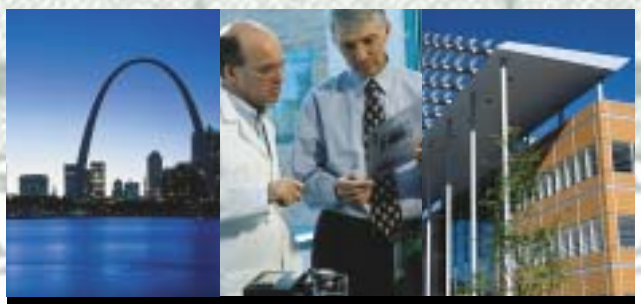
Donald Danforth Plant Science Center



ANNUAL REPORT 2001

The Donald Danforth Plant Science Center is named in honor of Donald Danforth, formerly president and chairman of the board of St. Louis-based Ralston Purina Company. A company with close ties to American agriculture, Ralston-Purina began under the leadership of Mr. Danforth's father, William H. Danforth, as a feed grain supplier, and evolved into a major force in cereals and in feed for farm animals and pets.

Mr. Danforth led the company for 30 years, during which time he built Ralston Purina into a national and international leader. In addition to his role as a successful business leader, Mr. Danforth led a number of community service and charitable activities.

During the Danforth Center's formal dedication ceremony on November 2, 2001, Mr. Danforth's son, the Honorable John C. Danforth, talked about reasons for naming the Center in honor of his father: "First, throughout Donald Danforth's lifetime, Ralston Purina was an agricultural business … many of its employees were from farm backgrounds … the purpose of their business was, as my father often said, 'to feed the world.' This is also the mission of the Donald Danforth Plant Science Center … It could not be more in keeping with the legacy of Donald Danforth."

The second reason the name of the Center is fitting, he said, "has to do with St. Louis. Donald Danforth lived all his life in St. Louis. This was his place, and he was committed to it. He would have gained enormous pleasure knowing that St. Louis would become a world leader, especially in what was so close to his heart – feeding the world."

Dr. William H. Danforth, son of Donald Danforth and chairman of the board of the Danforth Center, has said, "His children believe that this noble institution, tied to agriculture and dedicated to improving the human condition, is a fitting remembrance of their revered father. It means a lot to us that it is in his hometown, St. Louis, the city he loved." Mr. Danforth's other children are Dorothy Danforth Miller and the late Donald Danforth Jr. Each of his three remaining children lives in St. Louis.

The mission of the Donald Danforth Plant Science Center is to:

- *increase understanding of basic plant biology*
- *apply new knowledge for the benefit of human nutrition and health and improve the sustainability of agriculture worldwide*
- *facilitate the rapid development and commercialization of promising technologies and products*
- *contribute to the education and training of graduate and postdoctoral students, scientists and technicians from around the world*

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Come my friends.
'Tis not too late to seek a newer world…
–Alfred, Lord Tennyson

Imperfect as we are, we can still create something wonderful.
–Czeslaw Milosz

These words by two poets – one English and one Polish – are a fitting introduction to the first annual report of an effort by imperfect human beings to turn a wonderful dream into something concrete. In the creation of the Danforth Center, we saw grand opportunities: to use science to benefit humankind, to help feed the hungry, and to protect the world's environment for our grandchildren and great-grandchildren; to provide discoveries that will help spark the next generation of science-based industry; and to collaborate with partners to make the Midwestern region a world center for plant science.

Now we have an institution designed to realize our goals – an institution with an outstanding board of trustees, a visionary leader in Roger Beachy, a building that is both functional and elegant, and, most importantly, a group of talented, multidisciplinary scientists already hard at work. I am thankful for all of those who made possible the progress reported herein, especially for our partner institutions, the Missouri Botanical Garden, Monsanto Company, Purdue University, the University of Illinois at Urbana-Champaign, the University of Missouri-Columbia, and Washington University in St. Louis, and for their people who were essential to getting us underway and on whose strengths we continue to rely. I am grateful to the members of our board for their wisdom and dedication; to our interim leader, Dr. Ernest Jaworski; to our able administrative staff without whom nothing could happen; and to our financial supporters who share our dream.

