. Aspinall	York University, Ontario, Canada
	George Whitney	Boston, MA
May 1991	Germain Puzo	CNRS, Toulouse, France
	Wolf-Dieter Reiter	MSU/DOE Plant Research Laboratory, East Lansing, MI
	Robert Rosenzweig	President of the American Association of Universities
	Kilian Dill	Dept. of Chemistry, Clemson University, Clemson, SC
	Olle Alsholm	Swedish Pulp and Paper Institute, Stockholm, Sweden
	Carol Arnosti	Woods Hole Oceanographic Institute, Woods, Hole, MA
	Sheilah Asher	Amgen, Inc., Thousand Oaks, CA
	Paul G. James	Glycomed, Inc., Alameda, CA
	Ludmila Novik	Miles, Inc., Cutter Biologicals, Berkeley, CA
	Mark Plucinsky	Centocor, Malvern, PA
	Maryline Sharp	Washington University School of Medicine, St. Louis, MO
	Joseph Andrade	Baxter Hyland, Duarte, CA
	Alexis Eberendu	Carrington Labs, Inc., Dallas, TX
	Sylvia Harwig	UCLA Center for Health Sciences, Los Angeles, CA
	David Hawke	Applied Biosystems, Foster City, CA

	Rebecca Robbins	University of California, Los Angeles, CA
	Gary Rogers	Amgen, Inc., Thousand Oaks, CA
	Dave Sertl	Ross Laboratories, Columbus, OH
	P.K. Tsai	Merck Sharp & Dohme, West Point, PA
	Kerstin Skoog	University of Lund, Sweden
	Lisbeth Olson	University of Lund, Sweden
	Sarah Hake	USDA, Plant Gene Expression Laboratory, Albany, CA
	Michael Spellman	Genentech, Inc., San Fransisco, CA
	Gerald Hart	The Johns Hopkins University School of Medicine, Baltimore, MD
	James Prestegard	Yale University, New Haven, CT
	A.L. Burlingame	University of California, San Francisco, CA
	David Bundle	National Research Council of Canada, Ottawa, Canada
June 1991	George Whitney	Boston, MA
	Peter Doerner	The Salk Institute for Biological Studies, La Jolla, CA
	Marcia Kieliszewski	MSU-DOE Plant Research Laboratory, East Lansing, MI
July 1991	Myron Williams	ICGEB, New Delhi, India
	Seiji Sanoke	Osaka University, Japan
	Koichi Mezuno	Osaka University, Japan
	Zhu Cheng	Department of Biology, Peking University
August 1991	Deborah Delmer	Hebrew University, Israel
	Vera Lozovaya	USSR Academy of Sciences
	George H. Whitney	Boston, MA
September 1991	Larry Griffing	Texas A&M University
October 1991	Fons Voragen	Wageningen Agricultural University, The Netherlands
	Phil Enriquez	Advanced Magnetics, Cambridge, MA
	Atsushi Komamine	Tohoku University, Sendai, Japan
	Isao Ishida	Kirin Brewery Company, Gunma, Japan
	Jürgen Ebel	Albert-Ludwigs University, Freiburg, Germany
	Bill Phillips	Kodak, Kingsport, TN
	George Whitney	Boston, MA
	Charles E. Hess	University of California, Davis
	Yongzheng Hui	Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences
	Yulin Wu	Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences
	Guanghui Wang	Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences
	Xiuwen Han	DaLian Institute of Chemical Physics, China
December 1991	G.P. Kaushal	University of Arkansas
	Varsha Kaushal	University of Arkansas
January 1992	Philip Floersheim	Sandoz Pharma Ag, Basel, Switzerland
	Dante Marciani	Cambridge Biotech, MA
	Don Eastman	Vice President for Development, University of Georgia
February 1992	Anders Sonnesson	Chemical Center, University of Lund, Sweden
	Lihua Huang	Clemson University, South Carolina
	Robert Goldberg	Thinking Machines, Chevy Chase, MD

	Yoshifumi Jigami	National Chemical Laboratory for Industry, Agency of Industrial Science and Technology, MITI, Japan
	Tetsuo Hitouji	Asahi Chemical Industry Co., Ltd., Japan
	Tadashi Makino	Mitsui-Toatsu Chemicals, Inc., Japan
	Hiroshi Ikenaga	Kirin Brewery Co., Ltd., Japan
	Makoto Takeuchi	Kirin Brewery Co., Ltd., Japan
	Katsumi Ajisaka	Meiji Milk Products Co., Ltd., Japan
	Tadayoshi Kawasaki	Pharmacia Biosystems K.K., Japan
	Ake Danielsson	Pharmacia LKB Biotechnology, Sweden
	Ernst Rietschel	Borstel Research Institute, Germany
March 1992	Zamas Lam	DuPont, Wilmington, DE
	Paul Johnson	University of North Dakota, Grand Forks, ND
	Ron Lou Doong	University of Florida
April 1992	Ron Orlando	Suntory Institute for Bioorganic Research, Osaka, Japan
	James Prestegard	Yale University
	James Paulson	Cytel Corporation, San Diego, CA
	Ole Hindsgaul	University of Alberta, Edmonton, Canada
	David Bundle	National Research Council, Canada
	Akira Kobata	University of Tokyo
	Ajit Varki	University of California, San Diego
	Gerald Hart	Johns Hopkins University
	David Puett	University of Miami
	F. Robert Tabita	Department of Microbiology, Ohio State University
	John Magnani	Gaithersburg, MD
	Dante Marciani	Cambridge Biotech, Cambridge, MA
May 1992	Takayoshi Higuchi	Wood Research Institute, Kyoto University, Japan

Appendix 1. CCRC Chronology, Advisers, Visitors, and Interactions

Center-Wide Weekly Research Seminar Speakers, 1991 - 1992

October 16, 1991

William S. York[1]	Structural and Conformational Analysis of Xyloglucans
Michael Pierce[9]	Cloning of GnT-V

October 23, 1991

Kelley Moremen[8]	Purification and Cloning of Glycoprotein Processing Enzymes
Jamie De Stefano[10]	The Characterization of the Cyst Wall of *Pneumocystis carinii*

October 31, 1991

Richard Cummings[3]	GMP140: A Lectin Involved in Neutrofil Adhesion
Jörg Puhlmann[5]	Characterization of Epitopes Recognized by Monoclonal Antibodies Against Cell Wall Polysaccharides

November 6, 1991

Raja Sterjiades[4]	Isolation and Characterization of Laccase from Sycamore Cell Cultures
Russell Carlson[2]	Characterization of Nodulation Factors in Nitrogen Fixation

November 13, 1991

Marianne Zsiska[6]	Conformational Calculations of the N-Terminal Sequence of Glycophorin A
Malcolm O'Neill[1]	Analysis of Bioactive Oligosaccharides and Plant Cell Wall Polysaccharides by High Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD)

November 20, 1991

Roberta Merkle[3]	Biosynthesis of the HIV Envelope Glycoproteins gp160, gp120 and gp41
Debra Mohnen[7]	Tobacco Thin Cell Layer (TCL) Morphogenesis: A Model Organogenesis System

December 4, 1991

J.-J. Cheong[5]	Elicitor-Binding Proteins
Michael Pierce[9]	Studies on Fucosyltransferase

January 15, 1992

Herman van Halbeek[10]	Glycobiology and Drug Design

January 22, 1992

Carl Bergmann[1]	Investigation of the Interaction of PGIP with EPG as a Model System for Plant Defense Against Pathogens

January 29, 1992	Karl-Heinz Ott[6]	The Structure and Dynamics of the LeuA Operator Analyzed by NMR Measurements and Molecular Dynamics Calculations
February 5, 1992	Debra Mohnen[7]	Biosynthesis of Polygalacturonides: Synthesis of UDP Galacturonic Acid
February 12, 1992	Jeff Dean[4]	Lignin Biosynthesis in Vascular Plants
February 19, 1992	Kelley Moremen[8]	Molecular Biology of Processing Mannosidases
February 26, 1992	Daotian Fu[10]	Structural Characterization Studies of gp120, the Envelope Glycoprotein of HIV-1
March 4, 1992	Kevin Brady[1]	The Possible Role of Glycine-Rich Proteins in the Protection of Tobacco Against TMV
March 11, 1992	Philip Buckhaults[9]	Oncogenes and Oligosaccharides
March 18, 1992	Peter Albersheim[1]	New Evidence that Oligogalacturonides are Important as Biological Regulators
April 1, 1992	Francois Côté[5]	Functional Cloning of the Gene for a Soybean Elicitor-Binding Protein
April 15, 1992	Ramadas Bhat[2]	Rhizobium Lipopolysaccharide Structure: Evidence for a Role in Symbiosis
April 27, 1992	John Magnani	The Tumor Markers Sialyl Le^a and Sialyl Le^x Function as Ligands in Cell Adhesion

[1]Albersheim/Darvill group
[2]Carlson group
[3]Cummings group
[4]Eriksson group
[5]Hahn group
[6]Meyer group
[7]Mohnen group
[8]Moremen group
[9]Pierce group
[10]Van Halbeek group

Appendix 1. CCRC Chronology, Advisers, Visitors, and Interactions

CCRC Industrial Interactions

Training Course participants have come from:
AMGEN, Inc.
Applied Biosystems
Baxter Healthcare Corporation (Hyland Division)
Carrington Labs, Inc
Centocor, Inc.
Eli Lilly
Glycomed, Inc.
Lederle-Praxis Biologicals
Merck Sharp & Dohme
Miles, Inc. (Cutter Biological Division)
Oncogen
Ross Laboratories
The Upjohn Company

Collaborations:
AMGEN, Inc.
Cambridge Biotech (formerly Cambridge BioScience)
DuPont
Eli Lilly
Genencor International
Genentech
Sandoz
Texaco

Analytical services (DOE Center):
ABC Labs, Inc.
Advanced Magnetics, Inc.
Cambridge Biotech
Eastman Chemical Company
ESCA Genetics Corp.
Immunsine Corporation (Orinda, CA)
Immunsine Corporation (San Leandro, CA)
Interchem
Merrill-Dow
Noramco, Inc.
PathoGene
Ross Laboratories
WRRC

Analytical services (Resource Center):
Berlex Biosciences
Biogen, Inc.
Centocor, Inc.
Stolle Research & Development Corp.

Samples/protocols sent to outside investigators at:
Bayer AG
Carlsberg Research Center
Sandoz

CCRC-trained students and postdocs hired by:
Alpha Beta Technology
Analytical Biochemistry Laboratories, Inc.
Applied Biosystems
BASF AG
Cambridge Neuroscience
Cytel Corporation
DuPont
Eli Lilly
Genencor International
Glycogen
Hewlett Packard Corporation
Miles Laboratories, Inc.
Nestle Research Centre
Philip Morris
Shell Oil Company

CarbBank users located at:
A.E. Staley (USA)
Agriculture Canada (Canada)
Ajinomoto Co., Inc.
Allelix Biopharmaceuticals (Canada)
American Type Culture Collection
Amgen (USA)
Analytical Biochemical Labs (USA)
Anver Bioscience Design
Apple Investments
Applied Biosystems
BASF (Germany)
BASF AG (Germany)
Baxter Healthcare (USA)
Bayer AG (Germany)
Bayer Yakuhin Ltd. (Japan)
Beckman (USA)
Becton Dickinson (USA)
Beecham Wulfing (Germany)
BioCarb Technology AB (Sweden)
Biochemisches Inst. (Germany)
Bioengineering News (USA)
Bio-Informatics (Switzerland)
BioGen (USA)
Biogene Source Ltd. (Philippines)
Biostructure (France)
Biostructures Unit (Italy)
Biosym Technologies (USA)
Biotechnology Research Institute (Canada)
Boehringer Mannheim GmbH (Germany & USA)
Bristol Myers (USA)
British Bio-technology Ltd. (U.K.)

CA Pharmaceuticals (Greece)
Carrington Labs (USA)
Chemical Abstracts Service (USA)
Ciba-Geigy (U.K.)
CID SCIC (Spain)
CSIRO (Australia)
Cytel (USA)
Cytogen (USA)
DIAMED (USA)
Dionex (USA)
DNASTAR
E. Merck (Germany)
Eli Lilly
ENZON (USA)
Enimont America, Inc. (USA)
Eurobio (France)
European Patent Office (The Netherlands)
FMC Corp. (USA)
Fundacion Campomar (Argentina)
GenBank (USA)
Genentech (USA)
General Foods USA (USA)
Genzyme (USA)
Glycomed
Health Scientific West (USA)
Hoffmann LaRoche (Switzerland)
Hollister-Stier Lab (USA)
ICI Americus (USA)
Iketon Farmaceutical (Italy)
Interbio Limited (Isle of Man)
Irdex (Sweden)
JIPID (Japan)
JPL (USA)
Japan Immunoresearch Labs (Japan)
Kelco (USA)
Kyowa Hakko Kogyo Co. (Japan)
Lab Biochemical Structurale (France)
Lab Cassenne (France)
Lab Goemar (France)
Makor Chemicals Ltd. (Israel)
Martek Corp. (USA)
Master Chu Foods (USA)
Merrell-Dow Research Institution
Metsa-Seria Oy (Finland)
MicroCarb
Miles Cutter Lab (USA)
Mitsubishi Kasei (Japan)
Molecular Design Limited (USA)
Monsanto (USA)
Nature (U.K.)
Nestle (Switzerland)
Novo Nodisk
Nutrasweet (USA)
OMEC International (USA)
Oncogen (USA)
Oxford Glycosystems (U.K.)

PFI (Norway)
Pfizer, Inc. (USA)
Philip Morris
PLIVA (Yugoslavia)
Polybios (Trieste, Italy)
Roussel Uclaf (France)
Sales Consultants of Tucson (USA)
Sandoz Agro AG
Schwarz Pharma
Science (USA)
Smith Line & French Labs (USA)
Squibb Inst (USA)
SRI International
Styrechale (U.K.)
Symbicom AB (Sweden)
Taisho Pharmaceutical Co.
Takara Shuzo
TNO, Inc.
Tranel (USA)
Tripos (USA)
The Upjohn Company
Union Chemical Labs (Taiwan)
UNIVAX Biologics (USA)
UOP (USA)
UWCC (U.K.)
Wellcome Research Labs (U.K.)

Grants/contracts from:
Digital Equipment Corporation
Gold Kist
Sandoz Ltd.

The Center for Plant and Microbial Complex Carbohydrates at the University of Georgia Complex Carbohydrate Research Center

1987-92 Five-Year Report and 1993-97 Renewal Application

Appendix 2: Research Accomplishments 1987-April 1992

Collaborative Projects at the CCRC

Publications and Abstracts Citing the Plant Science Center Grant

Graduate Student Research Projects Supported by the Plant Science Center Grant

Faculty Research Interests

May 1992

Appendix 2. Research Accomplishments, 1987 - April 1992

Total Collaborative Projects at the Complex Carbohydrate Research Center

[*indicates projects sponsored by the Plant Science Center Grant]

__Collaborations within the CCRC__:

*__Collaboration between Michael Hahn, Peter Albersheim, and Alan Darvill.__ Generation of monoclonal antibodies that recognize plant cell wall polysaccharides and characterization of the epitopes recognized by those antibodies. *(Ongoing)*

*__Collaboration between Michael Hahn, Herman van Halbeek, and Bernd Meyer.__ Studies of the solution conformation(s) of the hepta-β-glucoside elicitor using theoretical conformation calculations and high-field NMR spectroscopic methods. *(Ongoing)*

Collaboration between Bernd Meyer and Herman van Halbeek. The three-dimensional structure of sialyl oligosaccharides. *(Ongoing)*

Collaboration between Bernd Meyer and Herman van Halbeek. Infrared spectroscopy and artificial neural networks for the identification of sulfate groups in oligosaccharides. *(Ongoing)*

Collaboration between Bernd Meyer and Herman van Halbeek. Artificial neural networks for interpretation of the ^{1}H-NMR spectra of glycoprotein carbohydrate chains. *(Ongoing)*

*__Collaboration between Bernd Meyer, William York, and Jan Thomsen.__ Applications of cluster analysis to the interpretation of Metropolis Monte Carlo calculations. *(Ongoing)*

*__Collaboration between Bernd Meyer, William York, Jan Thomsen, and Leszek Poppe.__ Studies of the conformational properties of cyclic β-1,2-glucans by conformational energy calculations and NMR spectroscopy. *(Ongoing)*

*__Collaboration between Debra Mohnen, Alan Darvill, and Peter Albersheim (Joint graduate student - Brent Ridley).__ Label biologically active oligogalacturonides and use these labeled oligogalacturonides to identify oligogalacturonide-binding proteins (i.e., putative receptors) and their corresponding genes. *(Ongoing)*

*__Collaboration between Debra Mohnen, Stefan Eberhard, Alan Darvill, and Peter Albersheim.__ Developmental commitment of tobacco TCLs for organogenesis in response to plant hormonal and α-1,4-linked oligogalacturonides. *(Ongoing)*

Collaboration between Kelley Moremen and Michael Pierce. Regulation of metastasis by cell-surface glycosylation. *(Ongoing)*

Collaboration between Michael Pierce and Richard Cummings. Expression of polylactosamine and carbohydrate-binding proteins on cell surfaces. *(Ongoing)*

Collaboration between Michael Pierce and Herman van Halbeek. Structures of glycopeptides. *(Ongoing)*

*__Collaboration between Herman van Halbeek, Peter Albersheim, Alan Darvill, and Richard Cummings.__ The structures of HIV and CD4 carbohydrates. *(Ongoing)*

Collaboration between Herman van Halbeek and Richard Cummings. The interaction of sialyllactose and MAL (*Maackia amurensis* lectin) studied by NMR spectroscopy. *(Ongoing)*

*__Collaboration between Herman van Halbeek and Russell Carlson.__ Structure of a streptococcal adhesion carbohydrate receptor. *(Completed)*

*__Collaboration between Herman van Halbeek and Russell Carlson.__ NMR of rhizobia lipopolysaccharides. *(Completed)*

*__Collaboration between Herman van Halbeek, Peter Albersheim, William York, and Alan Darvill.__ The structure of plant cell wall xyloglucan polysaccharides. *(Completed)*

__Collaborations with Investigators from The University of Georgia:__

Collaboration between Richard Cummings and Milton J. Cormier (Department of Biochemistry, The University of Georgia). Enhanced detection of glycoconjugates and glycosyltransferase activities using recombinant bioluminescent proteins as detecting labels in ELISA-type assays. *(Ongoing)*

Collaboration between Karl-Erik Eriksson, Jeff Dean, and Melvin Fuller (Department of Botany, The University of Georgia). Gold labeling of enzymes and monoclonal antibodies for detection of enzymes and wood components. *(Ongoing)*

Collaboration between Karl-Erik Eriksson and David Whitmire (Department of Agricultural Engineering, The University of Georgia). To model, design, construct, and operate a process for xylanase-aided pulp bleaching. *(Ongoing)*

Collaboration between Karl-Erik Eriksson and Jim Rice (School of Forest Resources, The University of Georgia). Development of a glue for fiber boards from waste lignins. *(Ongoing)*

Collaboration between Michael Pierce and David F. Smith (Department of Biochemistry and CCRC Adjunct Faculty, The University of Georgia). Expression of novel glycosyltransferase activities in CHO and COS cells. *(Ongoing)*

Collaboration between Herman van Halbeek and Richard Fayrer-Hoskin (College of Veterinary Medicine, The University of Georgia). Analysis of glycosaminoglycan content of equine oviductal fluid and its role in sperm-egg interaction. *(Ongoing)*

Collaboration between Herman van Halbeek, E. Will Taylor and David C.K. Chu (Department of Medicinal Chemistry, The University of Georgia). NMR spectroscopic studies of the conformation of AZT analogs and other potential anti-AIDS drugs. *(Ongoing)*

Collaboration between Herman van Halbeek and James Travis (Department of Biochemistry, The University of Georgia). The structure and function of the neutrophil proteinases elastase and cathepsin G involved in lung emphysema. *(Ongoing)*

Collaboration between Herman van Halbeek and Barney Whitman (Department of Microbiology, The University of Georgia). Structural analysis of leucine and isoleucine metabolites in *Methanococcus voltae*. *(Ongoing)*

*__Collaboration between Peter Albersheim, Alan Darvill, Bernd Meyer, William York and Jeffrey Sellers, and Torben Hansen and Donald Nute (Artificial Intelligence Program, The University of Georgia).__ Identification of the ^{1}H-NMR spectra of complex oligosaccharides with artificial neural networks. *(Ongoing)*

Collaboration between Herman van Halbeek and Richard Hill (Department of Chemistry, The University of Georgia). Structural analysis of camphor oxime fragmentation products. *(Completed)*

Collaboration between Herman van Halbeek and Irv Honigberg (College of Pharmacy, The University of Georgia). NMR studies on selectivity in association of albuterol and ractopamine with β_2- and β_1-adrenergic peptide sequences. *(Completed)*

Collaboration between Herman van Halbeek and Lars Ljungdahl (Department of Biochemistry, The University of Georgia). Structural analysis of a menaquinane from *Clostridium thermoautotrophicum* and *Clostridium thermoaceticum*. *(Completed)*

Collaboration between Herman van Halbeek and Charles Stammer (Department of Chemistry, The University of Georgia). NMR and conformational analysis of biologically active peptides. *(Completed)*

Collaboration between Herman van Halbeek and David F. Smith (Department of Biochemistry and CCRC Adjunct Faculty, The University of Georgia). Structural analysis of a novel sialyl-hexasaccharide from human milk. *(Completed)*

Collaborations with Outside Investigators:

Peter Albersheim and Alan Darvill

***Collaboration between Peter Albersheim, Alan Darvill, Roberta K. Merkle and Izabella Poppe, and Wim J. Broothaerts (Laboratory of Plant Pathology, Katholieke Universiteit, Leuven, Belgium).** Characterization of the self-incompatibility glycoproteins from *Petunia hybrida*. *(Ongoing)*

***Collaboration between Peter Albersheim, Alan Darvill and Carl Bergmann, and Felice Cervone and Giulia De Lorenzo (University of Rome, Italy).** Studies on the polygalacturonase-inhibiting proteins and their effect on pectic enzymes from microbes and plants. *(Ongoing)*

***Collaboration between Peter Albersheim, Alan Darvill, and Bernard Fritig (CNRS-IBMP, University of Strasbourg, France).** Characterization of the glucan elicitor of the GRP gene in *Nicotianae*. *(Ongoing)*

***Collaboration between Peter Albersheim, Alan Darvill, Malcolm O'Neill and Mark Spiro, and Jean Guern (CNRS Plant Science Institute, Gif sur Yvette, France).** Oligogalacturonide uptake in cultured tobacco cells. *(Ongoing)*

***Collaboration between Peter Albersheim, Alan Darvill, and Jean Guern (CNRS Plant Science Institute, Gif sur Yvette, France).** Structural requirements of the oligogalacturonide for K^+ efflux to establish whether oligogalacturonides are internalized and what effect this process has on plasma membrane potential. *(Ongoing)*

***Collaboration between Peter Albersheim, Alan Darvill, Bernd Meyer, Rainer Stulke-Prill and William York, and Samuel Levy and L. Andrew Staehelin (University of Colorado, Boulder).** Three-dimensional structure of xyloglucans; mechanism of binding to cellulose. *(Ongoing)*

***Collaboration between Peter Albersheim, Alan Darvill, and Ben Lugtenberg (University of Leiden, The Netherlands).** Studies of *Rhizobium* extracellular polysaccharides. *(Ongoing)*

***Collaboration between Peter Albersheim, Alan Darvill, Carl Bergmann, William Friedman (Department of Botany, The University of Georgia), and Felice Cervone and Giulia De Lorenzo (University of Rome, Italy).** Investigation of the molecular basis of a plant defense response. *(Ongoing)*

Collaboration between Peter Albersheim, Alan Darvill and Chris Somerville (MSU-DOE Plant Research Laboratory, Michigan State University, E. Lansing). Characterization of *Arabidopsis* cell wall mutants. *(Ongoing)*

Collaboration between Peter Albersheim, Alan Darvill, Michael Hahn, and L. Andrew Staehelin (University of Colorado, Boulder). Immunolocalization of epitopes recognized by rhamnogalacturonan I-specific and xyloglucan-specific monoclonal antibodies. *(Ongoing)*

***Collaboration between Peter Albersheim, Alan Darvill, Sheng-Cheng Wu, and Barbara Valent (E.I. DuPont de Nemours & Company, Wilmington, Delaware).** Studies of β-xylanases in host-pathogen interactions. Mapping the *M. grisea* 22kd xylanase gene. *(Ongoing)*

***Collaboration between Peter Albersheim, Alan Darvill, John Glushka and William S. York, and Adam Kondorosi (CNRS Plant Science Institute, Gif sur Yvette, France).** Studies of rhizobia nod factors. *(Ongoing)*

***Collaboration between Peter Albersheim, Alan Darvill, Marly Eidsness, and Adam Kondorosi (CNRS Plant Science Institute, Gif sur Yvette, France).** Studies of *endo*polygalacturonases in rhizobia. *(Ongoing)*

***Collaboration between Peter Albersheim, Alan Darvill, Carl Bergmann, and Dierk Scheel (Max-Planck Institute, Köln, Germany).** Studies of *endo*polygalacturonases in host-pathogen interactions. *(Ongoing)*

***Collaboration between Peter Albersheim, Alan Darvill, and Michael McNeil and Cecil Stushnoff (Department of Microbiology, Colorado State University, Fort Collins).** Effects of oligosaccharides on cold hardening of plant tissues. *(Ongoing)*

***Collaboration between William S. York, John Glushka, and Herman Spaink and Ben Lugtenberg (University of Leiden, The Netherlands).** Determination of the structure of the biologically active lipooligosaccharide from *Rhizobium leguminosarum* by spectroscopic methods. *(Ongoing)*

***Collaboration between Peter Albersheim, Alan Darvill and Christopher Augur, and Nicole Benhamou (Université Laval, Québec, Canada).** Localization of α-fucosidase in pea tissues. *(Ongoing)*

***Collaboration between Peter Albersheim, Alan Darvill, Carl Bergmann and Yuki Ito, and Nicole Benhamou (Université Laval, Québec, Canada).** Localization of polygalacturonase-inhibiting protein (PGIP) in bean tissues. *(Ongoing)*

***Collaboration between Peter Albersheim, Alan Darvill and Christopher Augur, and Tomoya Ogawa (RIKEN Institute, Japan) and Pierre Sinaÿ (École Normale Supérieure, France).** Investigation of the structure/function activities of xyloglucan oligosaccharides. *(Ongoing)*

***Collaboration between Peter Albersheim, Alan Darvill, and Texaco, Inc. (Beacon, New York).** Study of the structures of two bacterial polysaccharides with interesting rheological properties. *(Completed)*

__Russell W. Carlson__

*__Collaboration between Russell Carlson and Nick Brewin (John Innes Research Institute, Norwich, United Kingdom).__ Structural characterization of an LPS epitope that is specific in *Rhizobium leguminosarum* bv. *viciae* bacteroids. *(Ongoing)*

*__Collaboration between Russell Carlson and Adam Kondorosi (CNRS Plant Science Institute, Gif sur Yvette, France).__ Analysis of the LPSs and a novel capsular polysaccharide from *R. meliloti* mutants that are symbiotically defective (*fix23* mutants). *(Ongoing)*

*__Collaboration between Russell Carlson and Dale Noel (Department of Biology, Marquette University, Milwaukee).__ Characterization of the lipopolysaccharides from symbiotic mutants of *R. leguminosarum* bv. *phaseoli*. *(Ongoing)*

*__Collaboration between Russell Carlson and Gary Stacey (Department of Microbiology, University of Tennessee, Knoxville).__ Characterization of the lipopolysaccharide from a symbiotically defective mutant of *Bradyrhizobium japonicum*. *(Ongoing)*

*__Collaboration between Russell Carlson, V. Puvanesarajah (formerly with the CCRC), and Gary Stacey (Department of Microbiology, University of Tennessee, Knoxville).__ Structural determination of flavonoid glycosides from soybean which differentially activate *nod* genes from *B. japonicum*. *(Completed)*

*__Collaboration between Russell Carlson and Gary Stacey (Department of Microbiology, University of Tennessee, Knoxville).__ Structural determination of nodulation factors produced by *B. japonicum*. *(Ongoing)*

*__Collaboration between Russell Carlson and John Streeter (Department of Agronomy, The Ohio State University, Columbus).__ Structural analysis of a novel polysaccharide produced by *B. japonicum* bacteroids in soybean nodules. *(Ongoing)*

*__Collaboration between Russell Carlson and Hubert Mayer (Max-Planck Institute, Freiburg, Germany).__ Analysis of bacterial lipid As. *(Ongoing)*

*__Collaboration between Russell Carlson, Malcolm O'Neill, and Joyce Czop (Harvard Medical School, Boston).__ Structural characterization of a yeast glucan which activates the complement system. *(Completed)*

__Richard Cummings__

Collaboration between Richard Cummings and Caroline Enns (Syracuse University, New York). Glycosylation of the human transferrin receptor. *(Ongoing)*

Collaboration between Richard Cummings and David F. Smith, and John Lowe (Howard Hughes Medical Institute, Ann Arbor). Regulation of glycoprotein and glycolipid expression: characterization of glycoconjugates synthesized by Chinese hamster ovary cell lines permanently expressing cloned human glycosyltransferases. *(Ongoing)*

Collaboration between Richard Cummings, Roberta K. Merkle, and William Haseltine (Dana Farber Cancer Institute, Cambridge). Analysis of the carbohydrates of HIV and its receptor. *(Ongoing)*

Collaboration between Richard Cummings, Roberta K. Merkle and Izabella Poppe, and Marvin Tanzer, University of Connecticut Health Center, Farmington). Structural analyses of oligosaccharides of bioactive laminin glycopeptides isolated from mouse EHS laminin. *(Ongoing)*

Collaboration between Richard Cummings and Rodger McEver (Oklahoma Medical Research Foundation, Oklahoma City). Structure/function of glycoconjugate ligands for the endothelial selectin GMP-140. *(Ongoing)*

Collaboration between Richard Cummings and Caroline Enns (Department of Biology, Syracuse University, New York). Separation of animal cell-derived oligosaccharides. *(Completed)*

Collaboration between Richard Cummings and John Lowe (Howard Hughes Medical Institute, Ann Arbor). Enzymes involved in the synthesis of complex carbohydrates of animal origin. *(Completed)*

Collaboration between Richard Cummings, Roberta K. Merkle, and Michihiko Kuwano (Department of Biochemistry, Oita Medical School, Japan). The dysfunctional LDL receptor in Mon^{r}31 lacks some *O*-linked oligosaccharides. *(Completed)*

<u>**Karl-Erik L. Eriksson**</u>

Collaboration between Karl-Erik L. Eriksson, Jan Yang, and Rudy Singh (North Carolina State University, Raleigh). Development of a new enzyme-, oxygen-, ozone-, and hydrogen peroxide-based technique for pulp bleaching. *(Ongoing)*

Collaboration between Karl-Erik Eriksson, Jeff Dean, and Ron Dinus and David Webb (Institute of Paper Science and Technology, Atlanta). Studies of lignin biosynthesis in cell cultures of forest trees, manipulation of lignin biosynthesis. *(Ongoing)*

<u>**Michael G. Hahn**</u>

Collaboration between Michael G. Hahn, Per J. Garegg (University of Stockholm, Sweden) and Tomoya Ogawa (RIKEN Institute, Japan). Structure-activity studies of synthetic oligoglucosides structurally related to the hepta-β-glucoside elicitor. *(Ongoing)*

***Collaboration between Michael G. Hahn and Larry Griffing (Department of Biology, Texas A&M University, College Station).** Localization of immunogold-labeled glucan elicitor preparations in plant cells and protoplasts. *(Ongoing)*

***Collaboration between Michael G. Hahn and Keith Roberts (John Innes Institute, Norwich, Great Britian).** Immunolocalization of plant cell wall polysaccharides using monoclonal antibodies. *(Ongoing)*

***Collaboration between Michael G. Hahn and Chris Somerville (Michigan State University, E. Lansing).** Use of monoclonal antibodies to select for plant cell wall mutants. *(Ongoing)*

Bernd Meyer

Collaboration between Bernd Meyer and Inka Brockhausen (Toronto, Canada). Three-dimensional structure of *O*-type oligosaccharides. *(Ongoing)*

Collaboration between Bernd Meyer and S. Koepper (University of Oldenburg, Germany). Conformational analysis of kijanimycin. *(Ongoing)*

Collaboration between Bernd Meyer and Thomas Peters (Frankfurt, Germany). Three-dimensional structure of ribonuclease B. *(Ongoing)*

Collaboration between Bernd Meyer and Thomas Peters (Frankfurt, Germany). Development of Monte Carlo procedures. *(Ongoing)*

Debra Mohnen

***Collaboration between Debra Mohnen and Herman Spaink (Department of Plant Molecular Biology, Leiden University, The Netherlands).** Effect of *Rhizobium leguminosarum* extracellular oligosaccharide factors on tobacco thin cell-layer morphogenesis. *(Completed)*

Kelley Moremen

Collaboration between Kelley Moremen and Annette Herscovics (McGill Cancer Center, Montreal, Canada). Cloning of variant forms of $\alpha(1,2)$mannosidases. *(Ongoing)*

Collaboration between Kelley Moremen and Michiko Fukuda (La Jolla Cancer Research Foundation, San Diego). Characterization of the molecular defect in congenital dyserythropoetic anemia type II (HEMPAS). *(Ongoing)*

Collaboration between Kelley Moremen and John Schutzbach (Department of Microbiology, University of Alabama, Birmingham). Cloning and characterization of rabbit and murine $\alpha(1,2)$mannosidases. *(Ongoing)*

Collaboration between Kelley Moremen and Marilyn Farquhar (Division of Cellular and Molecular Medicine, University of California, San Diego). Subcellular localization of processing α-mannosidases. *(Ongoing)*

Collaboration between Kelley Moremen and Jürgen Roth (Department of Cell and Molecular Pathology, Institute of Pathology, University of Zurich Medical School, Switzerland). Characterization of the structural determinants for Golgi retention of glycosyltransferases and processing hydrolases. *(Ongoing)*

Michael Pierce

Collaboration between Michael Pierce and Mary Bunge (University of Miami Medical School, Miami). Regulation of cell interactions in the central nervous system. *(Ongoing)*

Collaboration between Michael Pierce and Gerhard Dahl (University of Miami Medical School, Miami). Asymmetric expression of cell-surface oligosaccharides on *Xenopus* oocytes. *(Ongoing)*

Collaboration between Michael Pierce and Nevis Fregien (University of Miami Medical School, Miami). Co-transformation of Lec1 CHO cells with *N*-acetylglucosaminyltransferase I activity and a selectable marker. *(Ongoing)*

Collaboration between Michael Pierce and Ole Hindsgaul (University of Alberta, Canada). Purification of glycosyltransferases. *(Ongoing)*

Collaboration between Michael Pierce and Henri Lichenstei (Amgen). Carbohydrate binding proteins in neural tissue. *(Ongoing)*

Collaboration between Michael Pierce and Vance Lemmon (Case Western Reserve University, Cleveland). Charaterization of an acetylated ganglioside in developing chick retina. *(Ongoing)*

Collaboration between Michael Pierce and Monica Palcic (University of Alberta, Canada). Kinetics of glycosyltransferases and cell uptake of glycosyltransferase inhibitors. *(Ongoing)*

Collaboration between Michael Pierce and Melvin Silberklang (Merck, Sharp, and Dohme, Co.). Studies on recombinant glycoproteins. *(Ongoing)*

Collaboration between Michael Pierce and Tien-Wen Tao (Pharmagenetics, Inc.). Regulation of metastasis by cell-surface glycosylation. *(Ongoing)*

<u>Herman van Halbeek</u>

Collaboration between Herman van Halbeek and Jack Alhadeff (Lehigh University, Bethlehem). Structural analysis of carbohydrate moieties of human liver α-L-fucosidase. Structural analysis of *N*-linked carbohydrates of glycoproteins. Structural analysis of the *N*-glycans of a recombinant hepatitis B surface antigen derived from yeast. Structural analysis of IgG_4 carbohydrates. Structural analysis of the carbohydrates of human ribonucleases. *(Ongoing)*

Collaboration between Herman van Halbeek and Robert Cherniak (Georgia State University, Atlanta). The structure of glucuronoxylomannans from *Cryptococcus neoformans*. *(Ongoing)*

Collaboration between Herman van Halbeek and Volker Dube (Medical College of Georgia, Augusta). The carbohydrate structures of ovarian cyst mucins. *(Ongoing)*

Collaboration between Herman van Halbeek and Edward Hogan (Medical University of South Carolina, Charleston). Structural analysis of a disialoganglioside of the globo-series from chicken skeletal muscle. Structural analysis of novel branched monosialogangliosides from bovine erythrocytes. *(Ongoing)*

Collaboration between Herman van Halbeek and Roger O'Neill (previously with Genencor, currently with Applied Biosystems, Foster City). The structure of the carbohydrate moieties of recombinant glycoproteins. *(Ongoing)*

Collaboration between Herman van Halbeek and John Pierce (University of California, Los Angeles). Structure and conformation of the pituitary glycoprotein hormone carbohydrates. *(Ongoing)*

Collaboration between Herman van Halbeek and Terrone Rosenberry (Case Western Reserve University, Cleveland). The structure of the glycosylphosphatidylinositol (GPI) anchor of human acetylcholinesterase (AChE). *(Ongoing)*

Collaboration between Herman van Halbeek and Anne Sherblom (Previously of University of Maine, currently with National Institutes of Health, Bethesda). Structural analysis of human and bovine uromodulin. *(Ongoing)*

Collaboration between Herman van Halbeek and B.N. Singh (State University of New York). Structural analysis of glycophosphosphingolipids of *Tritrichomonas foetus* and *Tritrichomonas vaginalis*. *(Ongoing)*

Collaboration between Herman van Halbeek and Michael Spellman (Genentech, San Francisco). The structures of HIV gp120 and CD4 carbohydrates, and of other recombinant glycoproteins. *(Ongoing)*

Collaboration between Herman van Halbeek and Eric Watson (AMGEN, Thousand Oaks). The carbohydrate structures of recombinant erythropoietin glycoproteins. Structural analysis of carbohydrates from granulocyte colony-stimulating factor (G-CSF). *(Ongoing)*

Collaboration between Herman van Halbeek, Carl Wilhelm Vogel and Channe Gowda (Georgetown University, Washington, DC). The structure of the carbohydrate chains of cobra venom factor (CVF). *(Ongoing)*

Collaboration between Herman van Halbeek and Betty Yan (Eli Lilly Research Laboratories, Indianapolis). The structure of the carbohydrate chains of recombinant glycoproteins. *(Ongoing)*

Collaboration between Herman van Halbeek and Edward C. Yurewicz (Wayne State University, Detroit). The structure of sulfated oligosaccharides from mucin glycoproteins. *(Ongoing)*

Collaboration between Herman van Halbeek and Michael McNeil (Colorado State University, Ft. Collins). NMR studies of acylated trehaloses. *(Ongoing)*

Collaboration between Herman van Halbeek and Leszek Poppe, and Guido Tettamanti and Sandro Sonnino (University of Milan, Italy). Conformational studies of gangliosides in micelles. *(Ongoing)*

Collaboration between Herman van Halbeek and Jamie De Stefano, and Peter D. Walzer (University of Cincinnati College of Medicine, Cincinnati). Surface carbohydrates of *Pneumocystis carinii*. *(Ongoing)*

Collaboration between Herman van Halbeek and Jacques Baenziger (Washington University, St. Louis). Structural analysis of the disulfated oligosaccharide from bovine lutropin. Structural analysis of the asparagine-linked oligosaccharides of bovine fetuin. *(Completed)*

Collaboration between Herman van Halbeek and Veer P. Bhavanandan (Hershey Medical Center, Pennsylvania State, University Park). The structure of a dimer of 6'-aldehydo-raffinose. *(Completed)*

Collaboration between Herman van Halbeek and Roland Bourrillon (Paris, France). Structures of the carbohydrate chains of membrane glycoproteins isolated from human hepatoma. *(Completed)*

Collaboration between Herman van Halbeek, Russell Carlson, and Fred Cassels and Jack London (National Institutes of Health, Institute of Dental Research, Bethesda). Structure of a streptococcal adhesin carbohydrate receptor. *(Completed)*

Collaboration between Herman van Halbeek and Jukka Finne (Medical Biochemistry, University of Turku, Finland). Structural analysis of novel polyfucosylated *N*-linked glycopeptides from human small intestinal epithelial cells. *(Completed)*

Collaboration between Herman van Halbeek and David Himmelsbach (U.S. Department of Agriculture, Athens, Georgia). Structural characterization of soluble carbohydrates in lima bean seeds, in the phenolic compounds from *Centipede* grass roots and in *Nicotiana glutinosa*, and the assignment of ^{1}H and ^{13}C spectra of a sucrose ester from tobacco. *(Completed)*

Collaboration between Herman van Halbeek and Stuart Kornfeld (Washington University, St. Louis). Structural analysis of high-mannose oligosaccharides of *Dictyostelium discoideum* glycoproteins. *(Completed)*

Collaboration between Herman van Halbeek and Bente Nilsen (Oslo, Norway). Structural analysis of glycoprotein allergen *Art v* II from the pollen of mugwort (*Artemisia vulgaris* L.). *(Completed)*

Collaboration between Herman van Halbeek and Philippe Roussel (INSERM, Lille, France). Structural analysis of oligosaccharides from respiratory mucus glycoproteins of patients suffering from bronchiectasis, chronic bronchitis and cystic fibrosis. *(Completed)*

Collaboration between Herman van Halbeek and Richard Stevens (Harvard University, Cambridge). Structure of the carbohydrate chains of human aorta proteoglycans in atherosclerosis. *(Completed)*

Collaboration between Herman van Halbeek and Peter Waterman (Phytochemistry Research Laboratories, University of Strathclyde, Glasgow, Scotland). Structural analysis of a highly acylated trirhamnoside from *Mezzettia leptopoda* (*Annonaceae*). *(Completed)*

Appendix 2. Research Accomplishments, 1987 - April 1992

Publications and Abstracts Citing the Plant Science Center Grant

Publications

1989

The Complex Carbohydrate Research Center of The University of Georgia. 1989. *Plant Molecular Biology Reporter* **7**(1):49-56.

Carlson, R.W., F. Garcia, D. Noel, and R. Hollingsworth. 1989. The structures of the lipopolysaccharide core components from *Rhizobium leguminosarum* biovar *phaseoli* CE3 and two of its symbiotic mutants, CE109 and CE309. *Carbohydr. Res.* **195**:101-110.

Cervone, F., G. De Lorenzo, R. D'Ovidio, M.G. Hahn, Y. Ito, A. Darvill, and P. Albersheim. 1989. Phytotoxic effects and phytoalexin-elicitor activity of microbial pectic enzymes. *In: Phytotoxins and Plant Pathogenesis* (A. Graniti, R.D. Durbin, and A. Ballio, eds.), NATO ASI Series, Vol. H27. Springer-Verlag, Berlin, Heidelberg. pp. 473-477.

Cervone, F., G. De Lorenzo, G. Salvi, C. Bergmann, M.G. Hahn, Y. Ito, A. Darvill, and P. Albersheim. 1989. Release of phytoalexin elicitor-active oligogalacturonides by microbial pectic enzymes. *In: Signal Molecules in Plants and Plant-Microbe Interactions* (B.J.J. Lugtenberg, ed.), NATO ASI Series, Vol. H36. Springer-Verlag, Heidelberg. pp. 85-89.

Cervone, F., M.G. Hahn, G. De Lorenzo, A. Darvill, and P. Albersheim. 1989. Host-Pathogen Interactions XXXIII. A plant protein converts a fungal pathogenesis factor into an elicitor of plant defense responses. *Plant Physiol.* **90**:542-548.

Darvill, A.G., P. Albersheim, P. Bucheli, S. Doares, N. Doubrava, S. Eberhard, D.J. Gollin, M.G. Hahn, V. Marfà, W.S. York, and D. Mohnen. 1989. Oligosaccharins--plant regulatory molecules. *In: Signal Molecules in Plants and Plant-Microbe Interactions* (B.J.J. Lugtenberg, ed.), NATO ASI Series, Vol. H36. Springer-Verlag, Heidelberg. pp. 41-48.

Doares, S.H., P. Bucheli, P. Albersheim, and A.G. Darvill. 1989. Fungal enzymes, plant cell wall fragments, and plant cell death: A possible mechanism for hypersensitivity in the rice blast system. *In: Signal Molecules in Plants and Plant-Microbe Interactions* (B.J.J. Lugtenberg, ed.), NATO ASI Series, Vol. H36. Springer-Verlag, Heidelberg. pp. 229-233.

Doares, S.H., P. Bucheli, P. Albersheim, and A.G. Darvill. 1989. Host-Pathogen Interactions XXXIV. A heat-labile activity secreted by a fungal phytopathogen releases fragments of plant cell walls that kill plant cells. *Mol. Plant Interac.* **2**:346-353.

Eberhard, S., N. Doubrava, V. Marfà, D. Mohnen, A. Southwick, A. Darvill, and P. Albersheim. 1989. Pectic cell wall fragments regulate tobacco thin-cell-layer explant morphogenesis. *The Plant Cell* **1**:747-755.

Hahn, M.G. 1989. Animal receptors -- Examples of cellular signal perception molecules. *In: Signal Molecules in Plants and Plant-Microbe Interactions* (B.J.J. Lugtenberg, ed.), NATO ASI Series, Vol. H36. Springer Verlag, Heidelberg. pp. 1-26.

Hahn, M.G., P. Bucheli, F. Cervone, S.H. Doares, R.A. O'Neill, A. Darvill, and P. Albersheim. 1989. The roles of cell wall constituents in plant-pathogen interactions. *In: Plant Microbe Interactions* (E. Nester and T. Kosuge, eds.), Vol. 3. McGraw-Hill Publishing Company. pp. 131-181.

Hollingsworth, R.L. and R.W. Carlson. 1989. 27-hydroxyoctacosanoic acid is a major structural fatty acyl component of the lipopolysaccharide of *Rhizobium trifolii* ANU843. *J. Biol. Chem.* **264**:9300-9303.

Hollingsworth, R.L.. R.W. Carlson, F. Garcia, and D.A. Gage. 1989. A new core tetrasaccharide component from the lipopolysaccharide of *Rhizobium trifolii* ANU843. *J. Biol. Chem.* **264**:9294-9299.

Kiefer, L., W. York, A. Darvill, and P. Albersheim. 1989. The Structure of Plant Cell Walls XXVII. Xyloglucan isolated from suspension-cultured sycamore cell walls is *O*-acetylated. *Phytochem.* **28**:2105-2107.

O'Neill, R.A., P. Albersheim, and A.G. Darvill. 1989. The Structure of Plant Cell Walls XXVIII. Purification and characterization of a xyloglucan oligosaccharide-specific xylosidase for pea seedlings. *J. Biol. Chem.* **264**:20432-20437.

Thomsen, J.U. and B. Meyer. 1989. Recognition of NMR spectra by neural networks. *J. Magn. Res.* **84**:212.

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Albersheim, P. and A. Darvill. 1990. The commercial potential of plant cell wall polysaccharides and oligosaccharins. *In: Proceedings of the Symposium "Towards A Carbohydrate-Based Chemistry."* Commission of the European Communities. Amiens, France, October 23-26, pp. 175-196.

Bucheli, P., S.H. Doares, P. Albersheim, and A. Darvill. 1990. Host-Pathogen Interactions XXXVI. Partial purification and characterization of heat-labile molecules secreted by the rice blast pathogen that generates cell wall fragments that kill plant cells. *Physiol. Mol. Plant Pathol.* **36**:159-173.

Carlson, R.W., B. Lakshmi, U.R. Bhat, and G. Stacey. 1990. Isolation and analysis of the lipopolysaccharides from a symbiotic mutant of *B. japonicum*. *In: Nitrogen Fixation: Achievements and Objectives* (Gresshoff, Roth, Stacey and Newton, eds.). Chapman and Hall, New York/London.

Carrion, M., U.R. Bhat, B. Reuhs, and R.W. Carlson. 1990. Isolation and characterization of the lipopolysaccharides from *Bradyrhizobium japonicum*. *J. Bacteriol.* **172**:1725-1731.

Cervone, F., G. De Lorenzo, R. Pressey, P. Albersheim, and A.G. Darvill. 1990. Host-Pathogen Interactions XXXV. Can *Phaseolus* PGIP inhibit pectic enzymes from icrobes and plants? *Phytochem.* **29**:447-449.

De Lorenzo, G., Y. Ito, R. D'Ovidio, F. Cervone, P. Albersheim, and A.G. Darvill. 1990. Host-Pathogen Interactions XXXVII. Abilities of the polygalacturonase-inhibiting proteins from four cultivars of *Phaseolus vulgaris* to inhibit the *endo*polygalacturonases from three races of *Colletotrichum lindemuthianum*. *Physiol. and Mol. Plant Path.* **36**:421-435.

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Mohnen, D., S. Eberhard, V. Marfà, N. Doubrava, P. Toubart, D. Gollin, T. Gruber, W. Nuri, P. Albersheim, and A. Darvill. 1990. Control of flower, vegetative shoot, and root organogenesis in tobacco thin-cell-layer morphogenesis. *Development* **108**:191-201.

O'Neill, M., P. Albersheim, and A. Darvill. 1990. The pectic polysaccharides of primary cell walls. *In: Methods in Plant Biochemistry* (D.M. Dey, ed.), Vol. 2. Academic Press, London. pp. 415-441.

O'Neill, M.A., A.G. Darvill, P. Albersheim, and K.J. Chou. 1990. Structural analysis of an acidic polysaccharide secreted by *Xanthobacter* sp. (ATCC 53272). *Carbohydr. Res.* **206**:289-296.

Sellers, J., W. York, P. Albersheim, A. Darvill, and B. Meyer. 1990. Identification of the mass spectra of partially methylated alditol acetates by artificial neural networks. *Carbohydr. Res.* **207**:C1-C5.

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Cheong, J.-J. and M.G. Hahn. 1991. A specific, high-affinity binding site for the hepta-β-glucoside elicitor exists in soybean membranes. *The Plant Cell* **3**:137-147.

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O'Neill, M.A., A.G. Darvill, and P. Albersheim. 1991. The degree of esterification and points of substitution by *O*-acetyl and *O*-(3-hydroxybutanoyl) groups in the acidic extracellular polysaccharides secreted by *Rhizobium leguminosarum* biovars *viciae*, *trifolii*, and *Phaseoli* are not related to host range. *J. Biol. Chem.* **266**:9549-9555.

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Appendix 2: Research Accomplishments, 1987-April 1992

Graduate Student Research Projects Supported by the Plant Science Center Grant

One of the major functions of the center grant is the training of graduate students in complex carbohydrate science. During the first four and one-half years of the Grant (years two through six and a half of the CCRC), five students who were funded by the Grant completed their graduate degrees. The theses abstracts of the three Ph.D. and two Master's students are given below together with summaries of the current graduate student research projects funded by the Grant.

Theses Abstracts of Students Who Have Graduated

1. **Structural characterization of primary wall polysaccharides isolated from suspension-cultured cells of Douglas fir, maize, and rice. Jerry Ray Thomas (Ph.D. in biochemistry)**

The goal of the research described is to determine the degree to which the structures of the primary cell wall polysaccharides of higher plants have been conserved. This determination could not previously be made, because the primary cell wall polysaccharides of gymnosperms, and the pectic polysaccharides of monocots had not been characterized. To rectify this situation, polysaccharides from primary walls of Douglas fir cells, and pectic polysaccharides of maize and rice were isolated and characterized.

The primary cell walls of the gymnosperm, Douglas fir, are described. Douglas fir walls were found to contain the two hemicellulosic polysaccharides (xyloglucan and glucuronoarabinoxylan) found in angiosperms (monocots and dicots), and the three pectic polysaccharides (homogalacturonan, rhamnogalacturonan I, and rhamnogalacturonan II) found in dicots. Like dicot and unlike graminaceous monocot cell walls, gymnosperm cell walls have a high content of pectic polysaccharides, and the predominant hemicellulose is xyloglucan. It was concluded that the primary walls of Douglas fir are more like those of dicots than graminaceous monocots.

The pectic polysaccharide rhamnogalacturonan I was isolated from cell walls of maize and rice. Comparison of the products of chemical cleavage with lithium in ethylenediamine indicated that monocot and dicot rhamnogalacturonan I have many of the same structural features. The oligosaccharides previously obtained by lithium treatment of dicot rhamnogalacturonan I were also derived from maize rhamnogalacturonan I.

Rhamnogalacturonan II, previously unreported in a monocot, was isolated from cell walls of rice. Rice rhamnogalacturonan II was found to contain all the unusual monosaccharides and glycosyl linkages present in its dicot counterpart. Furthermore, two disaccharides, an aceryl acid-containing heptasaccharide, and a 2-*O*-methyl xylosyl-containing octasaccharide, both of which are components of dicot rhamnogalacturonan II, were isolated from rice rhamnogalacturonan II.

It was concluded that angiosperms and gymnosperms have the same primary cell wall polysaccharides and that most of the structural features of rhamnogalacturonans I and II have been conserved in the primary walls of monocots and dicots. Conservation of the structures of primary cell wall polysaccharides suggests that they have important functions.

[Jerry Thomas is presently a senior research associate in the laboratory of Professor J.F.G. Vliegenthart at the University of Utrecht, The Netherlands.]

2. Structural studies of extracellular polysaccharides and cell wall rhamnogalacturonan-II of sycamore cells. Thomas Towne Stevenson (Ph.D. in chemistry)

The water-soluble polysaccharides (SEPS) secreted into the medium by suspension-cultured sycamore cells were examined to determine whether the polysaccharides were the same as those present in the walls of sycamore cells. The following polysaccharides were found: xyloglucan, arabinoxylan, at least two arabinogalactans, rhamnogalacturonan-II (RG-II) and a polygalacturonic acid-rich polysaccharide. Evidence was obtained for the presence of a rhamnogalacturonan-I like polysaccharide. All of the above polysaccharides have been isolated from or are believed to be present in sycamore cell walls. Furthermore, all of the noncellulosic polysaccharides known to be present in sycamore cell walls appear to be present in the SEPS.

RG-II was chosen for further structural characterization. A previously unknown sugar, 3-deoxy-D-*lyxo*-2-heptulosaric acid (DHA) was identified as a component of RG-II isolated from both Pectinol (a commercially available preparation of cell wall-degrading enzymes) and sycamore cell walls as a part of the disaccharide β-L-ara*f*-(1→5)-D-DHA*p*. The structure of DHA was determined by chemical and spectroscopic methods. DHA was found to be present in the primary cell walls of many higher plants, including several dicots, two monocots, and a gymnosperm, but was not detected in two bacterial lipopolysaccharides.

End-group analysis of RG-II showed that it contained about 30 glycosyl residues. RG-II was treated with lithium metal dissolved in ethylenediamine to degrade the glycosyluronic acid residues. The major product was shown to be the triglycosyl alditol α-Xyl-(1→3)-α-Fuc-(1→4)-β-Rha-(1 →3')-apiitol. This tetrasaccharide fragment has three residues in common with a previously characterized heptasaccharide that had been derived from RG-II by partial acid hydrolysis. RG-II was found to contain a large number of branched galactosyluronic acid residues that are undoubtedly part of an octagalactosyluronic acid fragment generated by partial acid hydrolysis. The results of sequential partial acid hydrolysis provided evidence that in RG-II the 3-deoxy-D-*manno*-2-octulosonic acid and DHA residues are attached to *O*-3 of 3,4-linked galactosyluronic acid residues, and that the apiofuranosyl residues are attached to *O*-2 of 2,4-linked galactosyluronic acid residues. These and previously published data suggest that RG-II has a highly branched structure, arranged around an α-(1→4)-linked galactosyluronic acid backbone.

[Tom Stevenson is presently a research scientist, specializing in complex carbohydrate analysis at ABC Technology in Worcester, Massachusetts.]

3. Structural characterization of oligosaccharides enzymatically released from xyloglucan. Laura Lynn Kiefer (M.Sc. in biochemistry)

Xyloglucan oligosaccharides were enzymatically released from purified cell walls isolated from suspension-cultured sycamore cells. The *O*-acetyl substitution pattern of these oligosaccharides was determined by ^{1}H-NMR spectroscopy. This analysis revealed that most of the 2-linked galactosyl residues of the decasaccharide and nonasaccharide fragments of cell wall xyloglucan are *O*-acetyl substituted, while the heptasaccharide fragment does not contain *O*-acetyl substituents. This acetylation pattern does not differ significantly from that of the same xyloglucan oligosaccharides enzymatically released from the xyloglucan secreted by suspension-cultured sycamore cells.

An arabinose-containing heptadecasaccharide generated by *endo*polygalacturonase digestion of the xyloglucan secreted by suspension-cultured sycamore cells was structurally characterized. The structure of the heptadecasaccharide was unambiguously determined through combined data generated by ^{1}H-NMR spectroscopic, fast atom bombardment mass spectrometric (FAB-MS), and glycosyl-composition and glycosyl-linkage analyses of the intact heptadecasaccharide and of oligosaccharide fragments of the heptadecasaccharide. 1-D and 2-D ^{1}H-NMR analyses provided the anomeric configuration of the glycosidic linkages, as well as information about the glycosyl-residue and glycosyl-linkage compositions of the heptadecasaccharide. FAB-MS provided the molecular weight and supplied critical information about the glycosyl composition and glycosyl sequence of the heptadecasaccharide. The heptadecasaccharide was found to be a combination of previously characterized nona- and heptasaccharide components of xyloglucan. The nonasaccharide was shown to be glycosidically linked to the heptasaccharide through C-4 of the β-glucosyl residue at the non-reducing end of the heptasaccharide component. An arabinosyl residue was glycosidically linked at C-2 of the same β-glucosyl residue at the non-reducing end of the heptasaccharide component of the heptadecasaccharide. Although the presence of arabinosyl residues in sycamore xyloglucan has been recognized since 1973, the location of the arabinosyl residues had not been ascertained.

[Laura Kiefer left the University of Georgia to join her fiancé in North Carolina. She is presently a Ph.D. student at Duke University in Durham.]

4. Studies on elicitors of plant cell death: the release by pathogen-secreted enzymes of plant cell wall fragments that kill plant cells. Steven Humphrey Doares (Ph.D. in biochemistry)

We have hypothesized that plant cell wall fragments are a trigger for hypersensitive cell death, a fundamental defense response of plants that have been challenged by potential pathogens. The goal of this research was to demonstrate that plant cell wall fragments released by plant pathogen-secreted enzymes can kill plant cells. The rice blast fungus, *Magnaporthe grisea*, was found to secrete, when grown on plant cell walls, a heat-labile activity that killed suspension-cultured maize cells. Further, this heat-labile killing activity was shown to release heat-stable maize cell wall fragments that killed maize cells.