What do I hope we will report in the future? I hope and expect that we will be able to report progress in providing better nutrition for the people of the world without the use of more land or more fresh water, that we can describe how the Donald Danforth Plant Science Center has contributed to sustainable agriculture that preserves the fertility of the land and a healthy environment for future generations, and that we can point to companies that have taken the discoveries of our Center and developed them for the benefit of both the United States and people around the world. I hope and expect that we can say that our Center and its partner institutions have been a blessing both to our region and to our world. Thus will our dream have been realized.

William H. Danforth

William H. Danforth, M.D.
Chairman of the Board of Trustees

"Welcome to the Donald Danforth Plant Science Center" was probably the sentence most used by Danforth Center employees during the final months of 2001 as visitors entered our extraordinarily functional and beautiful facility. The long hours of hard work and the dedication of a management team led by Sam Fiorello, senior vice president of administration and finance, ensured that the building was finished on time and on budget. The grand opening, held on November 2, 2001, celebrated the new building, and the remarks made by special guests reinforced the mission of the Center as established by its founders.

Research activities at the Danforth Center in 2001 expanded nearly three-fold as the number of principal investigators grew from four to eleven and the use of laboratories and core facilities expanded. Success was also reflected in research support received from a variety of federal research granting agencies, including the National Science Foundation, the National Institutes of Health, the U.S. Department of Energy, and the National Aeronautics and Space Administration, and from a variety of private companies. In addition, we are pleased that a large number of post-doctoral candidates have applied for positions at the Center; clearly, scientists around the world perceive our mission as a good addition to the field. Including the administrative and support staff, the total number of employees rose to about 120 in 2001. These numbers are expected to grow to more than 175 during 2002.

We are deeply gratified that the St. Louis community has warmly received the Danforth Center. Visitors from the local area and, indeed, around the world have toured the building, and we have hosted a number of events that have presented our mission to visitors. In 2001, a Friends of the Danforth Center group was initiated under the guidance of Ms. Jeannette Huey, vice president for development. This energetic group continues to grow as new members commit their support to the Center. Our interactions with small and emerging businesses, as well as larger companies from around the world have expanded substantially, and we are confident that the economic benefits of the Danforth Center to the St. Louis region's "BioBelt" will accelerate with time.

I hope that you enjoy the first annual report of the Danforth Center. It will tell you who we are, what we do, and how we expect our work to impact the region and the world. And, I hope that it reflects the excitement that we feel about having the opportunity to be engaged in research in the plant and life sciences at this point in history. We welcome you to visit the Center and to work with us for the benefit of humankind.

Roger N. Beachy, Ph.D.
President

2001: THE YEAR IN REVIEW

The Grand Opening

Nearly four years of planning and preparation for the Donald Danforth Plant Science Center came to fruition in 2001 with grand opening events highlighted by a formal building dedication on November 2. Attended by more than 600 business, civic and academic leaders, the dedication was held in the atrium of the Center's $75 million state-of-the-art research building in St.Louis.

Led by William H. Danforth, M.D., chairman of the Danforth Center's board of trustees, the event featured remarks by Bob Holden, Governor of Missouri; U.S. Senator Kit Bond (MO-R); U.S. Congressman Todd Akin (MO-R); Hendrik Verfaillie, president and CEO of Monsanto Company; and Richard L. Wallace, Ph.D., chancellor of the University of Missouri-Columbia. The keynote address, entitled "A Vision for the Future," was delivered by Center president Roger Beachy, Ph.D. In his remarks, Dr. Beachy said, "The Danforth Center's research initiatives and global outreach efforts, coupled with our interactions with partner institutions and other collaborators, will unite experts in the plant, agricultural and medical sciences, shortening the path to discoveries that will help the world better meet the food, health and environmental needs of the 21st century."