In an attempt to determine whether selected cell wall-degrading enzymes secreted by the fungus were responsible for the heat-labile killing activity, pectin lyase, pectin methylesterase, and a xylanase were purified to homogeneity from the culture filtrate of *M. grisea*. These enzymes individually or together did not kill suspension-cultured maize cells. However, the xylanase was able to release, to a limited degree, heat-stable maize cell wall fragments that killed maize cells.

The maize cell wall fragments solubilized by the fungal-secreted heat-labile killing activity were found to be composed primarily of arabinoxylan oligosaccharides, some of which were feruloylated. The killing activity of the wall fragments was destroyed by base treatment, suggesting that the feruloyl esters were necessary for the killing activity. Purification of the active component was not possible because of the nearly total loss of killing activity during attempted chromatographic separations. The loss of killing activity was shown to result from precipitation or from binding to the walls of glass vessels of a quantitatively minor component of the fragment mixture.

The results presented support the hypothesis that plant cell wall fragments are elicitors of the hypersensitive response. However, the fact that the killing activity of the cell wall fragments is due to a quantitatively minor component means that the structural nature of the cell wall fragment that possesses killing activity remains to be determined.

[Steve Doares is presently a postdoctoral research associate with Professor C. A. Ryan at Washington State University.]

5. **A chromatographic method for screening enzymes for the ability to fragment rhamnogalacturonan II, a plant cell wall pectic polysaccharide. Que Guo (M.Sc. in botany)**

High-performance anion-exchange chromatography with pulsed amperometric detection has been used to screen commercial enzyme preparations and culture medium of fungi isolated from soil for the presence of *exo-* and *endo*glycanases that are able to fragment rhamnogalacturonan II (RG-II), a cell wall pectic polysaccharide. RG-II-degrading rhamnosidase, arabinosidase, galactosidase, galacturonidase, and glucuronidase activities were found in four commercial enzyme preparations. Small amounts of oligosaccharides were released by treatment of RG-II with a Cellulase preparation from *Penicillium funiculosum*. GLC-MS (CI and EI) analyses of the methylated oligoglycosyl alditols derived from the released oligosaccharides provided evidence that the *P. funiculosum* Cellulase preparation contains an *endo*galacturonidase and an *endo*rhamnosidase. *Exo*galactosidase and *exo*galacturonidase activities were found in the culture medium of *Aspergillus nidulans* when that fungus was grown on RG-II as a carbon source. An enzyme extract of etiolated pea seedlings showed no RG-II-degrading ability.

[Que Guo left the University of Georgia to secure employment in order to seek permanent residence in the USA. He is presently a research scientist, specializing in the chromatography of complex carbohydrates, at Cambridge Neuroscience, Cambridge, Massachusetts.]

Descriptions of Current Graduate Student Research Projects

1. **Oligogalacturonides are able to induce flowers to form and to inhibit root formation on tobacco explants. Victòria Marfà.**

Oligogalacturonides (α-1,4-D-galactosyluronic acid oligomers) are fragments of the homogalacturonan component of the primary cell walls of higher plants. Treatment of cell walls with *endo*polygalacturonase (EPG) derived from microbes releases the polysaccharides rhamnogalacturonan-I (RG-I) and rhamnogalacturonan-II (RG-II) and variously sized oligogalacturonide fragments of homogalacturonan. The EPG-released sycamore cell wall components are able to regulate several morphogenetic processes in tobacco thin-cell layer (TCL) explants. We followed one of these morphogenesis-regulating activities, namely, the induction of flower formation on TCL explants, with the goal of purifying the biologically active component from EPG-released material. Saponification of the methyl and acetyl esters of the EPG-released material did not reduce the flower-inducing activity. However, EPG treatment of the de-esterified EPG-released material destroyed the flower-inducing activity, establishing that the active carbohydrates contained several, consecutive α-1,4-linked galactosyluronic acid residues that are required for the flower-inducing activity. The flower-inducing activity was purified by gel permeation and ion-exchange chromatographies and HPLC and shown to be α-1,4-linked oligogalacturonides with a degree of polymerization (DP) of 12 to 14, which exhibited half-maximum activity at approximately 0.4 μM. Smaller oligogalacturonides, RG-I, and RG-II did not, even at higher concentrations, induce flowers to form.

We have also shown that oligogalacturonides with DPs between 10 and 15 are able to inhibit the formation of roots of TCLs incubated on a root-inducing medium [15 μM indolebutyric acid (IBA) and 0.5 μM kinetin]. Our collaborators, Professor F. Cervone and his research group (University of Rome, Italy), have recently shown that oligogalacturonides also inhibit the formation of roots on tobacco leaf disk explants. Homogeneously sized oligogalacturonides with DPs from 8 to 20 are being purified by Dionex HPLC and will be tested individually on both the leaf disk and TCL explant bioassays to determine the exact DP range required for the inhibition of root formation. The ability of oligogalacturonides to stimulate the formation of flowers and inhibit root formation on tobacco explants provides further evidence of the pleiotropic nature of this oligosaccharin.

2. The generation of monoclonal antibodies specific for plant cell wall polysaccharide epitopes. Nancy Dunning.

This research is part of a project to generate monoclonal antibodies that bind to specific epitopes of plant cell wall polysaccharides. The goal of this research is to obtain a diverse panel of polysaccharide-specific antibodies for a variety of experimental applications including the purification of oligo- and polysaccharides, structural characterization of polysaccharides, localization of polysaccharides within plant cell walls and tissues, and selection of plant cell wall mutants.

Previous attempts to generate monoclonal antibodies through traditional spleen cell/myeloma fusion have resulted in limited success. Mice immunized with rhamnogalacturonan I (RG-I) isolated from the cell walls of suspension-cultured sycamore maple (*Acer pseudoplatanus*) cells yielded only low numbers of hybridomas secreting antibodies recognizing RG-I. Only 12 lines were obtained out of approximately 2,000 hybridomas screened. The antibodies secreted by these hybridomas fall into three reactivity groups, two of which were polysaccharide-specific. The low yield of positive secreting hybridomas results from the low immunogenicity of polysaccharides. Our laboratory is developing experimental approaches to increase the number and diversity of plant cell wall polysaccharide-specific antibodies available for our research.

One of our approaches involves the use of recombinant expression libraries producing antibody Fab fragments.[1] We propose to screen such libraries for immunoglobulin clones with the ability to bind to plant cell wall polysaccharides. We believe this approach will enable us to obtain many more useful monoclonal antibodies than would be possible through traditional hybridoma technology. The antibody cloning and expression system we will use was developed by Lerner's group[1] and is marketed by Stratacyte in kit form. In this procedure, antibody cDNAs corresponding to mRNAs are amplified from murine mRNA using primers to conserved areas on the $C_H1(\gamma1)$ and V_H regions of the heavy chain and $C_L(\kappa)$ and $V_L(\kappa)$ regions of the light chain. A λ phage expression vector is used to generate two cDNA libraries, one for the heavy chains and one for the light chains. These libraries are then combined randomly to form a third λ library that expresses antibody Fab fragments. This combinatorial library will be screened for antibody Fab fragments that bind carbohydrates by using a filter-binding assay analogous to a Western blot, but using labeled oligosaccharides as probes.[2] The advantage of this cloning procedure over conventional hybridoma technology is the ability to easily screen thousands of recombinant clones. In addition, a good immune response is not strictly required. This makes the technique particularly suited to carbohydrate antigens that tend to have low immunogenicity.

We are initially testing the utility of this technology by cloning the antibody-producing genes from the spleen cells of a well-immunized mouse and also using a hybridoma cell line (CCRC-M1) which produces an IgG_1 antibody that recognizes sycamore xyloglucan. The mouse was immunized with endopolygalacturonase (EPG), a microbial plant cell wall-degrading enzyme that was previously found to be highly immunogenic. Poly $(A)^+$ RNA from the spleen cells of this mouse will be used to test the amplification and cloning protocols of the Stratacyte kit. We will use the hybridoma cell line to develop techniques to screen phage expression libraries with labeled oligosaccharides. Any cloned immunoglobulin genes from this line will provide positive controls for subsequent experiments designed to clone immunoglobulins recognizing plant cell wall carbohydrates.

The following steps in this protocol have been carried out. Total RNA has been isolated from the spleen of a mouse immunized with EPG and from the hybridoma line, CCRC-M1. Poly $(A)^+$ RNA was purified by passing total RNA through an oligo dT column. The quality of the poly $(A)^+$ RNA was checked by northern blot using a commercially available gene probe to murine $C_L(\kappa)$ chain. The immunoglobulin mRNA present in the poly $(A)^+$ RNA was amplified by PCR. The PCR primers provided by Stratacyte failed initially to amplify the heavy chain of the hybridoma line (CCRC-M1), while a product of the proper length was readily obtained for the light chain. A PCR produce of the correct length was recently obtained for the heavy chain of CCRC-M1 by lowering the annealing temperature and using cDNA that had been prepared using a $C_H3(\gamma_1)$ primer instead of an oligo dT primer. A PCR product of the correct length for both heavy and light chains was obtained from EPG-immunized mouse spleen RNA. Libraries using all of these PCR products are currently being constructed.

We will continue our effort to clone the antibody-producing genes from the hybridoma (CCRC-M1). When functional antibody protein has been obtained from cDNA clones, the antibody Fab fragment produced from bacteria will be compared for binding affinities and specificity to the antibody protein produced by the hybridoma. Also, because the large size of an intact antibody molecule often limits its use in immunolocalizations, functional differences in immunolocalizations will be compared using the hybridoma-produced whole antibody molecule, the Fab fragment generated by papain digestion of the hybridoma-produced antibody, and the antibody Fab fragment produced in *E. coli.* The heavy chain Fab fragment from *E. coli* will be included in this comparison, provided the heavy chain displays specific binding in the absence of the light chain. A smaller molecule with the same specific binding of the large, whole antibody molecule may penetrate plant tissues more effectively resulting in more efficient and accurate immunolocalization studies.

Once these procedures are perfected, cDNA libraries from mice immunized with various cell wall carbohydrates will be constructed and screened for antibodies recognizing diverse plant cell wall carbohydrates. Those antibody Fab fragments displaying specificity to unique carbohydrate moieties will be characterized to determine the epitope structures recognized by the antibodies. The antibodies will be tested for their utility in immunolocalization studies, selection of cell wall mutants, and for the purification of oligo- and polysaccharides.

1. Huse, W.D., L. Sastry, S.A. Iverson, A.S. Kang, M. Alting-Mees, D.R. Burton, S.J. Benkovic, and R.A. Lerner. 1989. Generation of a large combinatorial library of the immunoglobulin repertoire in phage lambda. *Science 246*:1275-1281.

2. Cheong, J.-J. and M.G. Hahn. 1991. A specific, high-affinity binding site for the hepta-β-glucoside elicitor exists in soybean membranes. *The Plant Cell 3*:137-147.

3. Studies on xyloglucan oligosaccharins and on a cell wall α-fucosidase that is able to regulate xyloglucan oligosaccharin activity. Christopher Augur.

Oligosaccharide fragments (oligosaccharins) from bacterial, fungal and plant polysaccharides have biological regulatory activities. The demonstrated activities of oligosaccharins include the ability of xyloglucan oligosaccharides to inhibit auxin (2,4-dichlorophenoxyacetic acid)-induced growth of pea stem segments. Evidence in the literature suggests that xyloglucan oligosaccharides are involved in feedback inhibition of auxin-induced growth. Xyloglucan is a hemicellulose present in the cell walls of higher plants. This research has involved two complementary investigations into the ability of xyloglucan oligosaccharides to inhibit auxin-induced growth of pea stems. We have obtained new information about the structural requirements of xyloglucan oligosaccharides that inhibit auxin-induced growth of etiolated pea stem segments. We have also purified, from pea stems, an α-fucosidase that destroys the growth inhibiting activity of xyloglucan oligosaccharides. Using immunocytochemical methods, we were able to localize the α-fucosidase in the primary cell walls of pea tissues.

Treatment of xyloglucan with an *endo*-β-1,4-glucanase isolated from the fungus *Trichoderma viride* cleaves the β-1,4-glucan backbone at unbranched glucosyl residues, generating oligosaccharide subunits of the polymer. A nonasaccharide (XG9) from this digest has been shown to inhibit auxin-stimulated elongation of etiolated pea stem segments. The inhibiting activity of XG9 exhibits a concentration optimum around 10^{-8} M. A structurally related heptasaccharide (XG7) does not inhibit elongation. This established that the fucosyl-galactosyl side chain of XG9 is essential for biological activity. Therefore, it was important to determine the biological activity of related xyloglucan oligosaccharides containing the characteristic disaccharide side chain to determine if other xyloglucan oligosaccharide structural features are required for biological activity. We have found that an undecasaccharide (XG11) containing two fucosyl-galactosyl side chains (also isolated from *endo*glucanase-digested xyloglucan) consistently exhibits greater inhibition of auxin-stimulated growth than does XG9. We have also shown that the aldehyde-reduced XG9 and XG8', which lack the xylosyl residue farthest from the reducing end, are as effective as XG9 in inhibiting auxin-stimulated elongation. Furthermore, in collaboration with Professor T. Ogawa and his research group at the RIKEN Institute in Japan, we have shown that the inhibiting properties of chemically synthesized XG9 are indistinguishable from those of XG9 isolated from cell wall xyloglucan, thus confirming the activity of XG9 isolated from natural sources. This work has resulted in a paper accepted for publication in *Plant Physiology*.[1]

Oligosaccharins, such as XG11 and XG9 are likely important components in the control of auxin-induced growth. Since the terminal fucosyl residues of XG11 and XG9 have been shown to be essential for their biological activity, we looked for, detected, and purified from pea stems a cell wall-localized α-L-fucosidase that is able to remove the terminal fucosyl residue from the XG oligosaccharides, thereby destroying their inhibiting activity. The fucosidase has no activity on polymeric xyloglucan nor on a variety of model substrates such as para-nitro-phenol α-L-fucoside. We have purified the fucosidase to homogeneity. This is the first report on the purification of an α-fucosidase from plants. We have been able to obtain an *N*-terminal amino acid sequence of the enzyme. Interestingly, this sequence shows strong homology to trypsin inhibitors. Additionally, we were able to generate, by PCR, a partial 354 bp cDNA clone of the α-fucosidase which is presently being sequenced.

Monoclonal antibodies have been obtained for the α-fucosidase and have been used in a collaborative project with Professor Nicole Behamou (Laval University, Quebec, Canada) to localize the α-fucosidase in the cell walls of etiolated pea seedlings. No labeling was found in the cell corners, but heavy labeling was found in primary cell walls. Further, cell walls from two- and four-day-old epicotyls showed little if any labeling with gold-labeled antibody. This finding correlated with our inability to isolate α-fucosidase from this tissue. Eight-day-old tissue shows heavy labeling and is a good source of α-fucosidase. This data establishes that α-fucosidase activity is developmentally controlled in growing pea epicotyls.

Future work will include the isolation, sequencing and characterization of a genomic clone coding for the pea stem α-fucosidase. Genomic cloning will be achieved by using the partial cDNA clone as a probe to screen an existing pea (and/or *Arabidopsis*) genomic library. We are also preparing to study the levels of mRNA encoding the α-fucosidase to learn whether auxin rapidly affects these levels.

1. Augur, C., L. Yu, K. Sakai, T. Ogawa, P. Sinaÿ, A.G. Darvill, and P. Albersheim. 1992. Further studies of the ability of xyloglucan oligosaccharides to inhibit auxin-stimulated growth. *Plant Physiol.* In press.

4. Studies to identify and purify a receptor for biologically active oligogalacturonides. Mark D. Spiro

This project involves the isolation and characterization of the putative tobacco cell receptor protein of biologically active 1,4-linked α-D-oligogalacturonides (DP 10-15). Several biological activities of size-specific oligogalacturonides have been identified in tobacco cells and tissues.[1,2] In order to obtain a well-defined ligand with which to probe for the receptor, it was first necessary to isolate homogeneous, size-specific 1,4-linked α-D-oligogalacturonides. We have developed a method for purification of milligram quantities of homogeneous, size-specific tridecagalacturonide from α-1,4-*endo*polygalacturonase-digested polygalacturonic acid (Sigma). Oligogalacturonides with DPs greater than nine were selectively precipitated by the addition, to the *endo*polygalacturonase digest of polygalacturonic acid, of sodium acetate (to 50 mM) and ethanol (to 11%). Oligogalacturonides with DPs less than eight and structurally modified oligogalacturonides (known to be released by *endo*polygalacturonase treatment of polygalacturonic acid) remained soluble. The ethanol-precipitated oligogalacturonides were purified using Q-Sepharose fast-flow anion-exchange chromatography, followed by semi-preparative high-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD). The purified tridecagalacturonide was shown, by glycosyl-residue and glycosyl-linkage composition analysis, analytical HPAE-PAD, fast atom bombardment mass spectroscopy, electrospray mass spectrometry, and ^{1}H-NMR spectroscopy, to be homogeneous. Approximately 70 mg of homogeneous tridecagalacturonide can be purified from 10 g of enzymatically digested polygalacturonic acid. The purified tridecagalacturonide has been shown to elicit phytoalexin accumulation in soybeans and induce flower formation in tobacco thin cell layer explants.

We derivatized tridecagalacturonide to prepare a labeled ligand for probing for binding proteins, as the trigalacturonide falls in the middle of the biologically active size range and, therefore, is less likely to require the reducing end for its biological activity. We have attached a tyramine to the reducing end of the tridecagalacturonide by reductive (sodium cyanoborohydride) amination. The structure of the tyraminated tridecagalacturonide was confirmed by electrospray mass spectrometry. This conjugated tyramine should then be amenable to radio-iodination employing Na[^{125}I] and "Iodogen" reagent (Pierce Chemical Company). Using this procedure, we expect to obtain a specific radioactivity of 200,000 cpm per pM of ligand. The biological activity of the resulting radiolabeled ligand will be determined by its ability to induce K^+ efflux in suspension-cultured tobacco cells, as recently described by Mathieu *et al.*[1] Microsomal fractions of suspension-cultured tobacco cells will be prepared and screened for tridecagalacturonide binding activity. We propose to solubilize the receptor protein with detergent and purify the solubilized protein by conventional purification procedures.

1. Mathieu, Y., A. Kurkdjian, H. Xia, J. Guern, M.D. Spiro, M.A. O'Neill, P. Albersheim, and A. Darvill. 1991. Membrane responses induced by oligogalacturonides in suspension-cultured tobacco cells. *The Plant Journal* 1:333-343.

2. Marfà, V., D.J. Gollin, S. Eberhard, D. Mohnen, A. Darvill, and P. Albersheim. 1991. Oligogalacturonides are able to induce flowers to form on tobacco explants. *The Plant Journal* *1*:217-225.

5. Development of spectroscopic methods for the structural analysis of plant cell wall xyloglucans. William S. York.

Xyloglucans are hemicellulosic polysaccharides that play an important role in the growth and development of plant cells. Xyloglucans consist of a cellulosic (β-1,4-linked D-glucopyranosyl) backbone with α-D-xylopyranosyl residues attached at C-6. β-D-Galactopyranosyl, α-L-fucopyranosyl, α-L-arabinofuranosyl, and β-D-xylopyranosyl residues are also found in xyloglucans. The identity and attachment sites of these other types of glycosyl residues vary from species to species and from tissue to tissue, and affect the rheological properties of polymeric xyloglucans and the biological activity of xyloglucan-derived oligosaccharides.

The focus of this project is to identify the primary structures of as many as possible of the different types of *endo*-β-1,4-glucanase-released oligosaccharide subunits of xyloglucans isolated from different plant species and thereby compile a detailed set of diagnostic correlations between specific structural features of the oligosaccharides and specific features in their NMR and mass spectra. The initial structural characterization of these oligosaccharides has been accomplished by a combination of NMR spectroscopy and mass spectrometry, but, once their structures have been determined, these spectroscopic techniques provide a "fingerprint" for each oligosaccharide, making it far easier to identify these oligosaccharides in the future. The use of artificial neural network analysis may further simplify the identifications. Furthermore, the diagnostic (structural-spectral) correlations that are deduced in the course of the characterization of the xyloglucan oligosaccharides can be used to assign structures for related oligosaccharides that have not been previously characterized.

We have made good progress in collecting and characterizing a large set of xyloglucan oligosaccharides by working with several (past and present) members of the CCRC. For example, Makoto Hisamatsu, a visiting scientist from Mie University, Tsu, Japan, who is a specialist in the isolation of oligosaccharides by HPLC, purified seven novel oligosaccharides derived from the xyloglucan found in the culture filtrate of suspension-cultured *Acer pseudoplatanus* (sycamore) cells. These oligosaccharides contained from 17 to 21 glycosyl residues. Seven of these oligosaccharides were completely characterized by spectroscopic techniques[1] based on previous structural analysis of smaller xyloglucan oligosaccharides.[2] The novel substructures characterized included, for example, a β-xylosyl residue attached to *O*-2 of a backbone glucosyl residue that has an α-xylosyl residue attached to *O*-6. These results expanded our list of structural-spectral correlations. We are performing a similar study of ten additional xyloglucan oligosaccharides obtained from suspension-cultured sycamore cells that were purified by Dr. Hisamatsu and Dr. Giuseppe Impallomeni (a visiting scientist from Catania, Italy). Additional structural-spectral correlations have already been obtained from this study. We are also examining xyloglucans from other plant species, such as apple and tobacco, which contain arabinosyl residues attached to different positions than in sycamore xyloglucan. Study of xyloglucans from these other species is required to get a complete picture of the relationships between the structures of xyloglucans and their NMR and mass spectra, and, eventually, biological function.

The secondary focus of this research is to examine the conformation of xyloglucans and their oligosaccharide subunits. The molecular basis for the biological functions of xyloglucans and xyloglucan-derived oligosaccharides will be fully appreciated only when the three-dimensional conformations of these molecules are established. A preliminary theoretical study of the conformations of xyloglucans was performed by Drs. Sam Levy and Andrew Staehelin at the University of Colorado, Drs. Bernd Meyer and Rainer Stuike-Prill of the CCRC, and York.[3] York is currently working (in collaboration with Dr. Leszek Poppe of the CCRC) on the development and application of NMR methods to the analysis of the conformations of xyloglucans. The conformational properties of model xyloglucan oligosaccharides (some of which are already available and some of which are being prepared) will be examined by these NMR techniques. This experimental evidence will allow us to evaluate the conclusions of our theoretical conformational analyses and gain more knowledge of the conformation and dynamic behavior of xyloglucan and xyloglucan oligosaccharides.

[York is a long-time member of the CCRC. In 1990, he decided to work towards obtaining a Ph.D. degree in biochemistry. York divides his time between his graduate studies, serving as a considerably knowledgeable resource for CCRC members, and managing the mass spectrometry facilities.]

1. Hisamatsu, M., W.S. York, A.G. Darvill, and P. Albersheim. 1992. Characterization of seven xyloglucan oligosaccharides containing from seventeen to twenty glycosyl residues. *Carbohydr. Res. 227*:183-194.

2. York, W.S., H. van Halbeek, A.G. Darvill, and P. Albersheim. 1990. Structural analysis of xyloglucan oligosaccharides by ^{1}H-n.m.r. spectroscopy and fast-atom-bombardment mass spectrometry. *Carbohydr. Res. 200*:9-31.

3. Levy, S., W.S. York, R. Stuike-Prill, B. Meyer, and L.A. Staehelin. 1991. Simulations of the static and dynamic molecular conformations of xyloglucan. The role of the fucosylated sidechain in surface-specific sidechain folding. *The Plant Journal 1(2)*:195-215.

6. An investigation to determine whether pathogenesis-related (PR) proteins release fungal cell wall oligosaccharides that induce plant defense responses. Kyung-Sik Ham.

Infection of many plants with viruses, fungi, or bacteria, or treatment of the plants with any of several chemicals that stress plants frequently leads to the induction, in the plant tissues, of pathogenesis-related (PR) proteins.[1] Although PR proteins have been widely studied and some of their catalytic functions identified,[1,2] biological functions for them have been only inferred. We and others hypothesize that PR proteins release biologically active oligosaccharides (oligosaccharins) from fungal cell walls that lead to the induction of defense responses in plants.

The goals of this research are to determine whether PR proteins can release biologically active oligosaccharides from fungal cell walls and to identify the structures of those oligosaccharides. We have shown that PR proteins are induced in soybean leaves by fungal infection and by treatment with mercuric chloride. Both β-1,3-glucanase and chitinase activities increased following treatment with fungal infection or mercuric chloride, indicating that some of the induced PR proteins are β-1,3-glucanase and chitinase. We purified two basic proteins that released phytoalexin elicitor-active fragments from the cell walls of a fungal pathogen (*Phytophthora megasperma* f.sp. *glycinea*; Pmg). We have shown that these two proteins are PR proteins and that the proteins have β-1,3-glucanase activities (this work has recently been published).[3] These results support the hypothesis that PR proteins can release biologically active elicitors from fungal cell walls. We propose to purify the oligosaccharide elicitors solubilized from fungal cell walls by purified PR β-1,3-glucanase. We also propose to identify the structure of the smallest elicitor released and will compare its structure to that of the hepta-β-glucoside, the smallest β-glucan elicitor released from fungal cell walls using acid hydrolysis. We will also determine whether other β-1,3-glucanase isozymes found in soybean produce structurally similar elicitors or whether they release different oligosaccharides that could have an interactive effect in generating phytoalexin elicitors. We will also test the released oligosaccharides for other biological activities, e.g., protection of tobacco from virus, another known function of fungal β-glucan oligosaccharides.

We are also studying whether fungal pathogens produce inhibitor proteins of soybean β-1,3-glucanases. The presence of inhibitor proteins was first found in the anthracnose-causing fungal pathogen (*Collectotrichum lindemuthianum*) about 20 years ago.[4] Since then, no further studies have been carried out. Our studies have shown that Pmg both produces inhibitor proteins of plant β-1,3-glucanases and produces β-1,3-glucanases itself. We will study the inhibitors to determine whether they are effective on plant PR glucanases in general or whether the inhibitors specifically inhibit selective PR glucanases, and whether the fungal inhibitor also inhibits fungal β-1,3-glucanases. These studies will allow us to learn more about the function of PR β-1,3-glucanases in soybean, their interaction with β-1,3-glucanase inhibitors produced by Pmg, and the role of these two proteins in releasing oligosaccharins active in host-pathogen interactions.

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2. Bol, J.F., J.M. Linthorst, and B.J.C. Cornelissen. 1990. Plant pathogenesis-related proteins induced by virus infection. *Ann. Rev. Phytopathol. 28*:113-138.

3. Ham, K.-S., S. Kauffmann, P. Albersheim, and A.G. Darvill. 1991. Host-Pathogen Interactions XXXIX. A soybean pathogenesis-related protein with β-1,3-glucanase activity releases phytoalexin elicitor-active heat-stable fragments from fungal walls. *Mol. Plant-Microbe Interact. 4*:545-552.

4. Albersheim, P. and B.S. Valent. 1974. Host-Pathogen Interactions VII. Plant pathogens secrete proteins which inhibit enzymes of the host capable of attacking the pathogen. *Plant Physiol. 53*:684-687.

7. Is improper protein folding a trigger for plant defense responses? Ivana Gelineo.

Plants have methods of fighting infections, although they have no immune system similar to that known to exist in animals. Plant defenses against potential pathogens include the hypersensitive response, synthesis and accumulation of phytoalexins, accumulation of hydroxyproline-rich glycoproteins, deposition of lignin-like material and other wall-bound phenolics, deposition of callose, stimulation of the activity of certain hydrolytic enzymes such as *endo*-β-glucanases and chitinases (PR proteins), as well as the accumulation of proteinase inhibitors.[1] A plant's defense system can be induced by application of elicitors (oligosaccharins) from plant or microbe cell wall origin.

Professor Jean Guern's group (Gif sur Yvette, France; see highlight section 1 in the Overview) has shown that treatment of suspension-cultured tobacco cells with structurally defined α-1,4-D-oligogalacturonides induced a rapid and transient stimulation of K^+ efflux concomitant with membrane depolarization, alkalinization of the incubation medium, acidification of the cytoplasm, and an influx of Ca^{2+}.[2] If the plasma membrane of tobacco cells exhibits such dramatic modifications upon oligogalacturonide elicitor treatment, with concomitant disruptive ionic flows, it is possible to expect a similar effect on internal cellular membranes such as the endoplasmic reticulum and Golgi apparatus, the two organelles where protein glycosylation takes place.

Professor Robert Fluhr (Weizmann Institute, Rehovot, Israel) hypothesized that, upon exposure to elicitors or pathogens, plants are triggered to change their protein glycosylation and folding. In this hypothesis, the resulting misfolded glycoproteins bind increased amounts of chaperone 3 protein, and the reduced amount of free chaperone leads to activation of plant defenses. To study this hypothesis, a transgenic tobacco plant has been produced containing a 35S-PR1M prepeptide sequence -- chloramphenicol acetyl transferase construct. The CAT product from this constructed gene sequence is glycosylated and is secreted into the extracellular matrix. Due to the glycosylation near its active site, the CAT is secreted in an enzymatically inactive form, but, in the presence of tunicamycin, an antibiotic that inhibits glycosylation, an active form is secreted. Thus, the CAT protein can be used as a monitor of changes in glycosylation in response to stress. We propose to look for changes in the CAT activity and in the structure of its carbohydrate side chain when tobacco cells are treated with different elicitors of plant defenses, such as α-1,4-oligogalacturonides and β-1,4-*endo*xylanases. To identify potential changes in glycosylation, we will convert the CAT carbohydrate chain to a free, radiolabeled oligoglycosyl alditol and analyze it with various chromatographic purifications already developed at the CCRC and, if possible, by artificial neural network analysis.