Earlier in the dedication week, members of the Danforth Center's board of trustees, along with civic and academic leaders who were instrumental in the Center's conception, attended a preview of the new facility. The event featured a reception, dinner and tours of the building.

Other Special Events

Former U.S. President **Jimmy Carter** visited the Danforth Center in December. In his address to staff, President Carter said, "I don't know of any other research center that I have ever visited that has a greater potential contribution to the well-being of the world." This was President Carter's second visit to St. Louis in honor of the Danforth Center. In 1998, he presented the keynote address at a ceremony announcing its initial plans.

Awards & Honors

In May, the Israel-based Wolf Foundation awarded the **2001 Wolf Prize in Agriculture** to Dr. Beachy and Dr. James E. Womack of Texas A & M University for their "use of recombinant DNA technology to revolutionize the plant (Dr. Beachy) and animal (Dr. Womack) sciences." Moshe Katsav, president of Israel, presented the Wolf Prize, which includes a $100,000 award, at ceremonies held in Jerusalem.

Ernst & Young of St. Louis presented its **Supporter of Entrepreneurship Award** to the partner institutions that originally envisioned the Danforth Center. In announcing the award, Spencer Burke, managing director of investment banking at A.G. Edwards and Sons, said: "The collective efforts of the Danforth Center will change the landscape of St. Louis business as it builds a foundation for the economic future of the community."

Development Progress

The **Southwestern Bell Foundation** presented a $1.9 million grant to the Danforth Center in May to fund its high-tech, 300-seat auditorium, named in honor of the foundation. At ceremonies held at the Center in May, Jan Newton, president of Southwestern Bell Missouri, said her company "recognizes the vital role that technology plays in helping scientists from around the world share knowledge and enjoy real-time access to important scientific events. We are pleased that our contribution will have such a meaningful impact for the Danforth Center and the St. Louis region."

In October, under the leadership of Robert L. Virgil, Principal, Edward Jones, the Center formed a **Friends Committee,** consisting of 20 leaders from the St. Louis business and civic communities. Members of the Friends Committee have pledged to help increase the number of Friends, a group of supporters who annually contribute $1,000 or more to the Center. The effort is off to a great start with over 50 members committed by the end of 2001. All donors to the Friend's Program by June 30, 2002, will be designated as Charter Members and will be recognized on a plaque in the entry foyer.

Conferences, Seminars & Meetings

The Danforth Center participated in the **Second Biennial World Congress of the World Agricultural Forum**, held in St. Louis in May. The meeting provided a neutral platform for discussions by world leaders and decision-makers concerning 21st century agricultural challenges. Dr. Beachy presented a talk at the Forum, and the Center hosted an informational booth.

In October, **The Rockefeller Foundation** sponsored a three-day workshop at the Danforth Center on crop productivity in water-limited environments. Attended by plant scientists from the U.S., Mexico, and Europe, the workshop resulted in a genetics and breeding plan aimed at developing drought-tolerant crops. The workshop was followed by the Danforth Center's **Third Annual Fall Symposium**, which addressed the biology of plant roots, a topic complementary to the emphasis of the workshop. The symposium series features speakers from the partner institutions of the Danforth Center.

More than 150 research scientists from Asia, Africa, Europe, North America, Latin America, and the Caribbean attended the **Fifth International Scientific Meeting of the Cassava Biotechnology Network** (CBN), held at the Danforth Center in November. Founded in 1988, CBN focuses on the development of modern scientific technologies to improve the productivity of cassava – an important staple food crop in many tropical countries – by facilitating communication among scientists in the developed and developing worlds.

Staff News

In June, **Jeannette Huey** joined the Center's staff as vice president for development. Prior to her appointment, Ms. Huey was a 15-year member of the alumni and development staff of Washington University in St. Louis where she served most recently as director of its International Alumni and Development programs and as director of Parent Programs. Ms. Huey holds a bachelor's degree in political science and a master's degree in education, both from Vanderbilt University.

The Danforth Center made significant progress in 2001 towards its ultimate staff goal of 17 principal investigators and more than 200 scientists, technicians, post-doctoral fellows and graduate students. As of December, the Center had recruited 109 people, including 16 research scientists, 30 post-doctoral fellows, three graduate students, 36 support staff and 24 administration staff members – an increase of 63 people during the year.

Education Initiatives

As part of its commitment to the advancement of plant science, the Danforth Center continued to support St. Louis area students in 2001 through its second annual **Undergraduate Summer Research Internship Program**. Four students from area colleges (Saint Louis University, Southern Illinois University, Washington University in St. Louis and Webster University) each worked with an established Danforth Center scientist for ten weeks. The students learned techniques specific to their mentor's research and conducted related research projects of their own. Eight St. Louis companies provided funding for the 2001 internships.

Thirty post-doctoral fellows from 19 countries also benefited from an opportunity to work with Danforth Center principal investigators in 2001. Post-doctoral training, which enhances a scientist's development in specialized areas and enables them to eventually conduct independent research projects, is an important part of science education worldwide.

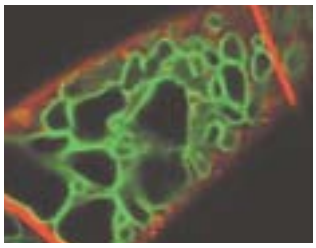
RESEARCH AT THE DANFORTH CENTER

The Biological Century

We like to say that the Danforth Center stands at the leading edge of plant science research. And while that may sound like the standard claim to fame, we have reason to believe it reflects an exciting reality. In the biological sciences, recent key advances in understanding basic molecular and genetic processes in living organisms have made it possible to proclaim the twenty-first century as the "biological century." This era will see a concentration of research and application in the plant and life sciences that will have profound and positive consequences for people worldwide. The Danforth Center will play a key role in expanding biological knowledge and technology in the new century.

The medical sciences have for many years been the focus of research and public interest; however, there is a new recognition that plant science research is just as vital to our future as medical research. The study of plants will provide us with a source of knowledge to enhance human health, agricultural productivity and environmental sustainability, and it will be a major component of the world economy and future development.

It is impossible to ignore plant science, because we, and, indeed, most life on earth, depend directly or indirectly on plants. We derive our livelihood, shelter and medicines from plants. Yet, even though plants are indispensable to us, science has far to go to reach a full understanding of how plants grow and develop, how they produce compounds we find useful, how they resist attack by pests and diseases, how they obtain nutrients, and how they sense and respond to their environment.

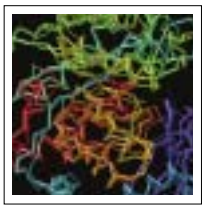


Fundamental Plant Science Research

The Danforth Center is dedicated to increasing knowledge in fundamental plant biology as well as to developing practical applications for new discoveries. Fundamental research lays the foundation for real world applications. The recent extraordinary revolution in biology has been steered by fundamental discoveries about the genetic code and how genes are translated to create proteins—the molecular building blocks and machinery of all living organisms. Technology resulting from genetic research has permitted scientists, like those at the Danforth Center, to manipulate genetic information in a wide variety of useful ways, giving them new tools for use in their research.

Scientific investigation into gene function and the structure and function of proteins lies at the heart of much fundamental research at the Danforth Center. Scientists working in these fields look at plants from the molecular level, studying the materials that comprise a cell in order to understand plant biology through the dynamics of the molecules responsible for living organisms.