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2. Mathieu, Y., A. Kurkdjian, H. Xia, J. Guern, A. Koller, M.D. Spiro, M. O'Neill, P. Albersheim, and A. Darvill. 1991. Membrane responses induced by oligogalacturonides in suspension-cultured tobacco cells. *The Plant Journal 1*:333-343.

8. Cloning and characterization of genes for α-1,4-oligogalacturonide binding proteins. Brent Ridley.

The goal of this project is to clone a gene for a receptor of biologically active α-1,4-oligogalacturonides (DP 12-14). The strategy is to identify proteins that bind labeled oligogalacturonides from cDNA expression libraries. This approach is based on the success of various researchers in identifying a variety of genes for proteins that bind specific ligands by screening cDNA expression libraries. For example, the genes for calmodulin-binding proteins,[1] the regulatory subunit of cAMP-dependent protein kinase,[2] and an enhancer-binding protein[3,4] have been cloned by screening λ-gt11 libraries with ^{125}I-labeled calmodulin, ^{32}P-labeled cAMP, and ^{32}P-labeled recognition site DNA, respectively.

The first phase of this project is to label biologically active oligogalacturonides with a ligand. Oligogalacturonides labeled with ^{125}I using a method being developed by Mark Spiro (see project 4 in this appendix) may be used to screen the expression library. However, we are also attempting to label the oligogalacturonides with biotin by formation of a hydrazone or hydrazide derivative. The model uronide, trigalacturonic acid, has been successfully labeled with biotin. Current efforts to label the pure tridecagalacturonide have yielded an HPLC-purified biotin and uronic acid-containing peak that has the expected ^{1}H-NMR signals for the oligogalacturonic acid biotin hydrazide. Attempts to obtain a ^{1}H-NMR spectrum of the product as well as assay for biological activity in the soybean cotyledon phytoalexin induction assay,[5] tobacco TCL morphogenesis bioassay[6] and tobacco cell suspension H^+ influx assay[7] are under way. Biotin labeling is advantageous because biotinylated oligogalacturonides can be used, due to their avidity for avidin, to attempt to identify mRNA populations that encode oligogalacturonide-binding proteins (OBPs). *In vitro* translated receptors from other systems have been shown to retain binding activity. An *in vitro* translated thyroid hormone receptor retained the ability to bind thyroid hormone with a high affinity.[8] Liu and colleagues[9] cloned an IgE-binding protein via the ability of the *in vitro* translated IgE-binding protein to bind IgE. Thus, *in vitro* translated *OBPs* may retain the ability to bind oligogalacturonides. We will *in vitro* translate mRNA isolated from a variety of tissues in the presence of ^{35}S-methionine and in the presence and absence of microsomes. The ^{35}S-labeled *in vitro* translation products will be incubated with biotinylated oligogalacturonide, and any protein-oligogalacturonide complexes will be precipitated with streptavidin-agarose and analyzed by SDS-PAGE.[10] These experiments will give us information about the number of different potential oligogalacturonide-binding proteins that may be expected from cDNA libraries made from various mRNA sources. In addition, detection of *in vitro* translated binding proteins will provide evidence for the feasibility of selecting genes for oligogalacturonide receptors by screening λ-gt11-cDNA expression libraries in *E. coli.*

A λ-gt11 expression library constructed from poly A$^+$ mRNA isolated from tobacco thin cell layers (TCLs) will be screened with labeled tridecagalacturonide.[6] Phage that express *OBPs* will be detected by transferring proteins from the plaques to nitrocellulose filters, blocking non-specific oligogalacturonide binding sites with oligosaccharides such as oligoguluronate, probing with radiolabeled oligogalacturonide in the presence of excess non-specific oligosaccharides, and washing the filter and exposing for autoradiography.[4] The success of this method requires that the expressed proteins fold in a conformation that retains the oligogalacturonide-binding domain and that non-specific binding is inhibited. If the receptor protein is an integral membrane protein it may not fold properly and no *OBPs* would be detected. In the event that no *OBPs* are detected in λgt11 libraries, cDNA will be cloned into a plasmid vector (e.g., *lam*B gene fusion protein vector) that allows the expression of receptors in the bacterial membrane.[11] Genes for *OBPs* would be detected by binding of oligogalacturonides to intact *E. coli* or membrane fractions. Such receptors have been shown to maintain native binding properties.[12] Clones selected by their production of a protein (which selectively binds the tridecagalacturonide) will be characterized in depth with the hope of identifying a clone that encodes a receptor for biologically active oligogalacturonides. It is possible that genes encoding enzymes that bind oligogalacturonides, such as *endo*galacturonases and pectin methylesterases, will also be identified using this method. These genes would be of interest since the enzymes they encode could release biologically active oligogalacturonides from the plant cell wall. However, we are more likely to identify the genes for receptors, since receptors generally have higher binding affinities than enzymes.

Clones that express oligogalacturonide-binding proteins will be sequenced with the hope that the function will be suggested by sequence homology to previously characterized receptor genes. Northern analysis will establish the temporal and tissue specificity of expression of the mRNAs of the clones. Future efforts to obtain other information about the physiological function of oligogalacturonide binding proteins may include the use of immunocytochemistry, anti-ligand binding site antibodies, and the production of transgenic plants that over- and under-express mRNA for putative oligogalacturonide receptors.

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9. Conformation analysis of the hepta-β-glucoside elicitor of phytoalexin accumulation. Veng-Meng Ló.

Many plants respond to microbial attack by accumulating small lipophilic molecules, called phytoalexins, that have antibiotic activity.[1-3] Molecules that signal plants to begin the process of phytoalexin synthesis and other plant defense responses are called elicitors.[4,5] The hepta-β-glucoside has been characterized as the smallest active fungal elicitor of phytoalexin accumulation.[6,7] To gain insight into the interaction of this elicitor with its putative receptor,[8] we are studying the energetically favored conformations of the hepta-β-glucoside. We also are studying a biologically related hexaglucoside, the backbone of which is identical to the hepta-β-glucoside except that the reducing residue is substituted with an α-linked allyl group. This hexaglucoside derivative has the minimum structure required for efficient phytoalexin elicitor activity.

The backbone of the heptaglucoside contains four β(1→6) linkages which allow great flexibility in the conformation of the molecule. To obtain information on the conformational preferences of the heptaglucoside, we have developed two interactive strategies. First, we use computer molecular modeling calculations to predict preferred conformations. Second, we use NMR spectroscopy to measure the average physical properties (e.g., NOEs, coupling constants) and compare those physical properties to the physical properties predicted by the theoretical calculations.

In the computer modeling theoretical approach to conformational analysis, we apply force field methods that do not require exorbitant amounts of computing effort and allow the calculation of conformations of relatively large oligosaccharides. We first used a variety of different potential starting conformations for the heptaglucoside and optimized their geometry by energy minimization using the GEGOP program.[9] As expected, we obtained a large number of local energy minima (> 35 conformations within 10 kcal/mole from the global minimum). Second, we used Metropolis Monte-Carlo calculations to characterize the dynamics of the molecule. We found that the $\beta(1\rightarrow3)$ branches and the ϕ angles of the backbone show, as expected, only moderate flexibility. However, the ψ and the ω angles around the $\beta(1\rightarrow6)$ linkages in the backbone show, also as expected, high flexibility with the ψ angles having values from 60° to 280°, while conformations around the ω angles include all three staggered rotamers.

It is important, in the Monte-Carlo calculation, to monitor whether the system reaches thermal equilibrium. Calculations at 300 K do not reach equilibrium, and, thus, the results are not reproducible. Therefore, we optimized the temperature parameter to allow the heptaglucoside to reach equilibrium, and found it to be 600 K.

The Monte-Carlo simulation generates an ensemble of conformers; however, it is difficult to visualize all of them. In order to group these conformers in classes of similar shapes, we used cluster analysis. Thus, we carried out Monte-Carlo simulations and cluster analysis of both the hexaglucoside and the tetraglucoside backbone of the hexaglucoside without the two $\beta(1\rightarrow3)$ branches. Interestingly, the fifth highest populated (4.2%) backbone conformation of the hexaglucoside is not populated at all by the tetramer backbone. Since the tetramer is not biologically active, it may imply that this hexasaccharide conformation is the biologically active conformation that binds to its cellular receptor. This conformer, in which $\beta(1\rightarrow6)$ and $\beta(1\rightarrow3)$ non-reducing end termini are located on the surface of the molecule, is in agreement with the biological experiments which have shown that chemical modification of the $\beta(1\rightarrow6)$ or $\beta(1\rightarrow3)$ non-reducing terminal significantly reduce the biological activity of the heptaglucoside.[8] Thus, it appears possible that the $\beta(1\rightarrow6)$ and $\beta(1\rightarrow3)$ non-reducing end termini of the heptaglucoside must occur in a certain spatial arrangement in order to form a surface that can bind to its host cell receptor. Any structural modification that causes steric hindrance or a conformational change would significantly reduce the biological activity of this molecule. The likelihood that this conclusion is correct has been strengthened by our theoretical studies of a structurally related but biologically inactive heptaglucoside; we found that this heptaglucoside cannot adopt the putative biologically active conformation of the active heptaglucoside.

The first step in NMR studies of the conformations of the active hepta-β-glucoside was to assign the signals in the 1-D ^{1}H-NMR spectra of the hexaglucoside and also the signals of the inactive analogues (synthesized by Ogawa *et al.*[11]) by using 1-D selective TOCSY experiments.[12] After assigning the ^{1}H-NMR spectra, we performed 2-D NOESY and ROESY experiments that have allowed us to measure the NOE interactions (i.e., through-space interactions) between pairs of individual protons. The results of these experiments should allow us to evaluate the results derived from the theoretical modeling. However, since many of the signals in the NMR spectra of the hexaglucoside overlap, it is difficult to identify a particular NOE interaction from one particular residue to another residue. In order to obtain higher resolution, we performed 3-D NOESY-TOCSY[13,14] and ROESY-TOCSY[15] experiments that resolved the overlap of spectra by expanding into an additional dimension. We are presently comparing these experimental data to the data derived from theoretical calculations.

The above experiments were done in D_2O solution. However, in order to observe hydroxyl group interactions (e.g., hydrogen bonds or NOEs between hydroxyl groups and other protons), we are conducting 1-D selective NOE experiments with water suppression in H_2O solution. Such experiment has already been carried out very successfully on gentiobiose, a part of the β-1,6 backbone of the hexaglucoside.[16] However, the hydroxyl groups of the various glycosyl residues of the hexaglucoside overlap; therefore, it will be a considerable challenge to observe individual NOE interactions involving OH protons, but we are continuing our efforts to interpret the spectra.

Our studies on the solution conformations of the heptaglucoside are continuing. Once the putative cell receptor for the heptaglucoside is isolated and sufficient amounts can be obtained by recombinant methods, we will be able to study the interaction between the elicitor and the receptor. We should be able to build a theoretical model using computer analysis and evaluate this by NMR experiments, where through-space interactions (NOEs) between the elicitor and the receptor can be measured.

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Appendix 2: Research Accomplishments, 1987-April 1992

FACULTY RESEARCH INTERESTS

1. Peter Albersheim, Research Professor of Biochemistry and Co-Director, CCRC
Oligosaccharins -- Carbohydrates Possessing Biological Regulatory Activities

Our laboratory has discovered that oligosaccharides are able to act as signal molecules in plants. Hormonal concentrations of biologically active oligosaccharides, called oligosaccharins, regulate growth and development as well as defense reactions by regulating gene expression. Most importantly, oligosaccharins have exquisitely specific structural requirements. The first oligosaccharide shown to possess biological activity was a hepta-β-glucoside isolated from the mycelial wall of a fungal pathogen of soybeans. The active hepta-β-glucoside, which at 10^{-9} M causes soybean cells to make phytoalexins (antibiotics), was the *only* active hepta-β-glucoside of about 300 different hepta-β-glucosides generated from the β-glucan by partial acid hydrolysis.

A mixture of large oligoglucoside fragments of the same mycelial wall β-glucan that contains the hepta-β-glucoside elicitor of phytoalexins has a different effect when it is sprayed on the leaves of tobacco plants. Only very small amounts of the oligoglucoside fragments need to be sprayed on the leaves to protect them against viral infection. But the fragment of the β-glucan that protects tobacco against viral infection is not the same hepta-β-glucoside that elicits the production of phytoalexins in soybeans, as that oligosaccharin has no ability to protect tobacco against viruses. Thus, two different oligosaccharide fragments of the same polysaccharide may signal activation of different defense mechanisms.

Oligogalacturonide fragments of plant cell wall homogalacturonans were the first oligosaccharin to be isolated from a plant cell wall polysaccharide. Linear α-1,4-D-oligogalacturonides containing between 12 and 14 galactosyluronic acid residues have the same biological effect as the active hepta-β-glucoside, that is, they elicit soybean seedlings to produce phytoalexins. Moreover, the hepta-β-glucoside (a pure neutral oligosaccharide of fungal cell wall origin) and oligogalacturonide elicitors (a pure polyanionic oligosaccharide of plant cell wall origin) act synergistically when activating the expression of the genes that encode the enzymes that synthesize phytoalexins to protect the plant, requiring the presence of less of each to stimulate phytoalexin production when both are present.

We hypothesize that other plant cell wall oligosaccharide fragments, solubilized by pathogen-secreted enzymes, trigger the widely observed and physiologically important hypersensitive resistance response. Hypersensitive resistance is evidenced by the death, in a resistant (incompatible) plant, of the first plant cells to come in contact with an infecting microbe. The enzymes secreted by a fungal pathogen of rice plants release fragments of plant cell walls that kill plant cells, and the active fragments appear to be part of arabinoxylan, a hemicellulose present in the cell walls of all higher plants.

Oligosaccharins are also involved in regulating growth and development of plants. The plant hormone auxin doubles the rate of elongation growth of excised segments of pea stems. We have demonstrated that a particular nonasaccharide fragment of xyloglucan, at a concentration of 10^{-8} to 10^{-9} M, inhibits auxin-stimulated growth of pea stem segments. A variety of other xyloglucan fragments, generated by a β-1,4-endoglucanase, are not active in inhibiting auxin-stimulated growth. A cell wall enzyme, an α-fucosidase, destroys the inhibitory activity of the nonasaccharide when it removes the terminal fucosyl residue. We are cloning the α-fucosidase and a β-1,4-endoglucanase (also of cell wall origin) that modify and generate the bioactive xyloglucan oligosaccharides in order to study their *in vivo* role in growth.

Other oligosaccharins inhibit the formation of roots and stimulate the production of flowers. The Albersheim/Darvill and Mohnen research teams developed a tobacco explant system to study the effect of wall fragments on morphogenesis. The explants we are studying are cut from the stems of flowering tobacco plants and are about 1 cm long, 1 mm wide, and 5 to 10 cells thick. We have found that we can determine the morphogenetic fate of the explants by regulating the concentrations of just two factors, the hormones auxin and cytokinin. Relatively high auxin and low cytokinin concentrations cause the explants to form only roots; at high cytokinin and low auxin the explants form only vegetative shoots; and at low auxin and low cytokinin, the explants form flowers. Plant cell wall fragments released by the action of an α-1,4-endopolygalacturonase inhibit the formation of roots and stimulate the formation of flowers. We have shown that the root-inhibiting and flower-stimulating oligosaccharin in the mixture of oligo- and polysaccharides released from the walls by the enzyme is linear α-1,4-D-oligogalacturonides with a degree of polymerization of 12 to 14. These are the same molecules that elicit phytoalexins to form in soybean seedlings, but it requires 100 times less oligogalacturonide (~10^{-7} M) to stimulate flowers to form than to stimulate phytoalexin production.

The mechanism of action of the oligogalacturonides is being studied. It is now established that within two minutes of exposure to the oligogalacturonides a variety of plasma membrane functions are transiently altered. These changes include a massive efflux of potassium ions and an influx of calcium ions and protons, as well as a major depolarization of the plasma membrane. Efforts are under way to determine whether these membrane effects lead to the physiological and morphological effects observed by applying oligogalacturonides to tissues.

2. Alan Darvill, Professor of Biochemistry and Co-Director, CCRC

Structures and Functions of Plant Cell Wall Complex Carbohydrates

Characterization of the non-cellulosic polysaccharides that constitute the primary cell walls of plants has been a major research goal of our laboratory for many years. We have emphasized the structural studies of five major non-cellulosic polysaccharides, namely, the pectic polysaccharides rhamnogalacturonan I (RG-I), rhamnogalacturonan II (RG-II), and homogalacturonan, and the hemicellulosic polysaccharides xyloglucan and arabinoxylan. We have characterized the structures of these polysaccharides isolated from dicot, monocot, and gymnosperm cell walls. We have come to realize that these polysaccharides are the predominant, and probably only, non-cellulosic polysaccharides of cell walls. These polysaccharides, although varying in quantitative amounts in the cell walls of dicots, monocots, and gymnosperms, are structurally conserved, indicating to us their importance in primary cell wall structure and function.

These cell wall, non-cellulosic polysaccharides represent a group of carbohydrates with considerable structural complexity that offer a major challenge to those wishing to decipher their structures. RG-II, for example, is probably the most structurally complex polysaccharide so far isolated from nature. RG-II was first discovered to be a component of the primary cell walls of plants by our laboratory in 1978. RG-II is a small polysaccharide consisting of approximately 30 glycosyl residues. However, this polysaccharide contains at least 11 different glycosyl residues linked together in over 20 different glycosyl linkages. RG-II is characterized by several unusual sugar components including 2-O-methyl fucose, 2-*O*-methyl xylose, apiose, 3-*C*-carboxy 5-deoxy-L-xylose (aceric acid), 3-deoxy-D-*manno*-2-octulosonic acid (KDO), and 3-deoxy-D-*lyxo*-2-heptulosaric acid (DHA). Both aceric acid and DHA have been found in no other natural source other than RG-II. We have characterized oligosaccharide fragments of RG-II that contain 28 of the possible 30 glycosyl residues of this pectic polysaccharide. We have obtained evidence that the backbone of RG-II consists of seven α-1,4-linked galactosyluronic acid residues to which two disaccharide, one heptasaccharide, and one octasaccharide side chains are attached. These side chains are attached to the backbone via the acid labile ketosidic and glycosidic linkages of KDO, DHA, and apiose. Defining the complete structural sequence of RG-II is a major research goal of our laboratory.

Research in our laboratory will continue to emphasize the structural characterization of the non-cellulosic polysaccharides of cell walls. The availability for the first time of cell wall synthesis mutants is assisting us in these structural studies. Structural analysis is accomplished by release of polysaccharides from cell walls and generation of defined polysaccharide fragments with purified enzymes or chemical treatments, chromatographic purification of the oligo- and polysaccharides, chemical structural characterization techniques, and a combination of several analytical techniques including gas-liquid chromatography, liquid chromatography, gas-liquid chromatography-mass spectrometry, liquid chromatography-mass spectrometry, fast atom bombardment mass spectrometry, and ^{1}H- and ^{13}C-NMR spectroscopy.

We are also collaborating with Dr. Michael Hahn in the production of monoclonal antibodies to the cell wall polysaccharides and oligosaccharides derived from these polysaccharides to be used in both purification of oligosaccharides and, more importantly, to begin to decipher the arrangement and location of the polysaccharides within the primary cell wall. Our goal over the next few years in this research is to propose a model for the structure of the primary cell wall of plants.

3. Karl-Erik Eriksson, Professor of Biochemistry and Eminent Scholar of Biotechnology

Genetic Manipulation of Lignin in Forest Trees, Lignin Biodegradation, Pulp Bleaching Techniques, and Purification of Waste Bleach Waters

My group is interested in elucidating the processes underlying lignin biosynthesis and biodegradation with the long-term goal of developing new processes for pulp bleaching and purification of waste bleach waters that decrease damage to the environment. We are attempting to use biotechnological techniques such as fermentation and enzyme production, genetic engineering and monoclonal antibody development to reach these goals.

Lignin, a phenolic polymer that accounts for 20 to 30% of wood biomass, provides compressive strength, stiffness, and pest resistance in woody plants. Lignin quantity and structure vary considerably among species, ages, developmental status, and cell types in trees, as well as between different regions of the cell wall. This variability causes differences in lignin degradation and removal during pulping and bleaching operations, making these operations less efficient. "Genetically engineering" trees to contain less or suitably modified lignin would reduce energy and chemicals required for pulping and minimize the need to bleach with chlorine and other harsh chemicals. Enzymes controlling the localization and timing of lignin biosynthesis in developing cells, differentiating tissues, and young trees are targets for genetic manipulation to reduce lignin quantity and structure in forest trees.

Before lignin biosynthesis can be modified, however, more precise data on how much and what type of lignin is deposited when and where during cell and plant development is needed. Toward these ends, we have assembled a collaborative team of specialists in chemistry, genetics, cell and tissue culture, biochemistry, immunology, microscopy, and spectroscopy. We are producing monoclonal antibodies to different lignin structures and to key enzymes in lignin biosynthesis so that, using light and electron microscopy as well as Raman microspectroscopy, we may document precisely the lignification process across a continuum of plant tissues -- from single cells in culture through developing plants.

One of the key elements of Earth's carbon cycle is the degradation of wood and other lignocellulosic materials to carbon dioxide, water, and humic substances. White-rot fungi are the only microorganisms capable of degrading lignin to any substantial degree. Studies of how they do so have led to a model of lignin degradation. It has long been realized that phenoloxidases are necessary for lignin degradation to take place. However, since the phenoloxidases produce phenoxyradicals that spontaneously polymerize, other enzymes are also necessary. These polymerization reactions must be prevented or reversed for complete depolymerization of the lignin polymer to take place. We do not yet know enough to construct a cell-free enzyme mixture that degrades lignin while preventing repolymerization. However, it seems clear that enzymes that neutralize phenoxyradicals are involved in the degradative process, and we are presently studying the mechanisms governing two such fungal enzymes.

Because they contain mutagenic, carcinogenic, acutely toxic compounds, high molecular mass chlorinated lignins, and sometimes dioxins, waste bleach waters released from conventional bleaching of chemical pulps into receiving waters is the most serious environmental problem created by the pulp and paper industry. For several years I have been involved in the development of a process for purification of waste bleach waters that reduces the amount of organically bound chlorine in these effluents. This process involves ultrafiltration of the alkaline-stage wastes, as well as fermentation of the permeate mixed with the chlorine-stage waste bleach waters in anaerobic and aerobic filters. The purification achieved with this process is far superior to that obtained with existing techniques such as aerated lagoons and activated sludge. We are working to improve the process so that it generates no waste or sludge whatsoever and so that waste waters can be completely recycled. The process has reached pilot plant scale, and work is now under way to develop it on a commercial scale.

Increasing environmental concern has also emphasized the need for new bleaching processes so that environmentally hazardous compounds are not produced. We have investigated the use of low molecular mass catalysts (such as porphyrins like hemoglobin) that can mimic the attack of lignin peroxidase on lignin. We have made great progress in using hemicellulases to facilitate pulp bleaching. The work is now focused on producing a specific xylanase free of cellulase activity so that the degree of polymerization of cellulose is not affected. Our goal is to obtain brightness ISO 86-90% without the use of chlorine or chlorine derivatives when xylanase is used as one of the bleaching stages. We are also studying other means to modify xylan to accelerate lignin removal without seriously affecting yield, viscosity, or pulp strength properties.

4. Michael G. Hahn, Assistant Professor of Botany

We are studying signal transduction mechanisms in plants using, as a model system, the induction of phytoalexin biosynthesis in soybean (*Glycine max*) cells by fungal cell wall-derived oligosaccharides. In a separate project, the structure of plant cell walls is being studied using monoclonal antibodies generated against polysaccharides purified from sycamore maple (*Acer pseudoplatanus*). These research projects rely primarily on chemical, biochemical, and immunological techniques.

Elucidation of Signal Transduction Mechanisms in Plant Cells. Accumulation of antimicrobial compounds called phytoalexins is an important defense response of plants against microorganisms. Phytoalexin synthesis and accumulation can also be induced in plant cells upon treatment with fragments of fungal cell wall-derived β-glucans (elicitor). The smallest biologically active β-glucan fragment whose structure has been completely determined is a branched hepta-β-glucoside that, at nanomolar concentrations, induces phytoalexin accumulation in soybean tissues. The goal of this research is the elucidation of plant cellular signaling mechanisms that lead to changes in gene expression in the affected plant cells, and the role that oligosaccharides play in these processes.

The present focus of our work is the isolation and characterization, from soybean tissue, of the receptor for the hepta-β-glucoside elicitor. Binding to such a receptor is the first step in the signal transduction pathway. We are using a fully active, radio-iodinatable derivative of the hepta-β-glucoside in binding assays to demonstrate the presence of a specific, high-affinity binding site for the elicitor in soybean membranes. We are also preparing a derivative of the hepta-β-glucoside that would be suitable for photo-affinity labeling the receptor. A number of chemically synthesized, structurally related oligoglucosides, whose elicitor activities vary over five orders of magnitude of concentration, show that this binding site is specific for the hepta-β-glucoside elicitor. The membrane-localized binding protein has been solubilized, and current research is aimed at purifying the binding protein by affinity chromatography. Biochemical and recombinant DNA techniques will then be used to demonstrate that the purified binding protein is the physiological receptor for the hepta-β-glucoside elicitor. This project will eventually examine the remainder of the signal transduction pathway (*e.g.*, identity and role of second messengers).

Studies of Plant Cell Wall Polysaccharides Using Monoclonal Antibodies. Plant primary cell walls are composed of a number of structurally complex polysaccharides, including hemicelluloses (*e.g.*, xyloglucan) and pectins (*e.g.*, rhamnogalacturonan I). Chemical studies have elucidated many structural features of these polysaccharides, but tell us very little about the distribution of specific polysaccharides in cells and tissues. We are developing monoclonal antibodies against plant cell wall polysaccharides as tools to address this question.

A number of secreting hybridomas have been generated using as immunogen the pectic polysaccharide rhamnogalacturonan I from suspension-cultured sycamore maple cells. Two of the three groups of antibodies secreted by these hybridomas recognize epitope(s) that are polysaccharide specific. One monoclonal antibody recognizes a xyloglucan epitope that contains a terminal fucosyl residue. Another antibody recognizes an epitope containing arabinosyl residues. Current research focuses on further delineation of the epitope structures recognized by the library of monoclonal antibodies.

We are interested in enlarging the library of specific antibodies reactive with plant cell wall polysaccharides to include antibodies reactive with xyloglucans, glucuronoarabinoxylans, and rhamnogalacturonan II. We are (i) testing a variety of methods for coupling oligo- and polysaccharides to proteins to improve polysaccharide immunogenicity and thereby the yield of positive secreting hybridomas; and (ii) generating a combinatorial expression library of monoclonal Fab fragments in *Escherichia coli*. Our long-term goal is to build a library of well-characterized antibodies that are specific for particular polysaccharides and/or particular structural motifs within those polysaccharides as tools for structural studies on plant cell wall polysaccharides, for the immuno-localization of the polysaccharides in plant cells and tissues, for the selection of mutant plants with altered cell wall polysaccharides, and for studies of the biosynthesis of the polysaccharides.

5. Bernd Meyer, Associate Professor of Biochemistry

3-D Structures and Biological Functions of Oligosaccharides and Glycoconjugates. Optimization of Research Information Using Artificial Neural Network Techniques

Oligosaccharides are carriers of biological information. They can be specific for a cell type or a species. Recognition of this information by a protein can trigger responses and initiate further action, like defense mechanisms against an invading bacterium may begin or cell growth may stop upon contact with another cell. The biological information is encoded into the 3-D structure of oligosaccharides. When oligosaccharides interact with proteins, the protein "reads" the information encoded into the 3-D structure of the oligosaccharide. We want to elucidate the

3-D structure of oligosaccharides as isolated species in solution as well as their preferred structures when they are interacting with proteins.

We try to correlate the 3-D structure of the carbohydrates with their biological function. To elucidate the 3-D structure we combine two techniques. We are developing software and using existing programs to predict the 3-D structure of the molecules theoretically. NMR spectroscopy is then used to obtain experimental evidence for the predicted 3-D structure. The combination of both techniques renders a picture of the static structure as well as the dynamic properties of the molecules.

It is known that oligosaccharides are vital to the biological functions of glycoproteins. For example, a slight alteration in the arrangement of the carbohydrates of immunoglobulin G_1 -- the absence of galactosyl residues normally present -- is correlated with the presence of rheumatoid arthritis. The carbohydrate content of glycophorin A, the major glycoprotein anchored into the membrane of erythrocytes, is about 60% of the total glycoprotein or about 75% of the extracellular portion, suggesting an important role in its bioactivity. In another project, we have found that although the oligosaccharide bound to ribonuclease B does not change its 3-D structure compared to the unbound state, it is positioned such that it can interact with RNA, the substrate of the enzyme, just outside the active site. Knowing the preferred ("normal") 3-D structural conformations of such glycoproteins, in particular their carbohydrate portions, will help us understand their bioactivity.

Molecules like heparin, chondroitin sulfate, dermatan sulfate or keratan sulfate form a family of sulfated polysaccharides with biological functions. These functions can be as diverse as playing a role in the blood coagulation cascade or providing stability to body tissues such as skin or cartilage. These molecules typically contain about two charged groups per monosaccharide unit, an extremely high clustering of charges. We want to understand the role of the charged groups (sulfates and carboxylates) in these molecules by using NMR spectroscopic techniques and computational methods to elucidate underlying principles on synthetic model oligosaccharides closely related to these polymers. Ultimately, we want to understand the interactions of the sulfated oligosaccharides with their protein receptors.