Because knowing the shape and function of proteins is central to current plant biology, many of our research programs are studying aspects of this question. In fact, two research groups are dedicated to elucidating the three-dimensional shape of protein molecules, via complementary methods: computer programs that predict shape and function from basic data, and protein crystallography, which measures protein shape in the crystal state. The three-dimensional shape of a protein reveals a great deal about its ultimate purpose and suggests methods through which we can inhibit or enhance its function.



Research is also underway to learn more about the way key enzymes (the proteins that drive biochemical reactions in cells) are controlled to shed more light on the biochemical processes of cells. Other projects tackle the identity of proteins that confer resistance to disease or examine the structure of protein components of viruses to learn basic information about the life cycle of these pathogens.

Other fundamental research at the Danforth Center targets certain aspects of plant development, such as the way plants grow, develop and produce specialized organs like leaves, roots and flowers. This is an area of plant biology about which relatively little is currently known, even though it is integral to the benefit we derive from plants.

Because plant science is universally important, the founders of the Danforth Center stipulated that the Center would use its scientific expertise to aid the people of the developing world. Implicit in the Danforth Center's mission is the understanding that our researchers will consider the research goals and agendas of developing countries as well as developed countries as they select research projects and implement training programs. Several of our laboratories conduct research and training for the benefit of the developing world.

Four Research Initiatives

Four primary initiatives guide the research undertaken at the Danforth Center and each is aimed toward practical application. These four program areas investigate plants for the benefit of human health, pest and disease resistance in plants, the role of roots in plant nutrition and response to stress, and the production of novel materials in plants.

Plants for the benefit of human health

Because plants are a major component of our diets, the quality and availability of the nutrients they contain have a tremendous impact on human health. New biotechnologies have enabled scientists to more efficiently develop plants that provide enhanced nutritional balance for people. Danforth Center scientists are working on several projects aimed at increasing the health benefits of plants. Researchers are studying the enzymes that produce folic acid, an important vitamin. They expect this work to lead to the development of plants with abundant folic acid that is easily absorbed by the body. Another project at the Center addresses the antioxidant content of soybeans in studies that examine the way isoflavones are produced. This research may lead to other legumes and non-legumes with enhanced isoflavone composition. Research on how seeds produce oil will lead to the development of crops with a more healthful oil content.

It is now possible to develop foods containing proteins that induce immunity to diseases. With this advance comes an opportunity to produce vaccines that are stable, easily transported and free of the contaminants sometimes found in today's vaccines. These new vaccines, which would be administered by health professionals, could be especially convenient in developing countries.

Pest and disease resistance in plants

Pests and diseases affect all agricultural plants and can reduce crop yields and decrease farm income. Worldwide, fungal diseases decrease overall crop yields by about 12 percent every year, and parasitic nematodes in the soil cause a loss of more than $77 billion annually. In some situations, chemicals are used to control the pests and pathogens that attack plants. In other cases, traditional plant breeding has produced varieties that resist some diseases or pests, but this method is slow and the results uncertain. In contrast, research into the genetic control of a plant's response to disease has led to methods for rapid development of disease resistant plants. These crops rely on natural methods to control diseases, while they reduce the use of chemical insecticides in crops.

Danforth Center researchers are studying pest and disease resistance from several directions. Two groups have developed plants that are better able to withstand viral disease using genetic transformation to improve their resistance. Such virus-resistant plants offer a safe and economical way to increase plant yield. Two other groups are studying what happens to plants when they are attacked by parasites or pathogens. One group looks at the internal molecular signals that are generated at the site of attack to initiate a systemic defensive response throughout the plant. Another group identifies genes that become active in response to nematodes, which are microscopic parasites that attack plant roots. The information from both groups will provide ways to increase the defensive responses in plants. Yet another group studies the role of isoflavones in plant defense. Like many compounds that plants produce, isoflavones serve the plant as a defense mechanism and, coincidentally, have a beneficial effect on human health.