Artificial neural networks are very powerful tools for utilizing the information contained in spectra of oligosaccharides to identify chemical structures of complex carbohydrates. An artificial neural network is a computer model that recognizes and discriminates between closely related patterns (spectra). We have trained neural networks to recognize a molecule or its substructures from its spectrum. Also, if an artificial neural network is asked to identify a new spectrum that the network has not seen before, the network points to the spectra of the closest chemical relatives indicating relationship but not identity. Generally, neural networks utilize more of the information contained in a spectrum than scientists do. The neural network can be trained to be tolerant of the variations introduced into spectra by varying experimental conditions or instrument settings. We have shown that artificial neural networks can identify a complex oligosaccharide that has up to 20 glycosyl residues from its ^{1}H-NMR spectra, partially methylated alditol acetates from mass spectra, or presence of sulfate groups from IR spectra. We are also working on a large project that places artificial neural network-based pattern recognition into the core of a larger system so that scientists can combine the information contained in different kinds of spectral data and store and retrieve this information in a database.

6. **Debra Mohnen, Assistant Professor of Biochemistry**

Molecular Mechanisms of Plant Developmental Plasticity. Biosynthesis of Plant Cell Wall Polysaccharides

Plants are aggressive in their development. They can overcome severe injuries that would cripple other types of organisms, such as removal of an organ or decapitation, by forming new centers of cell division called meristems. These root or shoot meristems develop into roots, vegetative shoots, or flowers. The goal of my research group is to understand the molecular basis for this plasticity that allows differentiated cells to embark on new developmental programs.

We are taking three approaches to understand how plant cells change their developmental state. The first two approaches involve manipulating plant tissues to undergo new developmental programs and studying how this occurs. This includes studying changes in gene expression in tissues undergoing organogenesis and determining how morphogenetically active oligosaccharides regulate the type of organ that forms. The third approach involves studying how plant cell wall polysaccharides are synthesized, the rationale being that the formation of a new wall is intimately associated with the differentiation of plant cells. An understanding of the enzymes that catalyze wall formation and the mechanisms that regulate them will increase our understanding of how plant cells differentiate.

We have designed two strategies to unravel the biochemical mechanism(s) that regulate meristem formation in excised plant tissues. First, we are investigating how oligo-α-1,4-galacturonides (structurally distinct oligosaccharides from the plant cell wall) regulate morphogenesis and *de novo* meristem formation in tissue explants. Oligogalacturonides of degrees of polymerization of 12-14 inhibit root formation and induce flower shoot formation in tobacco thin-cell layer explants (TCLs) from the outer layers of tobacco floral branches. TCLs are model tissues for studying developmental plasticity because they can be induced by the phytohormones auxin and cytokinin to produce roots, shoots, or flowers within 24 days of culture on a defined medium.

To elucidate how cell wall-derived oligogalacturonides regulate morphogenesis in tobacco TCLs and what this regulation means *in planta*, we are searching for the oligogalacturonide receptors involved in the morphogenesis response. We are developing methods to label variously sized oligogalacturonides and other polyanion oligosaccharides with biotin, a label that can be detected at low (pmol) levels and that has high affinity with avidin or streptavidin. By using labeled oligogalacturonides as ligands, we hope to identify the oligogalacturonide-binding proteins and isolate putative oligogalacturonide receptors from cDNA expression libraries. Once isolated, we can characterize the genes for putative oligogalacturonide receptors, determine when the receptors are expressed during plant development, and study the effect of altered expression of the receptor gene on the morphogenesis of the transgenic plants.

A second line of investigation is to identify genes associated with root and shoot meristem formation in tobacco TCLs and to study the expression of these genes during root and shoot organogenesis. We know that the twelfth day of culture is the earliest time at which cell division centers in TCLs can be identified as putative root and shoot meristems. Messenger RNA isolated from such TCLs was used to construct cDNA libraries in the phage vector λgt10. Differential hybridization of clones with ^{32}P-labeled cDNA from the shoot or root organogenesis-specific libraries identified 17 cDNA clones, which represented 11 non-cross-hybridizing groups: four groups preferentially hybridized to cDNA from the shoot organogenesis library and seven to cDNA from the root organogenesis library. Two of the genes from the shoot organogenesis library share sequence homology with known genes, one with elongation factor 1a and the other with histone H3. A gene selected from the root organogenesis library shows sequence similarity with a gene expressed in potato tubers. Seven of the other groups of genes show no major sequence similarities with any known genes. Most of these genes are expressed in an organogenesis- and time-dependent fashion in TCLs cultured for various lengths of time in the presence or absence of oligogalacturonides. We are using genes whose expression in TCLs changes prior to the time when a specific type of organogenesis has been determined for the TCLs (~6 days in culture) or whose expression changes in response to oligogalacturonides to study the developmental and molecular changes associated with TCL morphogenesis.

A new research area in the lab is to understand how pectic polysaccharides are synthesized within the cell and the role of this synthesis in plant cell differentiation. Little is known about the sequence of glycosylation reactions required for the biosynthesis of pectic polysaccharides. We will first identify, purify, and characterize the galacturonosyltransferases involved in the synthesis of homogalacturonans (oligo-α-1,4-galacturonides), one of three types of pectic polysaccharides found in the wall. Once isolated, antibodies will be generated against the enzyme, and the gene will be cloned. These tools will be used in cell biology studies on the localization of the galacturonosyltransferases within the organelles of the ER and Golgi and studies on the level of expression of the galacturonosyltransferases in different cell types at different times during development.

7. Kelley W. Moremen, Assistant Professor of Biochemistry

The Endogenous Enzymes of the Golgi Complex and Oligosaccharide Processing

The endogenous enzymes of the Golgi complex, particularly the α-mannosidases that are involved in the early stages of Asn-linked oligosaccharide processing, determine the fate of the oligosaccharides on newly synthesized glycoproteins by controlling the extent of processing from high-mannose to complex-type structures. We are developing cellular, molecular, and biochemical techniques to study the regulation, localization, structure, and function of these enzymes to elucidate their role in the glycosylation pathway. We use cloned genes for the α-mannosidases as a model system in several projects.

Localization studies. A basic question of cell biology is how does a cell maintain organelle structure and architecture? This question is critical to the Golgi complex since it maintains an apparently static steady state architecture and rigid localization of endogenous enzymatic activities despite an enormous flux of biosynthetic intermediates across the organelle cisternae. The mechanism by which endogenous enzymes are retained within discrete sub-Golgi compartments is the subject of one line of studies. The α-mannosidase cDNAs are being expressed as wild type, truncated, mutagenized, and fusion protein constructs in order to determine the minimum sufficient structures required for correct localization. Biochemical and genetic approaches will then be used to isolate Golgi enzyme-specific binding components that may be involved in the retention mechanism.

Regulation. Although we know that some of the terminal glycosyltransferase genes are expressed in specific developmental stages or have differential tissue distributions, very little is known about the regulation of the entire glycosylation pathway. Are all of the early and intermediate enzymes constitutively synthesized in all tissues? Is there a concerted regulation of sequential enzymes in the pathway at the enzyme or gene level? Are the same genes used throughout development or are distinct isozymes or promoters used at specific developmental stages? These questions can now be answered with the probes for many of the processing enzymes that are available. By determining the relative expression levels of mRNA, polypeptide, and active enzyme for the α-mannosidase genes in various experimental systems, regulatory aspects of the pathway can be determined at the level of transcription, translation, or post-translation. Tissue-specific and developmental regulation of the α-mannosidases will be tested both by Northern blots and *in situ* hybridization to determine the variation in cell-specific expression levels. Competitive inhibitors of the processing hydrolases are also being tested to determine if compensatory changes in expression can be detected.

Gene structure and function. Several unique experimental systems are available for the study of the expression and function of mannosidase structural genes. The human autosomal recessive disease, HEMPAS, characterized by acute anemia, results from a reduced expression of one of a series of N-linked oligosaccharide processing enzymes, and, in one case, from a reduced level of mannosidase II expression.

The cause of this reduced expression involving the mannosidase genes is the focus of another line of research. Recent work suggests that removal of α1,2-mannosyl residues from the $Man_9GlcNAc_2$ processing intermediate may result from a collection of similar enzymes. Identification and cloning of these individual enzymes and comparison with the mannosidase I cDNA cloned in our lab should determine if these enzymes represent a multigene family for processing the high-mannose intermediates. The possibility of multiple mannosidase II genes and developmental expression is also suggested by the postnatal onset of HEMPAS symptoms.

Protein structure and function. The sequences of mammalian α-mannosidases show several similarities with other processing enzymes from mammalian and yeast sources. The mammalian mannosidase I polypeptide sequence shows a high degree of similarity to the yeast processing α-mannosidase despite significant differences in their ability to trim the $Man_9GlcNAc_2$ structural intermediate. We will use mutagenesis and domain swapping experiments in conjunction with protein expression systems to define the structural features responsible for these differences in substrate specificity. Mannosidase II also has regions of sequence similarity to GlcNAc transferase I (probably involved in substrate recognition) and the ER α-mannosidase and yeast vacuolar α-mannosidase (probably involved in the active site). Large-scale recombinant expression of wide type, mutant, and domain-specific constructs of these enzymes will allow a far more detailed view of protein structure and function than could be accomplished with enzymes purified from animal tissues. The long-term goal of these studies is to isolate sufficient quantities of the pure enzymes in order to make more detailed molecular studies and to understand the nature of enzyme-substrate interactions.

8. Michael Pierce, Associate Professor of Biochemistry

Cell-Surface Molecules and the Regulation of Cell Interactions

The focus of my laboratory is on understanding how changes in particular cell-surface molecules regulate the interaction of an animal cell with its environment. This interaction can be mediated by, for example, receptors on other cell surfaces or extracellular matrices, or it may involve soluble molecules such as growth factors and hormones. One "language" of cell-surface molecules in which my laboratory specializes is that of the complex carbohydrates, oligosaccharides that are attached to proteins and lipids embedded in the plasma membrane. Many lines of evidence demonstrate that changes in specific cell-surface oligosaccharides can regulate diverse cellular behaviors, including adhesion to other cells and matrices, responses to growth factors, and recognition of cells by the immune system.

V-*src* transformation of fibroblasts specifically affects GlcNAc-T V activity. Oncogenic transformation of cells by tumor viruses or several isolated oncogenes causes specific changes in the expression of cell-surface oligosaccharides. We are interested in a particular change in oligosaccharide expression that occurs when fibroblasts are transformed by the oncogene v-*src*, making these cells tumorigenic and, in some cases, metastatic. After transformation, a significant increase in asparagine-linked oligosaccharides with the structure -[GlcNAcβ(1,6)Man]- occurs. This increase is caused by an increase in the specific activity of the glycosyltransferase GlcNAc-T V, which catalyzes the synthesis of this structure. In addition to these results from *in vitro* transformation experiments, a recent study shows that the activity of GlcNAc-T V and its cell-surface product are over-expressed in 50% of human breast tumor biopsies, while benign and control samples show normal levels of expression. We are interested in determining the mechanism by which the v-*src* oncogene causes this unique effect on GlcNAc-T V, and we want to test the hypothesis that increases in -[GlcNAcβ(1,6)Man]- structures on the cell surface can directly affect the tumorigenicity or metastatic potential of a cell.

To do this, we have affinity-purified the enzyme from a tissue source and are determining its amino acid sequence in order to isolate a cDNA encoding the enzyme. This approach augments another technique of cDNA isolation by "expression cloning." The cDNA probe for the enzyme and an antibody specific for the enzyme will be used to measure mRNA levels, to test for possible post-translational modifications, and to study the structure of the GlcNAc-T V gene before and after malignant transformation. With this knowledge, we can design experiments to test the direct effects of -[GlcNAcβ(1,6)Man]- structures on tumorigenicity and metastasis by causing over-production of GlcNAc-T V in non-transformed cells and inhibiting the enzyme in metastatic cells by the introduction of antisense RNA followed by appropriate *in vivo* tumorigenicity and metastasis assays. With our collaborators, we developed many synthetic oligosaccharide substrates for the enzyme to map its active site in order to design an active site-directed, irreversible inhibitor that will cross cell membranes and inactivate the enzyme *in situ*. We also helped develop a sensitive ELISA assay for GlcNAc-T V activity to determine if levels of the enzyme in serum or biopsies can be used as an aid for tumor diagnosis or prognosis.

Mechanisms of oncogene effects on oligosaccharide expression. In addition to changes in GlcNAc-T V activity by v-*src* expression, transformation of several cell types by H-*ras* also appears to cause changes in this enzyme and perhaps other glycosyltransferases. A fundamental observation of transformed cells is that they display specific changes in oligosaccharide expression. Therefore, other studies in our laboratory focus on the various mechanisms of oncogene regulation of glycosyltransferases in order to answer these questions: Are the changes in activities caused by oncogenes also a response to a normal signaling pathway in cells? Do other oncogenes such as *raf* and *erb*-b2, which have distinct activities, as well as agents such as phorbol esters and growth factors cause similar effects in fibroblasts? Do these oncogenes and agents cause the same changes in glycosyltransferase activities in other cell types, such as epithelial cells? Are changes in oligosaccharide expression, oncogene expression, and degrees of tumorigenicity and metastasis clinically correlated, and can these changes be exploited in clinical assays of tissue and serum?

Two other animal glycobiology projects are under way in our laboratory. First, by experimentally manipulating the expression of glycosyltransferase genes, we want to understand how the expression of oligosaccharides on cell-surface glycoproteins can be specifically altered. This study is particularly relevant to biotechnology companies involved in expressing recombinant glycoproteins in cells engineered for hypersecretion. We are also collaborating with other research groups on studies to identify oligosaccharide-binding proteins in the central nervous system that can ultimately be used to facilitate nerve regeneration.

9. Herman van Halbeek, Associate Professor of Biochemistry

Structures, Dynamics, and Functions of Glycoproteins and Glycolipids in Solution

Glycoproteins and glycolipids are an important class of complex carbohydrates that act, for example, as blood-group determinants, tumor-associated antigens, receptors for hormones and toxins, and lubricants protecting body tissues against destructive influences. The long-term goal of our research is to determine the structures and dynamics of glycoproteins and glycolipids in solution to gain more knowledge about their biological functions. Often considered the "antennae" of cells, the carbohydrates of glycoproteins and glycolipids allow cells to communicate with their environment. Elucidating the complete structures of the carbohydrate chains of glycoconjugates is prerequisite to understanding the functions of these complex carbohydrates in biological interaction and recognition processes. Complete structural elucidation includes delineating the primary structure (glycosyl residue composition and glycosyl sequence, linkage types and locations) as well as the conformation or three-dimensional (3-D) structure of the molecule in solution.

Ongoing research projects focus on the structure and function of glycoprotein carbohydrates in relation to disease processes, such as (i) the relevance of the carbohydrate portion of the envelope glycoprotein (gp120) of the human immunodeficiency virus in the binding of gp120 to the T4 lymphocyte (AIDS infection), (ii) the structure and role of the carbohydrate chains in the biological activity and function of recombinant glycoprotein drugs (such as human erythropoietin, tissue plasminogen activator, CD4, hepatitis B surface antigen, etc.), (iii) the structure of respiratory, gastrointestinal, and ovarian cyst mucus carbohydrates and their role in the viscosity of the macromolecules, (iv) the structure of cell wall polysaccharides and membrane glycoproteins of pathogens such as *Cryptococcus neoformans* and *Pneumocystis carinii*, two of the leading killers in AIDS patients, and (v) the role of the carbohydrate portion of the glycosyl phosphatidyl inositol (GPI) membrane anchor of trypanosome variant surface glycoproteins in membrane integrity of the parasites and its potential as a target for chemotherapy of trypanosomiasis (sleeping sickness).

The heterogeneity and branched pattern of the carbohydrate moieties of glycoproteins, along with their unusual combination of rigidity and flexibility, present challenges to structure determination of the intact macromolecules in solution. This means that in proteins more than one carbohydrate structure is usually found attached to the same glycosylation site. We, therefore, need to know the complete primary structure of all oligosaccharide chains at each glycosylation site, followed by solution structure analysis of the isolated oligosaccharide prior to attempting to elucidate the 3-D structure of the glycoprotein as a whole. The method of choice to study the structure and 3-D solution conformation of a carbohydrate or glycoconjugate is high-resolution NMR spectroscopy in conjunction with molecular mechanics and dynamics simulations. Consequently, the development of NMR spectroscopic methods tailored to carbohydrates and fine-tuning of current methods to study conformations of biomolecules in solution and the interactions of these molecules with each other is another major emphasis of our research. We are developing techniques for determining the sites of glycosylation in glycoproteins and techniques for structural characterization of novel carbohydrates such as sulfated *N*-glycans, mucins, and GPI anchors. We also have available the technology to characterize bacterial polysaccharides and the carbohydrate portions of glycolipids.

For example, we have developed an HPAE method, using the Dionex BioLC system, for fingerprinting glycoprotein carbohydrates and successfully applied the procedure to identify the high-mannose oligosaccharides released from recombinant hepatitis-B surface antigen expressed in a yeast mutant and the *N*-acetyllactosamine-type oligosaccharides released from recombinant tissue plasminogen activator and recombinant CD4, the soluble form of the lymphocyte receptor of the HIV surface glycoprotein gp120. We have also developed considerable improvements in the techniques for sequential assignments of signals in the ^{1}H-NMR spectrum of a carbohydrate, by introducing 1-D TOCSY with double DANTE inversion, 2-D TOCSY with DIPSI-2 mixing, and combined ROESY-TOCSY experiments. Another revolutionary concept for sequencing carbohydrates by NMR methods is based on the use of long-range proton-carbon couplings across glycosidic linkages. We have worked out the key experiment and the necessary assignment strategies for ^{1}H- and ^{13}C-NMR spectra and successfully applied the method to the structural characterization of a bacterial adhesion receptor hexasaccharide and for locating a multitude of acyl substituents in plant oligosaccharides. We also developed a method suitable for the accurate measurement of such long-range ^{1}H,^{13}C couplings, yielding a valuable alternative way to obtain information on the conformation of glycosidic linkages besides only nuclear Overhauser effects.

In addition to studying the intramolecular interaction of glycoprotein oligosaccharides with the protein to which they are attached, we are studying intermolecular interactions such as the recognition and binding of glycoprotein carbohydrates by lectins, and we plan to study the recognition of carbohydrates by the enzymes involved in their biosynthesis and catabolism and by specific antibodies.

CCRC Technical Directors

1. **Russell W. Carlson, Associate Research Biochemist and Technical Director - Plant and Microbial Complex Carbohydrates**

Rhizobium-Legume Symbiosis and the Molecular Basis of Nitrogen Fixation

We are interested in understanding the molecular basis by which nitrogen-fixing *Rhizobium* bacteria infect their host legumes. The nitrogen-fixing symbiosis between rhizobia and legumes is a complex infection process between a bacterium and a eukaryotic cell. An exchange of signal molecules triggers a highly coordinated regulation of gene expression on the part of both the *Rhizobium* symbiont and the host legume resulting in the formation of a root nodule containing nitrogen-fixing *Rhizobium* bacteria called bacteroids.

These rhizobia bacteria are host symbiont specific (e.g., *Rhizobium leguminosarum* biovar *viciae* infects peas but not alfalfa, while *R. meliloti* infects alfalfa but not peas). Bacteria attach to emerging root hairs of the host legume and invade the root hair membrane. An infection thread forms which transports bacteria to the inner cortex cells. The cortex cells are stimulated to divide and form a nodule on the root. The infection thread penetrates the cortical cells, and the bacteria, still surrounded by the infection thread membrane (now called a peri-bacteroid membrane), are released into the cortical cells. The bacteria differentiate into bacteroids, now altered in size and shape as compared to the bacteria, and produce nitrogenase that reduces dinitrogen to ammonia.

Work in many laboratories around the world has resulted in identifying the genetic and molecular basis for some of these observable phenomena. Many of the *Rhizobium* genes required for nodulation have been identified. My research group has been working to characterize the major outer membrane component of *Rhizobium* called the lipopolysaccharide (LPS). This *Rhizobium* molecule is essential for the development of the infection thread in some cases and for release of the bacteria into the root cortical cells in other cases. The LPS molecule is a highly complex molecule with three structural regions: a lipid (called lipid A), a core oligosaccharide, and an *O*-antigen polysaccharide consisting of a repeating oligosaccharide (*O*-chain). The LPS can exist with all three structural regions intact or in its incomplete form in which it lacks the *O*-chain. We are involved in collaborative projects to analyze the structure of the LPSs of *R. leguminosarum* mutants defective in symbiosis wherein infection thread development aborts. We showed that the mutants were missing the *O*-chain and also were defective in the structure of their core oligosaccharide region. We obtained similar results for the *Bradyrhizobium japonicum*-soybean system where the *B. japonicum* mutant failed to nodulate or, with some soybean cultivars, formed nodules that contained neither bacteria nor infection threads. The LPSs from this mutant are also missing the *O*-chain.

We have made significant progress in determining the structure of LPSs from strains of *R. leguminosarum.* These strains represent three different biovars: *viciae* that forms a symbiosis with pea, *phaseoli* a symbiont of bean, and *trifolii* a symbiont of clover. We found that one of the components of the lipid A from the LPSs of all three biovars is a unique, very long-chain fatty acid which serves as a phylogenetic marker for gram-negative bacteria and is thought to add extra stability to the bacterial outer membrane. This stability may be indispensable to the survival of the *Rhizobium* within the plant cortical root cell.

We have purified two oligosaccharides which comprise the core region of *R. leguminosarum* LPSs. The *O*-antigen polysaccharides from *R. leguminosarum* LPSs are highly variable in their structure. However, they all have KDO at their reducing end. We are currently investigating the arrangement of the various components (the *O*-antigen, the core tetrasaccharide and the trisaccharide, and the lipid A) in the complete LPS molecule.

Using monoclonal antibodies, others have shown that subtle changes in the LPS structure occur and are important in the formation of nitrogen-fixing bacteroids. My group is studying the structural changes that occur during differentiation of a *Rhizobium* bacteria into its nitrogen-fixing bacteroid. Present data indicate that in *R. leguminosarum* biovar *phaseoli* the bacteroid-specific epitopes are found in the *O*-chain polysaccharide. Further work using recombinant genetics techniques is under way in collaboration with other laboratories to characterize these bacteroid-specific LPS structures . Once these LPS structural changes are identified, it should be possible to identify the gene and gene products required for these alterations and determine how their expression is regulated by the host plant.

Knowledge gained in understanding the molecular basis for *Rhizobium*-legume symbiosis may lead to improving the yield of important legume groups and increase the fertility of soil for non-legume crops. These studies also have a bearing on how the plant's defense mechanism is regulated so that the growth of the bacteria is controlled by the host such that a symbiotic rather than a pathogenic relationship is established. Since the LPSs from enteric bacteria have potent biological activities (such as cytokine production), which may have important medical uses but are toxic, *Rhizobium* LPSs offer a variety of new structures to test in order to determine if they are medically useful but not toxic.

2. Roberta K. Merkle, Technical Director for Biomedical Complex Carbohydrates

Animal Cell-Surface Carbohydrates and Cellular Interactions

I am studying the role of animal cell-surface carbohydrates in cell-cell interactions. In particular, I have been investigating the role of the mammalian carbohydrate-binding protein L-14, a soluble S-type lectin. While the existence of these lectins has been known for many years, their biological functions are not understood. In studying the carbohydrate-binding specificity of this lectin, we found that it preferentially binds to poly-*N*-acetyllactosamine sequences contained in animal cell glycoproteins. To elucidate the biological role of this lectin, we attempted to identify its endogenous ligand(s) and found that laminin, a glycoprotein known to contain poly-*N*-acetyllactosamine sequences, binds selectively to L-14. Since laminin is a component of the basement membrane, it is possible that the S-type lectins are involved in cellular attachment or movement on basement membranes.

Another area of my research studies the structure and biosynthesis of animal cell glycoproteins. We are examining the regulation of biosynthesis of poly-*N*-acetyllactosamine chains in animal cells and investigating the structure and biosynthesis of the oligosaccharide chains contained on the envelope glycoproteins of the AIDS virus. The oligosaccharides of the envelope glycoproteins are believed to be involved in the process of viral infection or other viral interaction with the T-cell. Metabolic radiolabeling and other analytical methods are used in this work that allow the analysis of very small amounts of material. These approaches have enabled us to determine that terminal glycosylation of the envelope precursor glycoprotein gp160 is completed before it is proteolytically cleaved to the envelope glycoproteins gp120 and gp41 in virally infected human T-cells.

The Center for Plant and Microbial Complex Carbohydrates at the University of Georgia Complex Carbohydrate Research Center

1987-92 Five-Year Report and 1993-97 Renewal Application

Appendix 3: Analytical Services and Training

May 1992

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Summary of Analytical Services Activities, 1987 - April, 1992

Four routine analytical services are offered by the Plant and Microbial Carbohydrate Center: glycosyl-residue composition analysis, glycosyl-linkage composition analysis, one-dimensional NMR spectroscopy, and fast atom bombardment-mass spectrometry (FAB-MS). The availability of these services was advertised in the scientific journals *Bio/Technology*, *Cell*, *Journal of Biological Chemistry*, *Science*, and the newsletters for the American Society of Plant Physiologists and the American Society for Microbiology. Additionally, posters with attached inquiry cards are taken to meetings attended by CCRC scientists. These advertisements result in inquiries about the services themselves and also in requests for advice from non-carbohydrate scientists working with glycoconjugates (see following pages for information on where we advertised, costs and sample ads).

An interpretation of the data is made for each sample analyzed. The investigator receives a detailed written report of the results of analyses accompanied by copies of instrument print-outs. The amount of expertise and instrument usage invested in the analysis of each sample is highly dependent on the type of sample submitted. However, glycosyl-residue composition analysis requires a minimum of two separate procedures: the preparation of alditol acetate derivatives and the preparation of trimethylsilyl methylglycosides. Both types of derivatives are analyzed both by gas chromatography (GC) and by combined GC-mass spectrometry (GC-MS). Both the sample and a standard sugar preparation are run for each sample. A single composition analysis usually requires four GC or GC-MS runs.

Similarly, a single glycosyl-linkage composition analysis requires the preparation of partially methylated alditol acetates followed by GC-MS analysis. Frequently, two or three runs of a single sample must be made. In addition, if amino sugars are present, the sample is analyzed by GC-MS using a different column. Therefore, each glycosyl-linkage analysis requires a minimum of two GC-MS runs per sample, or at least 18 additional runs on our GC-MS instruments.

FAB-MS analyses are also quite labor-intensive and frequently require several runs on the VG Analytical Zab instrument in order to obtain an acceptable spectrum. Several matrices must often be tried, and runs must be made in both the negative and positive mode. Again, a single sample requires a minimum of two or three runs. This service has been the least requested analysis.

NMR analyses are performed on our Bruker AM-250 and AM-500 instruments. The instrument time for each sample is highly variable, from as little as 15 minutes to as much as 24 hours. However, the average time would be about 2.0 hours.

Glycosyl-residue and glycosyl-linkage composition analyses and the derivatizations required are performed by a technician (Dr. Ramadas Bhat). NMR analyses are performed by Dr. Russell Carlson. The data from these analyses are reviewed by the technical director, Dr. Carlson, who prepares an individual report including the data and an interpretation of the results for each "client."

To date several publications have resulted that use the data generated by service work done by the Plant and Microbial Carbohydrate Center (a list of these publications is included in this appendix). Given the time required for the data to reach publication, it is likely that this number will increase significantly in the next year.

The submission of samples for analytical services has led to the formation of some collaborative projects with investigators outside the CCRC, as the results obtained from the analytical service work have, in some instances, led to inquiries regarding the establishment of a more in-depth collaborative research project. The Technical Director discusses the project and refers the investigator to those CCRC members who are most likely to be interested in pursuing the project. It is up to the outside investigator and the interested CCRC staff member to establish a collaborative agreement.

Advertisements for Analytical Services

	Ad Size	Issue	Cost
1988			
C & E News	small display ad	March	$ 885.40
Science	1/4-page	April	1,670.00
Molecular Plant-Microbe Interactions and	full-page	April	
Phytopathology News	full-page	July	775.00*
ASM News	1/4-page	July	700.00
ASPP	small display ad	August	FREE
			$4,030.40

*Total cost for placement in *Molecular Plant-Microbe Interactions* and *Phytopathology News*

1989			
Nature	1/4-page	June	1,250.00
ASM	1/2-page	July	$ 828.75
ASPP	small display ad	July/August	FREE
Bio/Technology	1/4-page	July	700.00
Cell	1/4-page	July 28	225.00
LC/GC	1/3-page	July	2,132.75
Science	1/4-page	July 14	1,760.00
			$6,896.50
1990			
Cell	1/2-page	February 23	510.00
Science	1/4-page	February 23	1,850.00
Journal Biological Chemistry	1/4-page	February 25	580.00
			$2,940.00
1991			
ASPP	small disply ad	Nov./Dec., 1990	FREE
Bio/Technology	1/4-page	February	901.00
Science	1/4-page	February 8	1,871.00
Cell	1/4-page	February 22	295.00
Journal Biological Chemistry	1/4-page	February 25	625.00
ASM	1/4-page	March	$ 743.75
			$4,435.75

Sample services ad 1988-91

Sample 1991 ad

ANALYTICAL SERVICES

provided by the

COMPLEX CARBOHYDRATE RESEARCH CENTER (CCRC)

The CCRC of The University of Georgia has been designated a Resource Center for Biomedical Complex Carbohydrate Science (sponsored by the NIH) and a Plant Science Center for the study of Plant and Microbial Carbohydrates (sponsored by the USDA, DOE, and NSF). These centers provide analytical assistance for scientists in university, industrial, and government laboratories carrying out nonproprietary research on the complex carbohydrates derived from animal, plant and microbial sources. The routine services that are offered include (1) glycosyl composition analysis, (2) glycosyl linkage analysis and (3) molecular weight determinations. The CCRC instrumentation used to provide these services includes gas liquid chromatography, gas liquid chromatography-mass spectrometry, fast atom bombardment-mass spectrometry, 250- and 500-MHz ^{1}H-NMR, and ^{13}C-NMR spectroscopy. Investigators may wish to establish a collaboration with scientists in the Resource Center to carry out more extensive characterization of their samples such as glycosyl sequence analysis. Those wishing to use the services of the CCRC should contact the Technical Directors for further information regarding the criteria and cost for sample analysis. Please do not submit samples without prior agreement. Address inquiries to: Dr. Roberta K. Merkle, Technical Director for Biomedical Carbohydrates, or Dr. Russell W. Carlson, Technical Director for Plant and Microbial Carbohydrates, Complex Carbohydrate Research Center, 220 Riverbend Road, The University of Georgia, Athens, Georgia 30602. Facsimile: 404-542-4412. Phone: 404-542-4405.

Analytical Service Announcements Posted/Distributed at Meetings

1991

Meeting Dates	Title of Meeting	Location
June 17 - 21	Gordon Conference on Plant Molecular Biology	Andover, NH
June 30 - July 5	11th International Symposium on Glycoconjugates	Toronto, Canada
July 22 - 26	Gordon Conference on Biological Structure and Gene Expression	Plymouth, NH
July 28 - August 1	American Society of Plant Physiologists	Albuquerque, NM
August 17 - 21	5th International Fungus Spore Conference	Unicoi State Park Helen, GA
August	4th International Congress/ Phycological Society of America	Duke University Duke, NC
August 4 - 8	42nd AIBS Annual Meeting of Scientific Societies and Mycological Society of America	San Antonio, TX
August 5 - 9	Gordon Conference on Medicinal Chemistry	New London, NH
October 16	Applied Biosystems Symposium	Atlanta, GA

1992

Meeting Dates	Title of Meeting	Location
January 5 - 8	Third Gatlinburg Symposium on Plant Responses to the Environment	Gatlinburg, TN
January 13 - 19	Keystone Symposium on Endothelial Cell	Keystone, CO
January 14 - 20	Keystone Symposium on Host-Parasite Interaction	Park City, UT
March 21 - 27	Keystone Symposium on Glycobiology	Park City, UT
July 11 - 15	11th Annual Scientific Meeting of the American Society for Virology	Ithaca, NY

Poster and slide for advertisement at scientific meetings

Analytical Services for Carbohydrates

provided by the

COMPLEX CARBOHYDRATE RESEARCH CENTER

A DOE/NSF/USDA Plant Science and NIH Biomedical Resource Center

Routine services available:

- ▶ Glycosyl composition analysis
- ▶ Glycosyl linkage analysis
- ▶ One-dimensional NMR analysis
- ▶ Molecular weight determinations

Techniques utilized:

- ▶ Gas liquid chromatography
- ▶ Gas liquid chromatography-mass spectrometry
- ▶ Fast atom bombardment-mass spectrometry
- ▶ 250- and 500-MHz ^{1}H-NMR, and ^{13}C-NMR spectroscopy

For further information on the services available, the criteria and cost for sample analysis, and prior to submitting a sample, please fill out the attached form or contact:

Technical Director for Plant and Microbial Carbohydrates
or
Technical Director for Biomedical Carbohydrates
Complex Carbohydrate Research Center
The University of Georgia
220 Riverbend Road
Athens, Georgia 30602 USA
Tel.: 404-542-4405 Facsimile: 404-542-4412

1991 services flyer and reply card sent to scientific meetings

Carbohydrate Analytical Services

provided by the

COMPLEX CARBOHYDRATE RESEARCH CENTER

A DOE/NSF/USDA PLANT SCIENCE AND NIH BIOMEDICAL RESOURCE CENTER

Routine services available:

- Glycosyl-composition analysis
- Glycosyl-linkage analysis
- One-dimensional NMR analysis
- Molecular weight determinations

Techniques utilized:

- Gas-liquid chromatography
- Gas-liquid chromatography-mass spectrometry
- Liquid chromatography-mass spectrometry
- Fast atom bombardment-mass spectrometry
- 250-, 500-, and 600-MHz ^{1}H-, and ^{13}C-NMR spectroscopy

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A UNIQUE CONCENTRATION OF INTERDISCIPLINARY EXPERTISE AND STATE-OF-THE-ART INSTRUMENTATION

Send the attached card for further information on the services available (the cost for sample analysis, how to prepare your sample, etc.). Contact the appropriate technical director prior to submitting any samples. Non-routine services and more extensive collaborative investigations can be set up on a case-by-case basis.

Dr. Russell W. Carlson, Technical Director-Plant & Microbial Carbohydrates
Dr. Roberta K. Merkle, Technical Director-Biomedical Carbohydrates

Complex Carbohydrate Research Center, The University of Georgia
220 Riverbend Road, Athens, Georgia 30602 USA
Tel.: 404-542-4402 Facsimile: 404-542-4412

Complex Carbohydrate Research Center • The University of Georgia
220 Riverbend Road, Athens, Georgia 30602 USA
Telephone: 404-542-4402 Facsimile: 404-542-4412

Please send me information on the analytical services available at the Complex Carbohydrate Research Center.

Name:______________________________

Institution/Department/Company:____________________

____

Mailing Address:__________________________

____

Telephone No.:________________ Facsimile:________________

I am working in ☐ plant/microbial ☐ animal cell carbohydrates and am interested in____________

____

☐ I would also like to receive information on the content, capabilities, and purchase cost of the Complex Carbohydrate Structural Database and its search program, CarbBank.

1992 services flyer and reply card sent to scientific meetings

Carbohydrate Analytical Services
provided by the

COMPLEX CARBOHYDRATE RESEARCH CENTER

A UNIT OF THE USDA/DOE/NSF PLANT SCIENCE CENTER PROGRAM SPONSORED BY THE DEPARTMENT OF ENERGY

AND A NATIONAL INSTITUTES OF HEALTH BIOMEDICAL RESOURCE CENTER

Routine services available:

- Glycosyl-composition analysis
- Glycosyl-linkage analysis
- One-dimensional NMR analysis
- Molecular weight determinations

Techniques utilized:

- Gas-liquid chromatography
- Gas-liquid chromatography-mass spectrometry
- Liquid chromatography-mass spectrometry
- Fast atom bombardment-mass spectrometry
- 250-, 500-, and 600-MHz ^{1}H- and ^{13}C-NMR spectroscopy

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Send the attached card for further information on the services available (the cost for sample analysis, how to prepare your sample, etc.). Contact the appropriate technical director prior to submitting any samples. Non-routine services and more extensive collaborative investigations can be set up on a case-by-case basis.

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Complex Carbohydrate Research Center, The University of Georgia
220 Riverbend Road, Athens, Georgia 30602-4712 USA
Tel.: 706-542-4402 Facsimile: 706-542-4412

. . . .

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220 Riverbend Road, Athens, Georgia 30602-4712 USA
Telephone: 706-542-4402 Facsimile: 706-542-4412

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Name:____________________________________

Institution/Department/Company:________________________

Mailing Address:______________________________

Telephone No.:________________ Facsimile:________________

I am working in ☐ plant/microbial ☐ animal cell carbohydrates and am interested in

☐ I would also like to receive information on the content, capabilities, and purchase cost of the Complex Carbohydrate Structural Database and its search program, CarbBank.

List of Publications Resulting from Service Work

Brillouet, J.-M., Z. Gunata, S. Bitteur, R. Cordonnier, and C. Bossso. **1989**. Terminal apiose: A new sugar consitutent of grape juice glycosides. *J. Agric. and Food Chem. 37*:910-912.

Byrd, W. and S. Kadis. **1989**. Structures and sugar compositions of lipopolysaccharides isolated from seven *Actinobacillus pleuropneumoniae* serotypes. *Infection and Immunity 57*:3901-3906.

Sonnewald, U., A. Sturm, M. J. Chrispeels, and L. Willmitzer. **1989**. Targeting and glycosylation of patatin the major potato tuber protein in leaves of transgenic tobacco. *Planta 179*:171-180.

Datta, K., A. Schmidt, and A. Marcus. **1989**. Characterization of two soybean repetitive proline-rich proteins and a cognate cDNA from germinated axes. *Plant Cell. 1*:945-952.

Robertsen, B. **1989**. Pectate lyase from *Cladosporium cucumerinum*, purification, biochemical properties and ability to induce lignification in cucumber hypocotyls. *Mycol. Res. 94*:595-602.

Arrendale, R.F., R.F. Severson, V.A. Sisson, C.E. Costello, J.A. Leary, D.S. Himmelsbach, H. van Halbeek. **1990**. Characterization of the sucrose ester fraction from *Nicotiana glutinosa*. *J. Agric. Food Chem. 38*:75-85.

Austin, E.A., J.F. Graves, L.A. Hite, C.T. Parker, and C.A. Schnaitman. **1990**. Genetic analysis of lipopolysaccharide core biosynthesis by *Escherichia coli* K-12: Insertion mutagenesis of the *rfa* locus. *J. Bacteriol. 172*:5312-5325.

De Stefano, J.A., M.T. Cushion, V. Puvanesarajah, and P.D. Walzer. **1990**. Analysis of *Pneumocystic carinii* cyst wall. II. Sugar composition. *J. Protozool. 37*:436-441.

Schnaitman, C.A. and E.A. Austin. **1990**. Efficient incorporation of galactose into lipopolysaccharide by *Escherichia coli* K-12 strains with polar *galE* Mutations. *J. Bacteriol. 172*:5511-5513.

Shedletzky, E., M. Shmuel, D.P. Delmer, and D.T.A. Lamport. **1990**. Adaptation and growth of tomato cells on the herbicide 2,6-dichlorobenzonitrile leads to production of unique cell walls virtually lacking a cellulose-xyloglucan network. *Plant Physiol. 94*:980-987.

Tully, R.E., D.L. Keister, and K.C. Gross. **1990**. Fractionation of the β-Linked glucans of *Bradyrhizobium japonicum* and their response to osmotic potential. *App. Env. Microbiol. 56*:1518-1522.

Xu, P., M. Iwata, S. Leong, and L. Sequeira. **1990**. Highly virulent strains of *Pseudomonas solanacearum* that are defective in extracellular-polysaccharide production. *J. Bacteriol. 172*:3946-3951.

Fredrikson, K., P. Kjellbom, and C. Larsson. **1991**. Isolation and polypeptide composition of 1,3-β-glucan synthase from plasma membranes of *Brassica oleracea. Physiol. Plant. 81*:298-294.

Manning, P.M., S.L. Erlandsen, and E.L. Jarroll. Localization of glucose in *Giardia muris* cysts. 43rd Annual Meeting of the Society of Protozoologists, Univ. of Maryland, June, **1990**.

Domozych, D. S., B. Wells, and P. J. Shaw. **1991**. Basket scales of the green alga, *Mesostigma viride*: chemistry and ultrastructure. *J. Cell Science 100*:397-407.

Ziemba, V. A. and S. T. Kellogg. **1991**. Isolation and Characterization of the exopolymer from a virulent strain of *Staphylococcus epidermidis*. Abstr. Annu. Meet. Am. Soc. Microbiol. D49, p.86.

Sturm, A. **1991**. Heterogeneity of the complex N-linked oligosaccharides at specific glycosylation sites of two secreted carrot glycoproteins. *Eur. J. Biochem. 199*:169-179.

Pesacreta, T. C., V. I. Sullivan, K. H. Hasenstein, and J. M. Durand. **1991**. *Protoplasma 163*:174-180.

Fredrikson, K., P. Kjellbom, and C. Larsson. **1991**. Isolation and polypeptide composition of 1,3-β-glucan synthase from plasma membranes of *Brassica oleracea*. *Physiol. Plant. 81*:289-294.

Van Huystee, R. B., P. A. Sesto, and J. P. O'Donnnell. Robertsen, B. **1989**. Pectate lyase from *Cladosporium cucumerinum*, purification, biochemical properties and ability to induce lignification in cucumber hypocotyls. *Mycol. Res. 94*:595-602.

1992. Number and size of oligosaccharides linked to peanut peroxidases. *Plant Physiol. Biochem.* In press.

Inzana, T. J., R. P. Gogolewski, and L. B. Corbeil. **1992**. Phenotypic phase variation in *Haemophilus somnus* lipooligosaccharide during bovine pneumonia and after *in vitro* passage. *Infect. Immun.* Submitted.

Petter, J. G. **1992**. Identification of *Salmonella enteritidis* field isolate lipopolysaccharide form variation. In preparation.

Fuller, M. S. **1992**. Studies of *Gonapodya prolifera* in axexnic culture. In preparation.

Van Etten, J. L., University of Nebraska. **1992**. In preparation.

Burks, A. W., L. W. Williams, C. Connaughton, G. Cockrell, T. J. O'Brien, and R. M. Helm. **1992**. Identification and characterization of a second major peanut allergen, Ara h II, utilizing the sera of patients with atopic dermatitis and positive peanut challenge. *J. Allergy and Clinical Immun.* Submitted.

Appendix 3: Analytical Services and Training

Total Number of Analyses and Samples Performed June 1988 - April 1992

	Number of Analyses Performed				
Analysis	88-89	89-90	90-91	91-92	Total
Composition	40	51	60	90	241
NMR	14	33	13	6	66
FAB	12	29	0	13	54
Methylation/Linkage	15	6	14	63	98
Total	81	119	87	172	459

Total Number of Samples

Year	Number of Samples
88-89	76
89-90	97
90-91	72
91-92	154
Total	399

Appendix 3: Analytical Services and Training
Samples Analyzed by the CCRC Plant and Microbial Carbohydrate Center
June 1988-April 1992

Name	Sample Type	Analysis Type	Number of Samples	Date
M. O'Connell Bio. Sci. Dublin, IRELAND	*Rhizobium* VF39 EPS M45 EPS	Composition	2	06/28/88
A. Sturm Dept. of Biology U. of California La Jolla, CA	Glycoprotein fractions	Composition	6	08/02/88
D. Delmer MSU-DOE Plant Sci. Ctr. Michigan St. U. E. Lansing, MI	UDP glucose activator	GC-MS, FAB	2	08/04/88
A. Marcus Inst. for Cancer Res. Philadelphia, PA	Soybean cell wall glycoprotein	Composition	1	08/10/88
S.N. Talhouk Dept. of Ento. OARDC-OSU Wooster, OH	Powdered birch leaf	Composition	1	09/27/88
F. Cassels Dental Research NIH Bethesda, MD	*Streptococcus sangis H1* capsule repeating unit	NMR	1	10/09/88
E. Ha Food Science Clemson U. Clemson, SC	*Palmella texenis* UTEX 1708 colloid	Methylation, TMS methyl glycoside	1	11/09/88
L. Falb GA Exp. St. Griffin, GA	Herbicide sample	NMR, FAB	1	11/22/88
V. Crescenzi U. "La Sapienza" Rome, ITALY	*R. trifolii* TA1	Linkage EPS	1	12/08/88
D. Keister USDA Beltsville, MD	*Bradyrhizobium japonicum* glucans	NMR	5	12/15/88
L. Sequeira U. of Wisconsin Madison, WI	*P. solanacearum*, CK60, *P. solancearum* CKD700, PI KD700, PI K60 EPSs	Composition	4	01/03/89

M. Snook USDA Athens, GA	Flavanoid glycoside	Methylation	1	01/17/89
B. Fraser-Reid Duke University Durham, NC	Synthetic glucan	FAB	1	01/26/89
D. Delmer Hebrew U. Jerusalem, ISRAEL	UDP-Glc:(1/)-ß-glucan synthetase	GC-MS, NMR	2	01/31/89
M. Janusz Harvard Med. School Boston, MA	Yeast glucan Maltoheptose control	^{1}H-NMR, FAB, Methylation	2	01/31/89
R. Arrendale USDA/ARS/TQSRU Athens, GA	Spermidine	FAB-MS	2	02/23/89
N. Nanavati NORAMCO Inc. Athens, GA	Synthetic ester	FAB-MS	1	02/23/89
D. Kasarda WRRC Albany, CA	Wheat glutenin	Composition	1	02/28/89
A. Murphy Dentistry U. of Pacific San Francisco, CA	Membranous ATPase	Composition	1	02/28/89
W. Whelan Dental Res. NIH Bethesda, MD	*Candida albicans* mannans	Composition	2	02/28/89
H. Bussey McGill U. Montreal, CANADA	Yeast glucans	Methylation	2	03/01/89
L. Sequeira Plant Path. Russell Lab. Madison, WI	*P. solanacearum* EPS	Composition	1	03/02/89
M. Steup Botanishes Inst. U. Munster FED. REP. GERMANY	Plant carbohydrates	Composition	4	03/02/89
C. Schnaitman Microbiology U. of Virginia Charlottesville, VA	*E. coli* LPS	Composition	7	03/21/89

B. Fraser-Reid Chemistry Duke University Durham, NC	Synthetic glucan	FAB	1	03/25/89
P. Lipke Biology Hunter College New York, NY	*Candida albicans* mannans	Methylation	2	03/25/89
D. Holty Interchem Atlanta, GA	Chitosan	Composition	4	03/27/89
J. Chatterton USDA Utah State U. Logan, UT	*Conium maculatum* trisaccharide	Methylation	1	04/14/89
F. Cassels Dental Research NIH Bethesda, MD	*S. sanguis* capsule repeating unit	NMR	1	04/17/89
E. McManus NIH Bethesda, MD	ß-glucan, *Candida albicans* mannan *Saccharomyces cerivisiae*	Composition	3	04/17/89
S. Turchi Chemistry Millersville U. Millersville, PA	*Streptococcus mutans* glucan	NMR	2	04/17/89
A. Murphy Dentistry U. of the Pacific San Francisco, CA	ATPase	Composition	1	05/03/89
J. Chatterton USDA Utah State U. Logan, UT	Lolium and tomato trisaccharides	Methylation	2	05/05/89
M. Snook USDA Athens, GA	Glycoside flavones	FAB-MS	2	05/05/89
D. Emerson Microbiology Cornell Ithaca, NY	*Leptothrix discophora* glycoproteins	Composition	2	05/08/89
B. Finnerty Athens, GA	Fatty acyl trehelose	Methylation	1	05/09/89

H. Bussey Microbiology McGill U. Montreal, CANADA	*S. cerevisiae* 7B & mutant 3 glucans	Methylation	2	05/31/89
W.D. Bauer Agronomy Ohio State U. Columbus, OH	*Erwinea stewartii* EPS	^{1}H-NMR, ^{13}C-NMR, Composition, Methylation	1	06/01/89
C. Schnaitman Microbiology U. of Virginia Charlottesville, VA	*E. coli* lipopoly- saccharides	Composition	5	06/01/89
Z. Marom Mt. Sinai Med. Ctr. New York, NY	Glycoprotein	Composition	1	06/09/89
D. Delmer DOE Plant Research Michigan State U. E. Lansing, MI	UDP-glc synthetase activator	GC-MS	4	07/20/89
D. Emerson Microbiology Cornell Ithaca, NY	Bacterial fibril	Composition	2	07/20/89
D. De Jong Bacteriology U. of Wisconsin Madison, WI	Glycoprotein from gliding bacterium	Composition	1	07/20/89
M. Janusz Cell Biology Merrill-Dow Cincinnati, OH	Yeast glucan fraction 3	Methylation	1	08/16/89
L. Barlow Plant Pathology U. of Wisconsin Madison, WI	*P. solanacearum* EPS	Composition	1	08/29/89
K. Nickerson Biological Science U. of Nebraska Lincoln, NE	*Bacillus thuringiensis* toxins	Composition	2	09/11/89
A. Waiss, Jr. Plant Development USDA Albany, GA	3-ß-glucopyranosyl perulactone	Composition	1	09/11/89
L. Falb GA Experiment Station Griffin, GA	Clethodim acid degradation products	FAB-MS, NMR	9	09/17/89

K. Garrett PathoGene Raleigh, NC	Congo Red binding bacterial poly-saccharide	Composition	1	09/18/89
M. Snook USDA Athens, GA	Flavanol glycoside	FAB-MS	1	09/20/89
W.D. Bauer Agronomy Ohio State U. Columbus, OH	*Erwinia stewarti* EPS	NMR, Composition, Methylation	4	09/21/89
L. Falb GA Experiment Station Griffin, GA	Clethodim product	FAB-MS, NMR	10	10/09/89
K. Garrett PathoGene Raleigh, NC	Congo Red and Aniline Blue binding material	Composition	2	10/18/89
L.S. Phillips Medicine Emory University Atlanta, GA	Serum growth inhibitor	Composition	2	10/31/89
J. De Stefano Anatomy & Cell Bio. U. of Cincinnati Cincinnati, OH	*Pneumocystis carinii* cell walls	Composition	2	11/03/89
L. Falb GA Experiment Station Griffin, GA	Clethodim photodegra-dation products	FAB-MS, NMR	8	11/15/89
D. Chu College of Pharmacy U. of Georgia Athens, GA	Nucleoside sugar	FAB-MS	1	11/21/89
W.R. Usinger Immunsine Corp. Orinda, CA	Gliding bacterial adjuvant	Composition	1	12/01/89
J. Streeter Agronomy Ohio State U. Wooster, OH	Soybean nodule slime	Composition	1	12/08/89
D. Delmer Botany Hebrew U. Jerusalem, ISRAEL	Tomato cell wall fractions	Composition	2	12/14/89
D. Shah NORAMCO Athens, GA	Synthetic samples	GC-MS	3	01/02/90

K. Nickerson Biological Sciences U. of Nebraska Lincoln, NE	*B. thuringiensis* toxin	Composition	5	01/08/90
P. Manning Biology Cleveland State U. Cleveland, OH	*Giardia muris* cyst walls	Composition	1	01/08/90
D. Shah NORAMCO Athens, GA	Synthetic samples	GC-MS	2	01/16/90
T. Pesacreta Microscopy U. of SW Louisiana Lafayette, LA	Thistle filaments	Composition	4	01/25/90
S. Kadis Medical Microbiology U. of Georgia Athens, GA	*Pasteurella* LPS	Composition	10	02/19/90
S. Kadis Medical Microbiology U. of Georgia Athens, GA	*Pasteurella* LPS	GC-MS	5	03/06/90
D. Vinal Pathogene Raleigh, NC	Congo Red binding material from *E. coli*	Composition	1	03/06/90
D. Delmer Botany Hebrew University Jerusalem, ISRAEL	Barley endosperm	GC-MS	1	03/06/90
Z. Marom Mt. Sinai Med. Center New York, NY	MGP glycoprotein	GC-MS	1	03/19/90
W. Usinger Immunsine, Inc. San Leandro, CA	Gliding bacterium glycoconjugate	Composition	1	04/03/90
D. de Jong Dept. of Bacteriology Univ. of Wisconsin Madison, WI	Glycoprotein samples from gliding bacteria	Composition	2	6/1/90
J. Streeter Dept. of Agronomy OH State Univ. Wooster, OH	Carbohydrate slime from soybean samples	Composition	5	6/1/90

J. Puhlman CCRC Univ. of GA Athens, GA	Maltoheptaaminitol	^{1}H- and ^{13}C-NMR	1	6/19/90
J. de Stefano Univ. of Cincinatti Medical Ctr. Dept. of Anatomy and Cell Biology Cincinnati, OH	Alditol acetates	GC/MS	23	6/21/90
D. Sertl Ross Laboratories Columbus, OH	Ross Lab.	Composition, methylation	2	8/2/90
A. Sturm Friedrich Miescher Institut Basel, Switzerland	Secreted carrot glycoprotein	Composition	10	8/20/90
P. Atreya Dept. of Plant Path. Univ. of Kentucky Lexington, KY	Glycoprotein from tobacco leaves	Composition	1	8/27/90
M. Fuller Dept. of Botany Univ. of Georgia Athens, GA	Fungal glucan	Methylation	1	10/4/90
J. Puhlman CCRC Univ. of Georgia Athens, GA	Synthetic glucan	NMR	1	10/11/90
J. Puhlman CCRC Univ. of Georgia Athens, GA	Maltoheptose	^{1}H- and ^{13}C-NMR	1	10/17/90
M. Nieder ESCA Genetics Corp. San Carlos, CA	Polysaccharide from plant tissue culture	Composition	2	10/22/90
J. Streeter Dept. of Agronomy Ohio State Univ. Wooster, OH	Soybean nodule slime	Composition	2	10/24/90
S. M. Lim Dept. of Plant Path. and Genetics Univ. of Illinois Urbana, IL	*Septoria glycines* phytotoxin	Composition, linkage, NMR	1	11/5/90

K. Fredrikson Dept. of Plant Biochemistry Univ. of Lund Lund, Sweden	Synthetic polyglucan produced by glucan synthase from cauli-flower	Methylation	1	11/7/90
R. van Huystee Dept. of Plant Sci. Univ. of Western Ontario London, Ontario Canada	Peroxidase oligosaccharides	Composition Linkage	2 1	12/6/90
A. Junkins Univ. of Wisconsin Dept. of Food Microbiology and Toxicology Madison, WI	EPS from *E. Coli*	Composition, linkage, NMR	2	12/10/90
D. Domozych Dept. of Biology Skidmore College Saratoga Springs, NY	Algal-scale	Composition, methylation	1	01/10/91
H. Prechel Biologie VI (Genetik) Universitat Bielefeld Germany	*Rhizobium legumino-sarum* biovar *viciae* lipopoly-saccharide	Composition	3	01/11/91
J. Hyatt Eastman Chemical Company Kingsport, TN	Glucomannin	Composition, methylation	1	11/14/90
H. Grimes Dept. of Botany Washington State Univ. Pullman, WA	Glycoprotein	Composition	1	02/19/91
J. Hyatt Eastman Chemical Co. Kingsport, TN	Commercial cellulose acetates	Composition	5	02/19/91
S. Kadis Dept. of Medical Microbiology Univ. of Georgia Athens, GA	*Pasteurella* lipopolysaccharides	Composition	2	02/21/91
L. Hadwiger Dept. of Plant Path. Washington State Univ. Pullman, WA	Fungal pathogen elicitor	Composition, NMR	4	03/06/91

T. Inzana Dept. of Pathobiology Virginia-Maryland Regional College of Vet. Med. VPI Blacksburg, VA	*Haemophilus somnus*	Composition, Fatty acid	4	03/06/91
J. Hyatt Eastman Chemical Co. Kingsport, TN	Fractions of a commercial cellulose acetate	Composition	5	03/20/91
C. Read Kensil Cambridge Biotech Worcester, MA	*Quillaja saponaria*	Linkage	1	03/20/91
S. Kellog Dept. of Bacteriology Univ. of Idaho Moscow, ID	Gram positive Bacteria	Composition	1	03/22/91
M. Brown Dept. of Botany Univ. of TX Austin, TX	In vitro synthesized glutens	Linkage	3	03/22/91
C. Reed Kensil Natural Products Cambridge Biotech Worcester, MA	Saponin	Composition	2	04/16/91
J. van Etten Dept. of Plant Pathology Univ. of NE Lincoln, NE	*Chlorella* virus protein	Composition	1	04/16/91
E. Shotts Dept. of Med. Micro. Univ. of GA Athens, GA	An Algo suspension	Glycosyl composition	1	04/24/91
J. A. Raymond Dept. of Biological Sciences Univ. of South AL Mobile, AL	*Nitschia stellata* glycoprotein	Glycosyl composition	1	04/24/91
K. Fredrikson Dept. of Plant Biochemistry University of Lunde Lunde, Sweden	Polyglucan	Glycosyl composition Linkage	4 4	05/08/91
E. Hyman 3525 Prytania Str. Suite 220 New Orleans, LA	Bacterial sample	Glycosyl composition	1	05/08/91

T. Inzana Dept. of Pathobiology Virginia-Maryland Regional College of Veterinary Medicine VPI Blacksburg, VA	Lipopolysaccharide	Glycosyl composition and fatty acid	1	05/08/91
R. Pressey Richard Russell Research Center Athens, GA	Glycoprotein	Glycosyl composition	1	05/08/91
N. Carpita Dept. of Botany and Plant Pathology Purdue Univ. West Lafayette, IN 47907	Polyglycuronic acid	NMR	1	05/24/91
J. Hyatt Eastman Chemical Co. Kingsport, TN	Cellulose acetate	Glycosyl composition	3	05/31/91
S. Kadis Dept. of Med. Micro. UGA Vet. Med. Athens, GA	Pastuerella LPS	Composition	3	05/31/91
R. Pressey USDA Russell Rsch. Ctr. Athens, GA	Plant glycoprotein	Glycosyl composition	2	05/31/91
F. Voragen Dep. of Food Sci. Wageningen Agricultural University P.O.B. 81296700 E.V. Wageningen, The Netherlands	Pectic cell wall fraction	Glycosyl composition	1	05/31/91
N. V. Padhye Food Research Institute 1925 Willow Dr. Madison, WI	Glycoprotein from *E. coli*	Composition	1	06/19/91
J. van Etten Dept. of Plant Path. Univ. of Nebraska Lincoln, NE	Capsid protein from six *Chlorella*-virus PBCB-10 types	Composition	6	06/19/91
V. Puvanesarajah ABC Labs, Inc. P.O. Box 1097 Columbia, MO	Herbicide metabolite	FAB-MS and GC-MS	1	07/10/91