Roots: plant nutrition and responses to stress

Roots supply the plant with water and nutrients. They serve as soil sensors, sending signals to the rest of the plant about the availability of water, nutrients and other soil conditions. The importance of roots in plant health makes them central to agricultural improvement. Unfortunately, agricultural practices often create soils that limit crop productivity. Irrigated soils, for example, tend to become highly saline over time, limiting the growth of most plants. In work aimed to increase crop tolerance to salinity, research at the Danforth Center is identifying and characterizing the protein molecules roots use to transport minerals like sodium and potassium. In the same laboratory, work is progressing to characterize a transporter protein that moves zinc (an essential mineral) into the plant through the roots so that it can be loaded into seeds. Throughout the world, many soils are zinc deficient, so increasing the activity of the zinc transporter will improve plant nutrition.

Novel materials

Plants naturally produce a wide range of different types of oils, many of which are beneficial for human health. Some plant oils have unique qualities that make them useful for industrial applications, such as solvents, plastics, lubricants or adhesives. Often these oils are found in plants that are not grown commercially. Transferring the genes that guide the production of industrially useful oils into a major crop plant, such as soybeans, may lead to more efficient and cost-effective sources for these raw materials. This advance may also provide farmers with potentially valuable alternative markets for their crops. Searching for genes that produce unusual oils is an important area of research at the Danforth Center.

2001 Honor Roll of Donors

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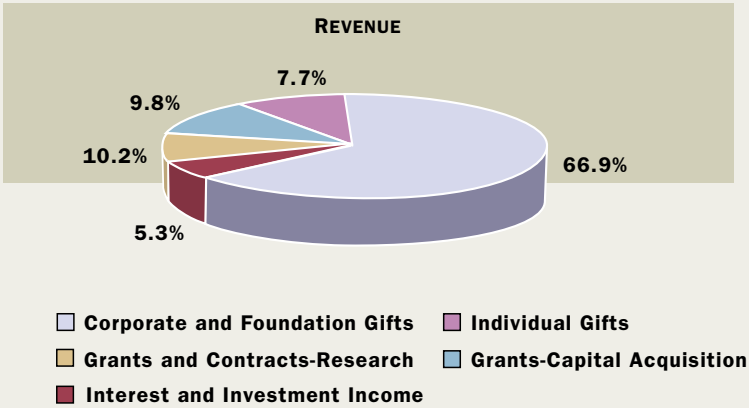
Donald Danforth Plant Science Center
Selected Financial Data
Fiscal Year Ended December 31, 2001

REVENUES AND EXPENDITURES (Dollars in Thousands)

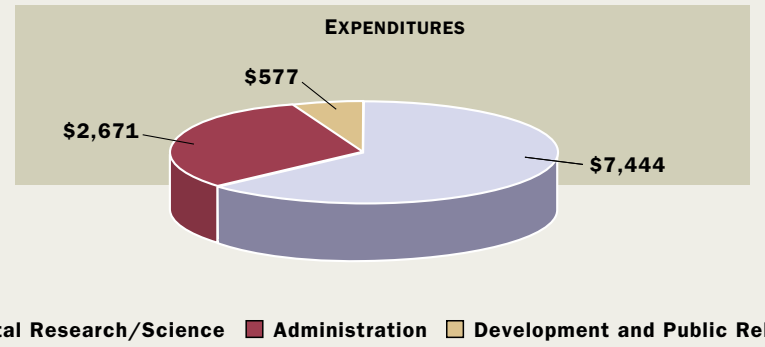
REVENUES (CASH BASIS)

	Revenue	Source %
Corporate and Foundation Gifts	$12,962	66.9%
Individual Gifts	1,036	5.3%
Grants & Contracts - Research	1,974	10.2%
Grants - Capital Acquisition	1,893	9.8%
Interest & Investment Income	1,498	7.7%
Total Revenues	**$19,363**	**100.0%**