A. Collmer Dept. of Plant Path. Cornell Univ. Ithaca, NY	Pectate lyase isozyme from *Erwinia chrysanthemi*	Composition	1	07/12/91
G. C. Daily Stanford Univ. Dept. of Biological Sciences Stanford, CA	Sap from a willow shrub	Composition	1	07/12/91
J. Chatterton USDA Northern Plains Area Forest and Range Rsch. Utah State Univ. Logan, UT	Tetrasaccharide from *Bromus tectorum*	Methylation and FAB-MS	1	07/15/91
C. R. Kensil Cambridge Biotech Worcester, MA	Triterpine glycoside	Glycosyl composition, linkage	1	07/15/91
K. E. Vogele The Wistar Institute Philadelphia, PA	Quilliac acid	Composition, linkage	1	07/24/91
D. L. Hendricks Western Cotton Rsch. Lab USDA-ARS Phoenix, AZ	Fractions from the honey dew of the sweet potato white fly *Bemisia tabaci*	Methylation	2	07/28/91
J. Hyatt Eastman Chemical Co. Kingsport, TN	Commercial cellulose acetate fractions	Glycosyl composition methylation	4 1	07/28/91
C. Bergmann CCRC Athens, GA	Endopolygalacuronase	Glycosyl composition	1	07/29/91
W. Burks Dept. of Pediatrics Univ. of Arkansas for Medical Sciences Arkansas Children's Hospital Little Rock, AR	Peanut glycoproteins	Glycosyl composition	2	07/29/91
J. Petter SE Poultry Rsch. Lab Athens, GA	Cell wall fraction from a chicken pathogen	Composition	1	07/28/91
R. V. van Huystee Dept. of Plant Sciences Univ. of Western Ontario London, Ontario Canada	Peanut peroxidase enzymes	Composition, linkage	2	07/29/91

Carl Bergman UGA, CCRC Athens, GA	EPG sample	Composition	1	08/22/91
Donald Hendricks USDA Western Cotton Research Lab Phoenix, AZ	Samples from the Western Cotton Research Lab	Methylation	4	08/22/91
Jean Petter SE Poultry Rsch. Lab. Athens, GA	*Salmonella enteridis* ECA samples	Composition	2	08/22/91
Charlotte Reed Kensil Cambridge Biotech Worcester, MA	Triterpine glycoside	Glycosyl Linkage	1	09/06/91
Nisha V. Padhye Food Research Institute Madison, WI	*E. coli* outermembrane protein	Composition	1	09/06/91
Jerry Chatterton USAD Northern Plains Area Utah State University Logan, UT	Samples from *Bomus tectorum*	Methylation and FAB-MS	3	09/12/91
Charlotte Reed Kensil Cambridge Biotech Worcester, MA	Triturpine glycoside (a saponin sample)	Glycosyl Linkage	1	09/16/91
Bonnie Reger USDA, Russell Rsch. Ctr. Athens, GA	A style glycoprotein	Composition	1	09/16/91
Katherine E. Vogele The Wistar Institute Philadelphia, PA	Triturpinoid glycosides	Composition and Methylation	2	09/16/91
R. Malcolm Brown, Jr. Dept. of Botany Univ. of TX at Austin Austin, TX	*In vitro* cotton synthesis products	Methylation	3	10/01/91
David Domozych Dept. of Biology, Skidmore College Saratoga Springs, NY	Mesostigma scales	Glycosyl Linkage	1	10/01/91
David Domozych Dept. of Biology Skidmore College Saratoga Springs, NY	Polysaccharide muciliage from a gliding desmid green alga	Composition	1	10/01/91
Park S. Noble Dept. of Biology Univ. of CA, Los Angeles Los Angeles, CA	Muciliage samples from *Opuntia* stems	Composition	3	10/01/91

Bonnie Reger USDA, Russell Rsch. Labs Athens, GA	A style sample	Composition	1	10/01/91
Rebecca Robbins UCLA Dept. of Biology University of California Los Angeles, CA	Syanil bacterial sheath	Composition	1	10/18/91
R. Malcolm Brown, Jr. Department of Botany Univ. of TX, Austin Austin, TX	Synthetic cotton products	Methylation	4	10/29/91
Jerry Chatterton USDA-ARS Forage and Range Research Lab Utah State Univ. Logan, UT	Fructose containing oligosaccharides from *Bromus tectorum*	Methylation and FAB-MS	10	10/29/91
Phillip H. Enriquez Advanced Magnetics Inc. Cambridge, MA	An arabinogalactan	Composition and Methylation (in triplicate)	1	10/29/91
David Sertl Ross Laboratories Columbus, OH	Soybean samples	Composition, Glycosyl Linkage, NMR	2 of 5 3 of 5 2 of 5	11/11/91
Carl Bergmann UGA, CCRC	EPG and PGIP proteins	Composition	2	11/20/91
Larry Dyck Biological Sciences Clemson University Clemson, SC	Sheath from the filamentous cyano bacterium *Lyngbya wollei*	Composition	1	11/20/91
Barry G. Harmon Dept. of Vet. Pathology UGA Athens, GA	LPS from *Pasteurella maltocida*	Composition	2	11/20/91
Park S. Noble Univ. of California Laboratory of Biomedical and Environmetnal Sciences Los Angeles, Ca	Mucilage samples	Composition	6	12/03/91
Phillip H. Enriquez Advanced Magnetics Inc. Cambridge, MA	Larch arabinogalactan	Proton and Carbon NMR	1	12/12/91
A. Gururaj Rao Pioneer Hybrid International Inc. Johnson, IA	Glycoprotein samples from *Bauhinia purpurea* and *Eranthis hyemalis*	Composition	2	12/16/91

Dr. Marwood Alpura Koreco AG Konolfingen (Schweiz) Switzerland	Soybean milk powder	Composition	1	12/30/91
Ron Clay UGA, Botany Department Athens, GA	Barley coleptile cell walls	Composition	1	01/24/92
David Livingston USDA-ARS Regional Pasture Research Lab University Park, PA	Fructane samples	Methylation	9	01/24/92
Carl Bergmann UGA, CCRC Athens, GA	*Fursarium* EPG	Composition	1	01/31/92
Phillip Enriquez Advanced Magnetics Inc. Cambridge, MA	Arabinogalactan samples	Composition	2	12/31/92
E. Mösinger Sandoz Basel Switzerland	Fungal and bacterial preparations	Composition	3	01/31/92
Sean Soltysik Cambridge Biotech Worcester, MA	Saponin samples	Methylation	2	01/31/92
Carl Bergmann UGA, CCRC Athens, GA	Fusarium EPG sample	Composition	1	02/12/92
Sean Soltysik Cambridge Biotech Worcester, MA	Saponin samples	Composition	2	02/12/92
Lynn Epstein Dept. of Plant Pathology Univ. of California Berkeley, CA	Sample from Helmintho-sporium	Glycosyl Linkage	1	02/14/92
Brent M. Peyton Montana State Univ. College of Engineering Bozeman, MT	Biofilm samples	Composition	3	02/29/92
Gary Strobel Dept. of Plant Pathology Montana State Univ. Bozeman, MT	Free monosaccharides in the water soluble portion of the inner bark of Pacific U	Composition	1	02/29/92
Deborah Bedore Cambridge Biotech Worcester, MA	Saponin sample	Composition	1	03/19/92

Carl Bergmann UGA, CCRC Athens, GA	Fusarium EPG glycoprotein	Composition	1	03/19/92
Rebecca Robbins UCLA Dept. of Biology Univ. of California Los Angeles, CA	Sheath polysaccharide from sianyl bacterium *Lyngbya aestuarii*	Composition	1	03/19/92
Gary Strobel Dept. of Plant Pathology Montana State Univ. Bozeman, MT	Maculosin	Composition	1	04/06/92
Lynn Epstein Dept. of Plant Pathology Univ. of California Berkeley, CA	An intracellular fraction from the pathogenic fungus *Cochliobolus victoriae*	Glycosyl Linkage	1	04/07/92
Stephen C. Fry Center for Plant Science Univ. of Edinburgh Edinburgh, U.K.	Synthetic disaccharide	NMR	1	04/07/92
Otto Geiger Botanical Laboratories Dept. of Plant Mol. Biol. Leiden University Leiden The Netherlands	Phospholipids from Bradyrhizobium USDA 135 and AN 122	Fatty Acyl Composition	2	04/09/92
R. Malcolm Brown Dept. of Botany Univ. of TX, Austin Austin, TX	Synthetic cellulose samples	Methylation	6	04/27/92

Appendix 3. Analytical Services and Training

Samples and Protocols Sent to Others, December 1986 - March 1992

Investigator	Sample(s) and Protocol(s) Sent	Date
Dr. Felice Cervone University of Rome Rome, Italy	Void glucan elicitor from *Phytophthora megasperma*	12/86
Dr. Osamu Kodama Ibaraki University Ibaraki, Japan	Void glucan elicitor from *Phytophthora megasperma*	12/86
Dr. Bernard Fritig CNRS - IBMP Strasbourg, France	~ 10 mg void glucan elicitor	12/86
Dr. Michel Thellier University of Rouen Mont-Saint-Aignan, France	Cell wall fraction B, *Endo*polygalacturonase solubilized cell wall	9/87
Richard C. Staples Boyce Thompson Institute for Plant Research Cornell University Ithaca, NY	~ 1 mg hepta-β-glucoside elicitor ~ 3 mg *P. megasperma* fragments	9/87
Dr. C.J. Coscia St. Louis University School of Medicine St. Louis, MO	~ 3 mg void glucan elicitor	9/87
Dr. Stephanie Mustaftchiev CBM 2 Marseilles, France	Cell wall fraction B	10/87
Dr. C.J. Coscia St. Louis University School of Medicine St. Louis, MO	~ 3.5 mg Pre-A glucan elicitor	2/88
Dr. Mike Beck Clemson University Clemson, SC	Protocol for isolation of total RNA from plant tissue	2/88
Dr. Robert Fluhr Weissman Institute Rehovot, Israel	~ 2 mg void glucan elicitor ~ 2 mg Pre-A glucan elicitor	4/88
Dr. David J. Meyer University of Nebraska Lincoln, NE	Aliquots of hybidoma supernatants (MH 4.4E4 and CCC 2.2E10)	4/88
Dr. Darryl L. Kropf Oregon State University Corvallis, OR	3 ml aliquot of hybridoma supernatant (MH 4.4E4)	4/88

Investigator	Sample(s) and Protocol(s) Sent	Date
Dr. Jean-Luc Montillet CENC Saint Paul Lez Durance, France	~ 1 mg void glucan elicitor	4/88
Dr. Benedikt Timmerman Cidade Universitaria Rio De Janeiro, Brazil	TCL protocol	5/88
Dr. Mauricio Bustos Texas A&M University College Station, TX	TCL regeneration protocol	6/88
Dr. Margaret K. Essenberg Oklahoma State University Stillwater, OK	TCL protocol	6/88
Dr. Nien-Tai Hu AGR Biotech Lab Taichung, Taiwan	TCL bioassay protocol	6/88
Dr. Eric Terzaghi Massey University Palmerston North, New Zealand	TCL protocol	6/88
Dr. Tim Nelson Yale University New Haven, CT	1-2 mg cell wall fragments (EPGaA4mix, Syc-B)	8/88
Dr. Frank Dazzo Michigan State University East Lansing, MI	LPS samples	8/88
Dr. Charles West University of California Los Angeles, CA	Void glucan elicitor	8/88
Dr. Marguerite Kopp CNRS-IBMP Strasbourg, France	β-glucan elicitor Oligogalacturonide elicitor	9/88
Dr. Kathleen Jeong University of California Berkeley, CA	~ 2 ml aliquot of MH 4.4E4 hybridoma supernatant	10/88
Dr. Werner Zitzmann ZF-F Biotechnologie Leverkusen, FRG	Culture of *Acer pseudoplatanus*	10/88
Dr. Darryl L. Kropf University of Utah Salt Lake City, UT	~ 3 ml aliquots of hybridoma supernatants (MH 3.2B4, MH 4.2A4, MH 4.3E5)	10/88
Dr. Bernard Fritig CNRS-IBMP Strasbourg, France	Glucan elicitor	11/88
Mr. Hongchao Li Beijing Plant Cell Bioengineering Laboratory PR China	~ 5 mg void glucan elicitor	12/88

Investigator	Sample(s) and Protocol(s) Sent	Date
Dr. Bernard Fritig CNRS-IBMP Strasbourg, France	Glucan elicitor	12/88
Dr. Larry Griffing Texas A&M University College Station, TX	~ 4.5 mg void glucan elicitor	1/89
Dr. Osamu Kodama Ibaraki University Ibaraki, Japan	~ 3 mg void glucan elicitor	1/89
Dr. Keith Roberts John Innes Institute Norwich, United Kingdom	RG-I and RG-II	2/89
Dr. Bernard Fritig CNRS-IBMP Strasbourg, France	Glucan elicitor	2/89
Dr. G.B. Fincher La Trobe University Australia	Xylosidase	7/89
Dr. David F. Kendra EniChem America, Inc. Monmouth Junction, NJ	Pectic fragment preparation (EPGaA4)	9/89
Dr. Felice Cervone University of Rome Rome, Italy	6 oligonucleotide probes	10/89
Dr. Bernard Fritig CNRS-IBMP Strasbourg, France	Void glucan elicitor from *Phytophthora megasperma*	11/89
Dr. Christopher J. Lamb The Salk Institute La Jolla, CA	Void glucan preparation	12/89
Dr. Everardo Lopez-Romero Universidad de Guanajuato Guanajuato, Mexico	Strains of *C. lindemuthianum* and *E. carotovora*	12/89
Dr. Eugene W. Nester University of Washington School of Medicine Seattle, WA	2 mg xylose equivalent of glucuronoarabinoxylan, 3 fractions of xyloglucan	2/90
Dr. Jean Guern CNRS-ISV Gif sur Yvette, France	Oligosaccharins	3/90
Dr. Sondra Glucksmann MIT Cambridge, MA	Void glucan elicitor from *Phytophthora megasperma*	3/90
Dr. A.G.J. Voragen Wageningen Agricultural University Wageningen, The Netherlands	RG-I and RG-II	3/90

Investigator	Sample(s) and Protocol(s) Sent	Date
Dr. Thomas Pesacreta University of SW Louisiana Lafayette, LA	EPG	5/90
Dr. Jean Guern CNRS-ISV Gif sur Yvette, France	Oligomannuronic acid	5/90
Dr. Beat Keller Eidg. Forschungsanstelt für Lendw-Pflanzenbau Zurich, Switzerland	4 mg active glucan from tobacco leaves	9/90
Dr. Egon Mösinger Sandoz Agro Ltd. Basel, Switzerland	~ 3-5 mg Pmg Pre-A glucan ~ 3-5 mg Pmg void glucan	10/90
Dr. Martin Parniske Phillips-Universität Marburg, Germany	~ 10 mg Pmg void glucan	10/90
Dr. Karl-Erik Eriksson The University of Georgia Athens, GA	250 ml sycamore cell wall enzymes (whole cell extract)	10/90
Dr. Andrew Staehelin University of Colorado Boulder, CO	2 mL MH 4.34 2 mL CCC 8.10A3 2 mL CCC 8.2E6 2 mL CCC 8.12H8	10/90
Dr. Keith Roberts John Innes Institute Norwich, United Kingdom	5.5 mg sycamore extracellular xyloglucan	11/90
Dr. Paul J.J. Hooykaas Clusius Laboratory Leiden, The Netherlands	Mixtures of oligogalacturonides (Pool 1, 3, 4) Xyloglucan oligosaccharides (SEPS XG7, SEPS XG9)	11/90
Dr. Vernon N. Reinhold Harvard School of Public Health Boston, MA	Xyloglucan oligosaccharides (XG7, XG8, TXG9)	11/90
Dr. Thomas M. Zinnen Northern Illinois University Dekalb, IL	Fungal glucan preparation	11/90
Dr. John M. Labavitch University of California Davis, CA	1 mL aliquots of hybridoma CCC 8.2E6 and CCC 8.12HB	11/90
Dr. Eugene Nester University of Washington Seattle, WA	3 pools (3-4 mg each) of oligogalacturonides with differing degrees of polymerization	1/91
Dr. Jacek Hennig Rutgers University Piscataway, NJ	~ 5 mg void glucan elicitor	1/91

Investigator	Sample(s) and Protocol(s) Sent	Date
Dr. Keith Roberts John Innes Institute Norwich, United Kingdom	Hybridoma supernatants (3 mL each)	2/91
Dr. Jean Dénarié/Dr. Georges Truchet CNRS-INRA Castanet-Tolosan, France	Large and small oligogalacturonides	2/91
Dr. Jean Guern CNRS-ISV Gif Sur Yvette, France	Sodium borotritiide-reduced tridecagalacturonide ~ 1mg 13mer, 13mer modified by treatment with uronic acid oxidase	2/91
Dr. W.J. Lucas University of California Davis, CA	10 mg void glucan	2/91
Dr. Ann G. Matthysee University of North Carolina Chapel Hill, NC	~ 2 mg sycamore extracellular xyloglucan	3/91
Dr. Erwin Heberle-Bors Althanstrasse 14 Vienna, Austria	Samples containing oligogalacturonides of different size ranges	3/91
Dr. Jean Guern CNRS-ISV Gif Sur Yvette, France	12 mg of pooled oligogalacturonides containing the range that are active in potassium efflux assay	4/91
Dr. Jonathan Arias The Salk Institute La Jolla, CA	~ 50 mg Void glucan elicitor ~ 5 mg Pre-A glucan elicitor	4/91
Dr. Friedman Marquette University Milwaukee, WI	~ 50 ml sycamore suspension culture	5/91
Dr. Mike Adams The University of Georgia Athens, GA	1 g sycamore cell walls	5/91
Dr. Lars Ljungdahl The University of Georgia Athens, GA	2 g sycamore cell walls	5/91
Dr. Russell Pressey USDA Athens, GA	3 mg sycamore RG-I	5/91
Dr. Keith Roberts John Innes Institute Norwich, United Kingdom	1 mg sycamore RG-I 3 mg sycamore RG-II	5/91
Dr. Wolf-Dieter Reiter MSU-DOE Plant Research Laboratory East Lansing, MI	CCRC-M1-hybridoma supernatant, IgG_1 CCRC-M2-hybridoma supernatant, IgM	5/91
Dr. Jean Guern CNRS-ISV Gif Sur Yvette, France	~ 14 mg oligogalacturonides DP ~ 1-7	5/91

Investigator	Sample(s) and Protocol(s) Sent	Date
Dr. Jonathan Arias The Salk Institute La Jolla, CA	~ 100 mg void glucan elicitor ~ 12 mg fraction C glucan elicitor	6/91
Dr. John Mundy Carlsberg Research Center Copenhagen, Denmark	~ 8 mg void glucan elicitor	6/91
Dr. Larry Griffing Texas A&M University College Station, TX	8 mg Pre-A glucan ~ 8-9 mg fraction C glucan 1.1 mg hepta-β-glucoside elicitor	6/91
Dr. Alexei V. Babakov Institute of Agricultural Biotechnology Moscow, USSR	~ 2 mg void glucan elicitor	6/91
Dr. Louise Brisson The Salk Institute La Jolla, CA	~ 100 mg void glucan elicitor	7/91
Dr. Roland Bourrillon Université Paris VII Paris, France	12 glycopeptide samples	8/91
Dr. Nam-Hai Chua The Rockefeller University New York, NY	2 oligogalacturonide samples	8/91
Dr. Mike McNeil Colorado State University Fort Collins, CO	10 mg sycamore EPG-released material	8/91
Dr. Marguerite Kopp CNRS-IBMP Strasbourg, France	200 mg Pmg cell walls	8/91
Dr. Felice Cervone University of Rome Rome, Italy	EPG, PG II and PGIP antibodies	9/91
Dr. Gary Strobel Montana State University Bozeman, MT	Glucan and oligogalacturonide samples	9/91
Dr. Felice Cervone University of Rome Rome, Italy	EPG, PG II and PGIP antibodies	10/91
Dr. Adam Kondorosi CNRS-ISV Gif sur Yvette, France	2.12 mg mixture of cyclic β-1,2-linked glucans	11/91
Dr. Peter Bezukladrikor Far-East Division of the Academy of Sciences of the USSR Vladivostok, USSR	~ 3 mg void glucan elicitor	11/91
Dr. Ralph Dean Clemson University Clemson, SC	EPG antibodies	11/91

Investigator	Sample(s) and Protocol(s) Sent	Date
Dr. Felice Cervone University of Rome Rome, Italy	EPG, PG II and PGIP antibodies	12/91
Dr. Nesterenko Academy of Sciences Moscow, FSU	50 ml sycamore cell suspension	1/92
Dr. Michael McNeil Colorado State University Fort Collins, CO	1 mg sycamore RG-II	2/92
Dr. Jean Guern CNRS-ISV Gif sur Yvette, France	40 µl of 1.3 mg/ml *A. niger* EPG 40 µl of 1.0 mg/ml *F. moniliforme* EPG 40 µl of 3.0 mg/ml *F. moniliforme* EPG	2/92
Dr. J. Vendrig Katholieke Universiteit Leuven Leuven-Heverlee, Belgium	~ 10 mg oligogalacturonides ~ 600 µg pure undecagalacturonide	3/92
Dr. Martha C. Hawes The University of Arizona Tucson, AZ	Oligogalacturonides (DPs 6-20) Oligogalacturonides (DPs 3-8)	3/92

Appendix 3: Analytical Services and Training

Summary of Training Courses

Two training courses were held at the CCRC, sponsored jointly by the USDA/DOE/NSF Plant and Microbial Carbohydrate Center and the NIH Biomedical Carbohydrate Resource Center. Course 1, Separation and Characterization of Oligosaccharides, was held May 21-26, 1990 and May 13-17, 1991, and Course 2, Structural Analysis of Oligosaccharides, was held May 29-June 2, 1990 and May 20-24, 1991. The first course concentrated on the separation and characterization of glycoprotein carbohydrates, and the second on the structural analysis of oligosaccharides. The lab manual was written specifically for these courses and included selected analytical techniques and a bibliography. The courses were organized and led by Drs. Roberta Merkle, Russell Carlson, and Richard Cummings. The following CCRC staff presented lectures and provided hands-on instruction during the courses:

Ramadas Bhat	Russell Carlson	Richard Cummings
Terry Dorman-Smith	Roberta Merkle	Malcolm O'Neill
Izabella Poppe	Donald Powell	V. Puvanesarajah
David Smith	Herman van Halbeek	

Participants in the first course were exposed to basic techniques for the isolation and characterization of oligosaccharides. Lectures and demonstrations covered techniques of carbohydrate analysis such as: sugar quantitation by colorimetric assays; lectin blotting, thin-layer chromatography of glycolipids; enzymatic desialylation of glycolipids; overlay assay of glycolipids; separation of individual components from mixtures of oligosaccharides derived from glycoproteins by chromatographic procedures such as amine adsorption HPLC, Dionex HPLC, paper chromatography; lectin affinity chromatography; composition and methylation analyses, gas liquid chromatography; fast atom bombardment-mass spectrometry; and ^{1}H-NMR spectroscopy.

Trainees in the second course learned the techniques of glycosyl-residue (composition) and glycosyl-linkage (methylation) analyses using gas-liquid chromatography-mass spectrometry. Lectures and demonstrations covered other techniques for structural analysis including: fast atom bombardment-mass spectrometry, ^{1}H-NMR spectroscopy, and methods for the separation and purification of monosaccharides and oligosaccharides using Dionex HPLC.

Both courses emphasize hands-on laboratory work and included demonstrations and lectures. Each course is limited to 10 participants, and experience with basic biochemical techniques is a prerequisite for participation. The cost of registration per course is $250 for individuals from non-profit institutions, $500 for others. The cost of travel, lodging, and food is not included. Participants are chosen on the basis of their potential for benefiting from the course, i.e., for application of the techniques learned to their own projects. We asked each group of participants to evaluate their course, and the responses were positive and enthusiastic.

The following pages in this appendix include a list of participants in each course and their affiliations, as well as summaries of participants' comments evaluating the courses. The course training manual is included as Appendix 5.

Participants in the 1990 and 1991 Training Courses

Course 1: Separation and Characterization of Glycoprotein Oligosaccharides

May 21 - 26, 1990

Dr. Ibrahim S. Barsoum, Research Scientist
Vanderbilt University School of Medicine
Department of Microbiology and Immunology
Nashville, TN

Dr. Ellen L. Berg, Postdoctoral Associate
Stanford University Medical School
Department of Pathology
Stanford, CA

Dr. Somsankar Dasgupta, Research Scientist
Department of Neurology
Medical University of South Carolina
Charleston, SC

Dr. Jamie A. De Stefano, Graduate Student
Department of Anatomy and Cell Biology
University of Cincinnati Medical Center
Cincinnati, OH

Dr. Michael L. Dumas, Research Scientist
Miles Inc. - Cutter Biological Division
Berkeley, CA

Dr. Hans Marquardt, Program Manager
Oncogen
Seattle, WA

Dr. Dennis Piszkiewicz, Research Scientist
Baxter Healthcare Corporation
Hyland Division
Duarte, CA

Dr. Therese Timmons, Research Scientist
Baylor College of Medicine
Department of Cell Biology
Houston, TX

Dr. Edward Yeh, Professor
Massachusetts General Hospital
Harvard Medical School
Arthritis Research
Charlestown, MA

Dr. C. David Zarley, Research Scientist
Lederle-Praxis Biologicals
American Cyanamid Company
Pearl River, NY

May 13 - 17, 1991

Ms. Carol Arnosti, Graduate Student
Woods Hole Oceanographic Institution
Chemistry Department
Woods Hole, MA

Dr. Sheilah Asher
Amgen Inc.
Amgen Center
Thousand Oaks, CA

Dr. Paul G. James, Research Scientist
Glycomed, Inc.
Alameda, CA

Dr. Ludmila Novik, Research Scientist
Miles Inc., Cutter Biological
Berkeley, CA

Dr.Mark C. Plucinsky, Research Scientist
Centocor
Malvern, PA

Dr. Maryline Sharp, Postdoctoral Research Associate
Washington University School of Medicine
St. Louis, MO

Joseph Andrade, Research Scientist
Baxter Hyland Division
Duarte, CA

Course 2: Structural Analysis of Oligosaccharides

May 29 - June 3, 1990

Dr. Paul Aeed, Research Scientist
The Upjohn Company
Biopolymer Chemistry
Kalamazoo, MI

Dr. Somsankar Dasgupta, Research Scientist
Department of Neurology
Medical University of South Carolina
Charleston, SC

Ms. Jamie De Stefano, Graduate Student
Department of Anatomy and Cell Biology
University of Cincinnati Medical Center
Cincinnati, OH

Dr. Rajan P. Nair, Research Scientist
Howard Hughes Medical Institute
University of Michigan Medical Center
Ann Arbor, MI

Mr. Brian O'Connell, Graduate Student
Department of Biochemistry
University of Rochester Medical Center
Rochester, NY

Dr. Nate Wardrip, Research Scientist
Department of Biochemistry and Biophysics
University of California
Davis, CA

Dr. S. Betty Yan, Program Manager and Research Scientist
Eli Lilly and Company
Department of Biochemical Research
Indianapolis, IN

May 20 - 24, 1991

Ms. Carol Arnosti, Graduate Student
Woods Hole Oceanographic
Institution
Chemistry Department
Woods Hole, MA

Dr. Alexis Eberendu, Research Scientist
Carrington Labs Inc.
Dallas, TX

Dr. Sylvia Harwig, Research Scientist
UCLA Center for Health Sciences
Los Angeles, CA

Dr. David Hawke, Research Scientist
Applied Biosystems
Foster City, CA

Dr. Paul James, Research Scientist
Glycomed, Inc.
Alameda, CA

Dr. Mark C. Plucinsky, Research Scientist
Centocor
Malvern, PA

Ms. Rebecca Robbins, Graduate Student
UCLA Dept. of Biology
Los Angeles, CA

Dr. Gary N. Rogers, Research Scientist
Amgen
Amgen Center
Thousand Oaks, CA

Dr. Dave Sertl, Research Scientist
Ross Laboratories
Columbus, OH

Dr. P. K. Tsai, Research Scientist
Merck Sharp & Dohme
 Research Laboratories
West Point, PA

Training Course Evaluation Questionnaire

We would like to make this course as useful as possible for future participants, and would, therefore, appreciate your evaluations of the course content, the lab manual, and the laboratory exercises. Please respond to the following questions.

1. Was the course taught at the proper level, too basic, or too advanced?

2. Which laboratory exercises were the most useful to you? Were there any you would prefer that we did not cover?

3. In what ways could this course be improved? Would you like to see particular techniques covered that we did not include?

4. Was the laboratory manual organized well? Were the procedures easy to follow?

5. Was the laboratory environment easy to work in? Did you receive enough help during the laboratory exercises? Too much help?

6. Was it useful to have lectures related to the lab exercises?

7. Did the lectures cover topics you were interested in? Would you like us to cover other topics?

8. Did you find it useful to have trainees work in pairs?

9. Please make any other comments regarding this course.

Summary of Evaluations for Training Course 1: Separation and Characterization of Glycoprotein Carbohydrates, May 21 - 26, 1990

The comments of the ten trainees on the course evaluation forms were very positive and enthusiastic. The trainees felt the course was taught at the proper level, that the laboratory exercises were useful, and the laboratory facilities well-organized and easy to work in. The trainees also commented on the good organization of the laboratory manual and the ease of following the procedures for the lab exercises. The trainees believed that it was useful to have a variety of people from different scientific backgrounds give the lectures. All the trainees found the course helpful and were enthusiastic that it should be continued in the future.