EXPENDITURES FROM CONTINUING OPERATIONS

	Expenditures	Expenditure %
Total Research/Science	$7,444	69.6%
Administration	2,671	25.0%
Development and Public Relations	577	5.4%
Total Expenditures from Continuing Operations	**$10,692**	**100.0%**
Capital Building Project and Capital Equipment	$41,830	



Donald Danforth Plant Science Center

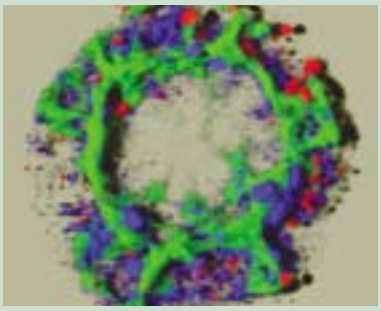
Science Report 2001

The goals of the research in my laboratory are to develop an understanding of the cellular and molecular basis of virus diseases and to develop strategies to limit infection, disease, and crop loss due to infection.

Roger Beachy, Ph.D., Member and Principal Investigator

Studies of tobamoviruses TMV and Cg

Tobacco mosaic virus (TMV) and Cg virus are members of the tobamovirus family and share many gene functions in common; TMV is a pathogen of the *Solonaceous* family and Cg is a pathogen of *Arabidopsis thaliana.* In studies of coat protein mediated resistance (CP-MR) we reported that a mutant of coat protein (CP) of TMV with a substitution of Thr for Trp at amino acid 42 (T42W) resulted in very high levels of resistance. Further studies revealed that wild type CP increases the production of 30 kDa movement protein (MP), a protein that is essential for cell-cell spread of infection. In contrast, the mutant CP-T42W significantly reduced production of MP and the formation of 'factories' of virus replication. We conclude that the effects of the mutant CP were responsible for reduced cell-cell spread of infection and concomitant high levels of CP-MR in transgenic plants. We are continuing studies of the cellular and structural mechanisms by which CP enhances production of MP and CP-T42W reduces MP production.



This image of the distribution of three TMV proteins in a single tobacco cell protoplast shows the power of confocal microscopy and 3D digital image reconstruction in analyzing the cell biology of infection, clearly showing the co-distribution of virus movement protein (green), coat protein (blue), and replicase (red).

In studies to determine how MP facilitates cell-cell spread of infection, we are applying live cell imaging techniques to visualize *A. thaliana,* tobacco, and BY-2 protoplasts following infection with viruses that produce MP-GFP (and related fluorescent proteins). The MP has characteristics of an integral membrane protein and is associated with endoplasmic reticulum. However, the nature and mechanism of insertion into the ER is not known, and we combine the use of genetics, biochemistry, and cell biology to determine the topology of the MP in membranes and to identify host factors that contribute to its function in cell-cell spread of infection.

Regulated expression of the promoter of rice tungro bacilliform virus

The promoter of RTBV is expressed solely in vascular tissues of both dicots and monocots. We isolated and characterized two b-ZIP proteins, RF2a and RF2b, that bind a *cis* element immediately upstream of the RTBV promoter. *In vitro* transcription reactions and *in vivo* (transient and transgenic) assays confirmed that RF2a and RF2b can act as homo- and hetero-dimers to regulate expression of the promoter. Each protein contains multiple regulatory domains, some of which interact in the dimers and/or interact with the rice TATA binding protein. Ongoing studies include determination of the nature of inter-protein interactions on regulating the promoter and developing strategies to use such information to develop rice plants that resist infection by RTBV.

Recent Publications

Bendahmane M, Chen I, Szecsi J, Berg RH, Beachy RN. 2002. Characterization of mutant tobacco mosaic virus coat protein which interferes with virus cell to cell movement. Proc Natl Acad Sci 99(6):3645-3650.

Brugidou C, Opalka N, Yeager M, Beachy RN, Fauquet C. 2002. Stability of rice yellow mottle virus (RYMV) and cellular compartmentalization during the infection process in *Oryza sativa* (L). Virology (in press).