Summary of Evaluations for Training Course 2: Structural Analysis of Oligosaccharides, May 29 - June 2, 1990

All the comments of the seven people in this course were generally very positive. The students felt the course was taught at the proper level (that it was not too basic or too advanced) and that the exercises were very good. The students commented that the manual was well-organized and easy to follow, and they praised the laboratory equipment and learning environment. The technical help for the laboratory exercises was excellent. The technical help for the laboratory exercises was excellent. The students remarked that the lectures and demonstrations on the GC-MS analysis of permethylated oligosaccharides, FAB-MS analysis, Dionex separations of oligosaccharides and monosaccharides, and NMR techniques in particular were excellent. The students did mention that they would have preferred the symposium (May 23) to have been scheduled between Courses 1 and 2 so that students from both courses could attend. The students emphasized that they would have liked even more laboratory work.

Generally, all the students thought that the course was very good. They would have liked to use some of their own samples in the laboratory exercises. They all thought that the course was very helpful to their research and that it should be continued. They stated that the faculty and staff were excellent: extremely knowledgeable, friendly, and helpful; and that the Complex Carbohydrate Research Center was an excellent atmosphere in which to work.

Summary of Evaluations for Training Course 1: Separation and Characterization of Glycoprotein Carbohydrates, May 13 - 17, 1991

The majority of the participants thought that the course was taught at an appropriate level, that the laboratory manual was well organized and would serve as a good reference source, and that the laboratory environment was easy to work in. They thought the procedures were easy to follow, that it was helpful to work in pairs, and that they received enough assistance while performing the laboratory exercises, and, in fact, some commented that they would like to have a broader range of topics included.

Suggestions for improvements included adding more exercises such as sequential exoglycosidase digestions and preparation of lectin affinity columns, and having more formalized follow-up discussions to some of the exercises.

Summary of Evaluations for Training Course 2: Methods for the Analysis of Complex Carbohydrates, May 20 - 24, 1991

Participants in this course thought that it was taught at the proper level, that the laboratory exercises were very useful, that the laboratory manual was well organized, the laboratory environment was good, the lectures were good, the topics covered were appropriate and useful, and that it was beneficial to work in pairs. The participants would have liked to have had laboratory exercises in NMR, FAB-MS, and DIONEX, more discussion after the laboratory exercises, and more lectures on NMR spectroscopy.

Advertisements for Training Courses

	Ad Size	Issue	Cost
1990			
Bio/Technology	1/2-page	March	$1,560.00
Science	1/2-page	March 23	3,180.00
			$4,740.00
1991			
ASPP	small display ad	March	FREE
Bio/Technology	1/3-page	March	$1,290.00
Cell	1/2-page	March 22	550.00
Science	1/3-page	March 15	2,361.80
			$4,201.80
1992			
ASPP	small display ad	March/April	FREE
Bio/Technology	1/3-page	March	$1,420.00
Cell	1/2-page	March 20	595.00
Journal Biological Chemistry	1/4-page	March 25	349.00
NIH Research Resources Reporter	1/3-page	March	800.00
Science	1/3-page	February 28	2,410.00
			$5,574.00

Sample Advertisement for 1992 Training Courses in Techniques for Separation and Characterization of Complex Carbohydrates

Courses in Techniques for Separation and Characterization of Complex Carbohydrates
June 8 - 12 and June 15 - 19, 1992

Two courses will be offered at the Complex Carbohydrate Research Center (CCRC) of the University of Georgia. The first course (*June 8-12, 1992*), "The Separation and Characterization of Oligosaccharides Isolated from Glycoproteins", is intended for scientists with no experience with carbohydrate analysis. The second course (*June 15-19, 1992*), "Structural Analysis of Oligosaccharides", is intended for scientists with some experience with glycoconjugates or for those who have completed the first course, and will focus on techniques of composition and linkage analysis. Both courses will consist of hands-on laboratory work, demonstrations and lectures. A lab manual including selected analytical techniques and references will be provided. Each course is limited to 10 participants. Experience with basic biochemical techniques is a prerequisite for participation. The cost of registration per course is $250 for individuals from non-profit institutions, $500 for others. Lodging and food expenses are not included in the registration fee.

The courses are supported jointly by the Plant and Microbial Carbohydrate Center (a unit of the USDA/DOE/NSF Plant Science Centers Program sponsored by the Department of Energy), and the NIH Biomedical Resource Center of the CCRC. For further information or to apply for the courses contact: Dr. Roberta K. Merkle, Technical Director for Biomedical Carbohydrates, Complex Carbohydrate Research Center, 220 Riverbend Road, The University of Georgia, Athens, Georgia 30602. Phone: 404-542-4402. Facsimile: 404-542-4412.

Appendix 3: Analytical Services and Training
Sample Training Course Syllabus from 1991

Course 1: May 13 - 17, 1991
Course 2: May 20 - 24, 1991

Course 1: Separation and Characterization of Glycoprotein Oligosaccharides

Monday, May 13

9:00 a.m.	Introductory lecture: Overview of carbohydrate structures and problems in analysis. Dr. Richard Cummings
10:30 a.m.	Laboratory: Colorimetric assays: Assay of a Glycoprotein for Neutral Hexose Content
12:00 noon	Lunch
1:15-2:00 p.m.	Lecture: Use of enzymes for preparation of glycopeptides and oligosaccharides from glycoproteins and for sequential exoglycosidase analysis. Dr. Roberta Merkle
2:00-4:30 p.m.	Laboratory: Use of *N*-glycanase to release oligosaccharide chains from a glycoprotein - Preparation of sample - Begin *N*-glycanase reaction - Size-exclusion chromatography of glycoprotein *before* enzyme treatment - Assay of column fractions for protein and neutral hexose
4:30-5:00 p.m.	Laboratory: Neuraminidase treatment of bovine brain gangliosides

Tuesday, May 14

9:00-9:15 a.m.	Laboratory: Termination of neuraminidase treatment of bovine brain gangliosides and of *N*-glycanase reaction of glycoprotein - Dilute reaction mixture of gangliosides - Heat reaction mixtures to kill enzymes - Dry down gangliosides on Speed-Vac
9:15-11:00 a.m.	Laboratory: Analysis of products of *N*-glycanase reaction products - Size-exclusion chromatography of reaction products of enzyme treatment - Assay of column fractions for protein and neutral hexose
11:00-1:00 p.m.	Demonstration: High-performance Liquid Chromatography - Separation of oligosaccharides by size - Separation of *N*-linked high-mannose oligosaccharides by amine adsorption HPLC using AX-5 column. Ms. Terry Smith, in Graduate Studies Research Center, UGA Campus
1:00 p.m.	Lunch

1:30-3:30 p.m.	Laboratory: Separation of radiolabeled, cell-derived glycopeptides by lectin affinity chromatography - Pour and equilibrate 1-ml Con A-Sepharose columns - Apply samples to Con A, collect fractions - Liquid scintillation counting of fractions
3:30-5:00 p.m.	Laboratory: Separation of small sized oligosaccharides and monosaccharides by descending paper chromatography - Spot unknowns and knowns, 24 h chromatography

Wednesday, May 15

9:00-10:00 a.m.	Laboratory: Detection of glycoproteins by lectin blotting - Prepare dot blots - Block blots - Wash blots - Probe blots with lectins (lunch during 1 h incubation period)
12:00 noon	Lunch
1:00-3:30 p.m.	Laboratory: Continuation of lectin blotting - Wash blots - Overlay with antibody-AP - Wash blots - Color development
3:30-5:00	Laboratory: Analysis of results of descending paper chromatography - Silver nitrate dip assay

Thursday, May 16

9:00-12:00 noon	Laboratory: Separation of glycolipids by TLC and identification by overlay assay - Preparation of solvents - Equilibrate tanks - Spot samples - Separate samples by TLC - Develop plates - Begin overlay procedure - Incubate plate with cholera toxin β-horseradish peroxidase conjugate (Lunch and lecture are scheduled during the 2 h incubation period)
12:00 noon	Lunch
1:00-2:00 p.m.	Lecture: Glycolipids. Dr. David Smith
2:00-4:00 p.m.	Laboratory: Complete glycolipid overlay assay - Wash TLC plates - Color development

4:00-5:00 p.m.	Lecture: Other carbohydrate analytical methods. Dr. Russell Carlson - Composition analysis, GC/MS - Glycosyl-linkage analysis - NMR

Friday, May 17

9:00-11:00 a.m.	Demonstration: Introduction to Dionex HPLC. Mr. Malcolm O'Neill - Separation of standard *N*-linked oligosaccharides by Dionex-HPLC - Analysis of *N*-glycanase-released oligosaccharides by Dionex-HPLC
11:00-12:00 noon	Course summary: question and answers, evaluation

<u>Course 2: Structural Analysis of Oligosaccharides</u>

Monday May 20, 1991

9:00-10:00 a.m.	Introduction Dr. Russell W. Carlson
10:00-11:00 a.m.	Lecture: Methods for composition analysis. Dr. Russell Carlson
11:00-12:00 noon	Lecture: Methods for linkage analysis. Dr. Russell Carlson
12:00 noon	Lunch
1:30-2:00 p.m.	Lecture: Introduction to GC/MS analysis. Dr. Russell Carlson)
2:00-3:00 p.m.	Laboratory: GC/MS analysis of alditol acetates and partially methylated alditol acetates
3:00-5:00 p.m.	Lecture and demonstration: GC/MS analysis of permethylated oligosaccharides. Dr. Ramadas Bhat

Tuesday, May 21

9:00-12:00 noon	Lecture: NMR analysis of carbohydrates. Dr. Herman van Halbeek
12:00 noon	Lunch
1:30-3:00p.m.	Lecture and demonstration: Analysis of oligosaccharides by FAB-MS. Mr. William York
3:00-5:00 p.m.	Lecture and demonstration: DIONEX separation of carbohydrates. Mr. Malcolm O'Neill

Wednesday, May 22

9:00-9:30 a.m.	Laboratory: Begin alditol acetate preparation
9:30-10:30 a.m.	Laboratory: preparation of dimethylsulfoxide anion
10:30-12:00 noon	Laboratory: Begin methylation analysis, continue alditol acetate preparation
12:00 noon	Lunch
1:30-4:00 p.m.	Laboratory: Complete alditol acetate preparation
4:00-5:00 p.m.	Laboratory: Continue methylation analysis, begin trimethylsilyl methylglycoside preparation

Thursday, May 23

9:00-10:00 a.m.	Laboratory: Purification of permethylated oligosaccharides. Completion of trimethylsilyl methylglycoside preparation
10:00-12:00 noon	Laboratory: Begin acetylation of methylated oligosaccharides, end GC analysis of alditol acetates and TMS methylglycosides
12:00 noon	Lunch
1:30-4:00 p.m.	Complete acetylation of methylated oligosaccharides. Complete GC analysis of alditol acetates and TMS methylglycosides.
4:00-5:00 p.m.	Lecture: Methods for the isolation and analysis of glycoprotein oligosaccharides. Dr. Roberta Merkle

Friday, May 24

9:00-11:30 a.m.	Laboratory: GC/MS analysis of permethylated alditol acetates
11:30-12:30 noon	Course summary, evaluation, and question and answer period

Appendix 3: Analytical Services and Training

Summary of Teaching and Training Activities of CCRC Faculty

The CCRC's regular faculty members are very active in teaching courses in the Biochemistry, Chemistry, and Botany Departments to UGA undergraduate and graduate students as well as continuing to direct the research of M.Sc. and Ph.D. students. Several of the CCRC faculty have participated in teaching Advanced Biochemistry (BCH 802), a graduate-level course taken by all graduate students in biochemistry, chemistry, botany, zoology, genetics, food science, and horticulture. Since 1987 half of the 802 course has been devoted to complex carbohydrates. Most CCRC faculty have taught BCH 810 ("Biochemistry Seminar") since 1986.

Dr. Van Halbeek has taught two lecture courses and one laboratory course each year since 1986 on the theory and use of NMR spectroscopy for the studies of macromolecule structures, including and emphasizing those of complex carbohydrates. These courses are CHM 617/417, "An Introduction to Modern NMR Spectroscopy," and CHM 618/418, "NMR Spectroscopy: Practical Aspects," which are available to both undergraduate and graduate students. He has also taught CHM 901 ("Advanced NMR Spectroscopy"), which is for graduate students who want to learn the most up-to-date NMR techniques. It would not have been possible to teach these courses without the NMR facilities of the CCRC.

Drs. Albersheim and Darvill (with Dr. Russell Malmberg) taught Biology 107 ("Introductory Biology" for undergraduate students with a chemistry prerequisite) in the fall quarters of 1990 and 1991. Each year the class enrolls over 100 students. Dr. Darvill has also taught a section of Biochemistry 804 ("Plant Biochemistry") describing plant cell wall structure and biosynthesis. In 1988-89, Drs. Cummings, Darvill, and Van Halbeek taught "A Mini-Course in Complex Carbohydrates" as part of Biochemistry 310 for undergraduate students. Dr. Mohnen (who joined the CCRC faculty in September 1990) was invited to give one week of lectures on "DNA Synthesis, Transcription, and Translation" to Profs. Black and Travis's BCH 310 undergraduate class ("Introductory Biochemistry"). In the spring of 1993 she will assume the teaching of BCH 310 in total. In the spring quarter of 1991, Dr. Meyer taught CHM 341/341L, an undergraduate laboratory and lecture course in "Modern Organic Chemistry." He taught CHM 631 ("Intermediate Organic Chemistry. Mechanisms") during winter quarter 1992 and participating in the organization and teaching of BCH 812A, "Molecular Modeling" (with Profs. Wampler [Biochemistry], Allinger [Chemistry], Bowen [Chemistry], and Taylor [Pharmacy]).

Dr. Cummings has taught BCH 801 ("Advanced Biochemistry," for graduate students) and participated in BCH 802 each year for the last five years. He has also taught BCH 811 ("Cell and Molecular Biology") for two quarters, and has been active in organizing and teaching in BCH 810 ("Biochemistry Seminar"). In 1990 Drs. Cummings and Smith organized a winter seminar series as part of BCH 811 ("Cell and Molecular Biology") which brought outstanding carbohydrate scientists from around the U.S. to speak at UGA. Dr. Eriksson taught BCH 812A ("Special Topics in Biochemistry") in the winter quarter 1991 entitled, "Biosynthesis and Biodegradation of Wood and Wood Components." Dr. Hahn taught BOT 881 ("Biochemistry of Plant Responses to Microbial Infection") in Winter 1990 and gave lectures in PAT 840 ("Host-Pathogen Interaction"), BOT 823 ("Monoclonal Antibodies as Research Tools") and BOT 465/665 ("Plant Taxonomy"). He taught BOT 882 ("Graduate Research Seminar") in the winter quarter 1991 and BOT 823 ("Monoclonal Antibodies as Research Tools") again in the spring of 1991 and 1992.

Drs. Carlson and Merkle, the technical directors for extramural services (responsible for plant and microbial and for biomedical complex carbohydrates, respectively) and CCRC adjunct faculty members have conducted two hands-on, two-week laboratory training courses for scientists from other academic and industrial organizations around the country (see other parts of Appendix 3). These training courses concentrate on the separation and characterization of glycoprotein carbohydrates and on the structural analysis of oligosaccharides. The 1990 courses had 17 trainees; the 1991 courses had 20 trainees. We expect to have another 20 trainees for the 1992 courses to be held in early June.

The CCRC faculty also teach the center's own students, postdoctoral research associates, visiting scientists, and technicians the skills required to do research in complex carbohydrate science. CCRC faculty have directed the research of 50 graduate students, 50 postdocs, and 9 visiting scientists since 1986 including the current research of 36 graduate students, 23 postdocs, and one visiting scientist. Seventy-three undergraduate students have worked under the direction of CCRC faculty since 1986, including 14 undergraduate students currently working at the CCRC. Additionally, CCRC personnel are strongly encouraged to help each other master the sophisticated techniques required for their research. The CCRC acts as a resource for the students, staff, and faculty of UGA and of neighboring schools and industries as well as universities and corporations throughout the country by providing information about the best procedures to analyze complex carbohydrates; sometimes the CCRC provides these individuals with analytical services and, when possible, makes space available in its plant growth rooms and greenhouses to other members of the UGA faculty.

CCRC faculty currently lead five weekly research seminar groups, four of which are organized around specific areas of interest. All the individual research groups have weekly or biweekly research meetings to discuss progress and problems in their various research projects. Dr. Cummings (with Dr. Claiborne Glover of the Biochemistry Department) has led a weekly journal club on cell and molecular biology since 1984. Dr. Meyer continues the fourth year of his weekly seminar group organized around topics in carbohydrate chemistry. Dr. Mohnen leads a biweekly plant complex carbohydrate science journal club. A CCRC-wide, weekly two-hour research seminar has been held throughout the year from 1986 to the present for all interested faculty, graduate students, staff, and postdocs. In 1991 the format for this center-wide seminar was revamped to be devoted to presentations of senior members of every research group (see speaker list in Appendix 1). This research seminar series is designed to keep the seven groups located in the CCRC building and the four located in the Life Sciences building abreast of each other's research directions and accomplishments and to allow for an exchange of research ideas and problems among the eleven CCRC groups. This center-wide seminar group is organized and led by Drs. Moremen and Pierce. These five forums provide opportunities for young scientists to present the results of their work, to critique the work of others, and to become familiar with the expertise and research problems of other CCRC members.

The faculty of the CCRC have begun to discuss the possibilities of obtaining a "training grant" in carbohydrate science to formalize our training of graduate students in this area. Such a grant will also provide some stipends for students of our faculty in the "biomedical" groups. Together with students whose stipends are paid by the Plant Science Center grant, we believe we will have a nucleus of students such that developing a training grant is a natural development of the teaching commitments of the CCRC. Most likely this would take the form of a specialized line of study for students who would enroll in the Biochemistry Department. Dr. Herman van Halbeek has agreed to take the lead in beginning to formulate our ideas for this "training grant."

Appendix 3. Analytical Services and Training

Graduate Students, Postdocs, Technicians, and Visiting Scientists Trained at the CCRC and Current Affiliation, 1985 - April 1992

Names and Position	Period	Current Affiliation/Degree
Former Postdocs:		
*LuAnn Aquino	1987-88	Postdoc, Department of Microbiology and Immunology, Emory University, Atlanta
*Ramadas Bhat	1989-92	Cutter Biologicals, Miles, Inc., Berkeley, CA
*Peter Bucheli	1987-89	Research Scientist, Nestle Research Centre, Vevey, Switzerland
Giulia De Lorenzo (NATO Fellow)	1987-89	Research Scientist, University of Rome, Italy
Keith R. Davis	1985-86	Assistant Professor, Ohio State Biotechnology Center, Columbus, OH
R. Scott Doubet	1984-87	CarbBank Director, CCRC
Daotian Fu	1990-92	Applied Biosystems, Inc., Foster City, CA
*Teresa Gruber	1989-91	Law Student
*Makoto Hisamatsu	1989-90	Associate Professor, Faculty of Bioresources, Mie University, Tsu, Japan
Serge Kauffmann (EMBO Fellow)	1989-91	Research Scientist, Centre National de la Recherche Scientifique, Institut de Biologie Moleculaire des Plantes, Strasbourg, France
*Alan Koller	1987-90	Graduate Student, Franklin Pierce Law Center, Concord, NH
Patrice Lerouge	1991	Research Scientist, CNRS, Toulouse, France
Roberta K. Merkle	1984-87	Technical Director, CCRC University of Georgia
*Debra Mohnen	1986-90	Assistant Professor, CCRC and Department of Biochemistry, University of Georgia
Bernd Müller (DFG Fellow)	1990-91	Research Scientist, LAT GmbH, Munich, Germany
Kwame Nyame	1987-89	Postdoctoral Fellow, Brussels
Jane E. Oates	1984-86	Research Scientist, Department of Bioorganic Chemistry, University of Utrecht, The Netherlands
*Roger O'Neill	1984-88	Scientist, Genencor, San Francisco, CA
*Vince Pozsgay	1989-90	Research Scientist, NIH, Bethesda, MD
*Velupillai Puvanesarajah	1987-90	Research Scientist, Analytical Biochemistry Laboratories, Columbia, MO
Tadashi Seguchi	1988	Oita Medical School, Japan
*Ivan Šimkovic	1991	Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Czechoslovakia
Anne-Marie Strang	1987-91	Cytel Corporation, San Diego, CA
Zakaria A. Teleb	1986-89	Research Scientist, Egypt
*Jan Thomsen	1988-89	Project Director of Computer Operations, CCRC, University of Georgia

Wolfgang Von Deyn	1986-87	Research Scientist, BASF, Akliengesellschaft, Ludwigshafen, FRG
Jan Yang	1989-	Senior Research Scientist, Dept. of Biochemistry, University of Georgia
Paolo Zatta	1988-90	Research Scientist, Italy
Current Postdocs:		
*Jinhua An	1991-	Ph.D. Oklahoma State University
*Carl Bergmann	1986-	Ph.D. Ohio State University
*Kevin Brady	1989-	Ph.D. Indiana University
Tong-Bin Chen	1992-	Ph.D. University of Iowa
Jamie De Stefano	1991-	Ph.D. University of Cincinnati College of Medicine
Francois Côté (NSERC Fellow)	1990-	Ph.D. Université Laval
*Marly Eidsness	1991-	Ph.D. University of Cincinnati
John Glushka	1991-	Ph.D. McGill University, Canada
Lihua Huang	1992-	Ph.D. Clemson University
Marcia Kieliszewski	1991-	Ph.D. Michigan State University
Karl-Heinz Ott	1991-	Ph.D. University of Frankfurt, Frankfurt, Germany
*Izabella Poppe	1990-	Ph.D. Warsaw Medical Academy
Leszek Poppe	1989-	Ph.D. Tech. University of Warsaw
*Jörg Puhlmann (DFG Fellow)	1990-	Ph.D. University of Munich, FRG
Jan Radomski	1991-	Ph.D. Warsaw University
Masahiro Samejima	1990-	Ph.D. University of Tokyo, Japan
Mohamed Shoreibah	1991-	Ph.D. The University of Miami Medical School
Jayanthi Srivatsan	1989-	Ph.D. Indian Institute of Science, Bangalore, India
Wolfram Steffan (DFG Fellow)	1991-	Ph.D. University of Rostock, Germany
Raja Sterjiades	1990-	Ph.D. University of Fourier, France
Nancy Stults	1987-89, 1991-	Ph.D. Johns Hopkins University
Timothy Thurlby	1992-	Ph.D. University of Bristol, UK
*Myron Williams	1991-	Ph.D. Massachusetts Institute of Technology
*Sheng-Cheng Wu	1991-	Ph.D. Hungarian Academy of Sciences
*Earl Zablackis	1992-	Ph.D. University of California, Santa Barbara
Marianne Zsiska	1990-	Ph.D. University of Oldenburg, FRG
Former Visiting Scientists:		
*Felice Cervone	1987-89	Associate Professor, University of Rome, Department of Plant Biology
*Tadashi Ishii	1990-91	Forestry & Forest Products Research Institute, Ibaraki, Japan
*Giuseppe Impallomeni	1989-90	CNR Instituto per la Chimica e La Tecnologia dei Materiali, Polimerici, Catania, Italy
*Lu Yu	1989-90	Cheng-du Institute of Biology, PRC
Marie-Louise Milat	1986-87	Research Scientist, INRA, Laboratoire des Mediateurs, Chimiques, France
Bente Nilsen (Fulbright Fellow)	3-10/1989	Scientist, University of Oslo, Norway
Current Visiting Scientist:		
Rafael Guillen (Fulbright Fellow)	1990-	Instituto de la Grasa, Seville, Spain
Zhiquan Zhang	1991-	Lecturer, Northeast Agricultural College, China

Former Graduate Students:

*Thomas T. Stevenson	Ph.D., 1987	Postdoc, University of Montana, Missoula, MT
*Jerry R. Thomas-Oates	Ph.D., 1987	Postdoc, University of Dundee, Dundee, Scotland
Heide Kogelberg	Ph.D., 1991	Biological NMR Center, University of Leicester, UK
*Laura L. Kiefer	M.S., 1989	Ph.D. student, Biochemistry Department, Duke University, Durham, NC
*Steven H. Doares	Ph.D., 1990	Postdoc, Institute of Biological Chemistry, Washington State University, Pullman, WA
Marshall Skelton	M.S., 1991	Eli Lilly, Indianapolis, IN
*Que Guo	M.S., 1991	Cambridge Neuroscience, Cambridge, MA
Kim Richie	M.S., 1991	Ph.D. Student, Department of Chemistry, University of Georgia
Rainer Stuike-Prill	Ph.D., 1991	Postdoctoral Research Associate, Department of Chemistry, Carlsberg Laboratory, Valby, Denmark
Christine Maakaru	M.S., 1991	Unknown, New York
*David Gollin		Current Graduate Student in the Department of Biochemistry, University of Georgia (former graduate student at CCRC)

Current Graduate Students:

Rob Alba (Ph.D.)
*Christopher Augur (Ph.D.)
Phil Buckhaults (Ph.D.)
Jong-Joo Cheong (Ph.D.)
Wen Wen Chien (Ph.D.)
Somi Cho
*Ivana Djelineo (Ph.D.)
Ki Young Do (Ph.D.)
Su-Il Do (Ph.D.)
*Nancy Dunning (Ph.D.)
Vladimir Galchev (Ph.D.)
Ajay Gupta (Ph.D.)
*Kyung Sik Ham (Ph.D.)
Yuki Ito (Ph.D.)
Sandeep Kalelkar (Ph.D.)
Seungwon Kang (Ph.D.)
Tim Kunkle (Ph.D.)
*Veng-Meng Ló (Ph.D.)
Gu Lou (Ph.D.)
Jianyun Lu (Ph.D.)
*Victoria Marfà (M.S.)
Jörg Pieper (Ph.D.)
Dorothy Plummer (Ph.D.)
Donald Powell (Ph.D.)
Bradley Reuhs (Ph.D.)
*Brent Ridley (Ph.D.)
Carlos Rivera (Ph.D.)
Shuqun Sheng (Ph.D.)
Ali Shilatifard (Ph.D.)
Paul Simon (Ph.D.)
*Mark Spiro (Ph.D.)
Sergio Tonen (Ph.D.)
Jennifer Williams (Ph.D.)

*Yeong-Tong Wu (Ph.D.)
Jiunn Chern Yeh (Ph.D.)
*William S. York (Ph.D.)
Chilik Yu (Ph.D.)
Qun Zhou (Ph.D.)

Former Technicians:

*Susan Bernard	1988-90	Student, Physical Therapy, Boston University
*Eva Bucheli	1988-89	Research Scientist, Switzerland
Sandra Cummings	1991	Homemaker
Ivana Djelineo	1989-90	Ph.D. student, Biochemistry, University of Georgia
Nancy Dunning	1990	Ph.D. student, Botany, University of Georgia
*Stefan Eberhard	1986-89	Reserach Coordinator, CCRC
*Sally Giles	1989-92	Internship, Agroecology Program, University of California, Santa Cruz
*Teresa Gruber	1987-89	Law student
Bhagya Lakshmi	1989-91, 1992	Homemaker
Hilda Rivera	1991	SeaLite Sciences, Inc., Athens, Georgia
*Sheryl Roberts	1988-89	M.S. student, University of Maryland
*Audrey Southwick	1987-88	Ph.D. student, Stanford University
Susanne Wilson	1987-89	Ph.D. student, Medicinal Chemistry, University of Georgia

Current Technicians:
*Barbara Burgers
*Stefan Eberhard
*Mona Hendricks
Sharon Mattox
*Sarah Pattison
Terry Smith
Michael Swain
Glenn Thomas
Nate Weymouth
*Andrew Whitcombe

Senior Research Scientists:

Jeffrey Dean	1990-
*Malcolm O'Neill	1985-
Jan Yang	1991-
*William S. York	1985-

Former Undergraduate Students:

Aparna Asaii	1990
Deborah Ceiler	1989-90
Joel M. Clement	1989
David Circle	1989
Amy K. Fowler	1991
Camilla H. Ulrich	1986
Amanda Graham	1990
Chad Greer	1991
Dane K. Gregory	1991
Anne Marie Haddock	1990
Karen Hickey	1990
Deborah S. Houser	1988
Kacey Human	1991
Arnout P. Kalverda	1990
Seana M. Lesher	1991
*Valerie Licon	1989-91
J. Michael Long	1990

Clint McEntyre	1991
Jacquelin A. Murphy	1990-91
Elizabeth Patel	1990
Reshma Patel	1990-91
Laleh Rezaei	1989, 1991
*Sheryl Roberts	1987-89
*Darrell Singer	1989-91
Cindy L. Smith	1989
John Thomas	1988-89
Carla K. Tucker	1988
Camilla H. Ulrich	1986
Shannon Vaughn	1991
Rana Welch	1990-92
Toni M. Williams	1991
Kevin Windom	1988

Current Undergraduate Students:
Ryan Adolphson
Kellie Baker
John Blumer
Randy Booker
Dwight Cates
*George Chambers
Frank I. Comer
Julia Crane
Ross Cubbon
Dawn Farmer
Stephen Feren
*Lisa Harvey
*Andrew Hinze
*Keith Kates
John Kim
Manley Kiser
Ralph Kline
Scott Kline
Jaquelyn Kwon
Angie Malone
*Susan Notte
Kelly Sanders
Vince Sorrentino
Anna Maria Sult
*Melissa Templeton
Chris Wilkes
Li-Min Wu
Ning Zhang

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END

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