Zhu Q, Ordiz MI, Dabi T, Beachy RN, Lamb C. 2002. Rice TATA binding protein functionally interacts with transcription factor IIB and the RF2a bZIP transcriptional activator in an enhanced plant *in vitro* transcription system. Plant Cell 14:1-10.

Kotlizky G, Katz A, van der Laak J, Boyko V, Lapidot M, Beachy RN, Heinlein M, Epel BL. 2001. A dysfunctional movement protein of tobacco mosaic virus interferes with targeting of wild-type movement protein to microtubules. Mol Plant-Microb Interact 14(7):895-904.

Petruccelli S, Dai S, Carcamo R, Yin Y, Chen S, Beachy RN. 2001. Transcription factor RF2a alters expression of the rice tungro bacilliform virus promoter in transgenic tobacco plants. Proc Natl Acad Sci 98:7635-7640.

Schubert Publications

Cao Y, Shubert KR. 2001. Molecular cloning and characterization of a cDNA encoding soybean nodule IMP dehydrogenase. Biochim Biophys Acta 1520:242-246.

Kersey R, Inoue K, Schubert KR, and Dixon RA. 1999. Immunolocalization of two lignin O-methyltransferases in stems of alfalfa *(Medicago sativa* L.*)*. Protoplasma 209:46-57.

Shah Publications

Gao A-G, Hakimi S, Mittanck C, Wu Y, Stark D, Shah DM, Liang J, Rommens C. 2000. Fungal pathogen protection in potato by expression of a plant defensin peptide. Nature Biotechnol 18:1307-1310.

Gene discovery and engineering of metabolic pathways: Karel Schubert, Ph.D., Domain Member

Our research has three primary goals: 1) to understand the pathways and regulation of carbon and nitrogen assimilation in plants, 2) to discover novel bioactive proteins and natural products from tropical plants, and 3) to engineer metabolic pathways to increase the nutritional quality of crops. Studies on carbon and nitrogen assimilation have concentrated on 1) the *de novo* synthesis of purine nucleotides and the biogenesis of ureides in root nodules of soybeans and developing seedlings, 2) the energetics of carbon and nitrogen assimilation, and 3) the role of compartmentalization of enzymes involved in synthesis of ureides and lignin precursors and metabolic channeling on the regulation and the control of metabolic fluxes through pathways. Recent studies have centered on 1) understanding the regulation and localization of IMP dehydrogenase, the rate-limiting enzyme in GTP biosynthesis and a key enzyme in the biogenesis of ureides, the primary organic form for the storage and transport of nitrogen in many plant species, and 2) enhancing the bioavailability and content of folate in key crops through metabolic engineering.

Role of antifungal defensin peptides in plant defense: Dilip M. Shah, Ph.D., Domain Member

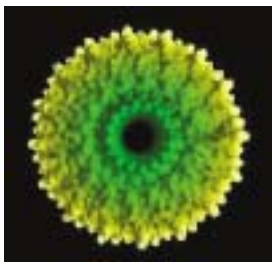
Plant diseases caused by fungal pathogens are responsible for substantial loss of crop yield worldwide, leading to pre-harvest losses estimated at 12 to 13 percent. Effective and sustainable control of fungal pathogens remains one of the most important challenges of modern agriculture. The introduction of novel genes for resistance to fungal pathogens into crops represents an exciting opportunity. Our lab is investigating the potential of small antifungal defensin peptides to confer disease resistance in crops.

It is well documented that defensin peptides play an important role in the defense against fungal pathogens. When a defensin peptide isolated from alfalfa seeds is constitutively expressed in potato, it confers strong resistance to a fungal disease called "early die." This resistance was found to be effective and sustainable in the field. Our lab is further characterizing the structure and function of this peptide with the aim of creating variants with enhanced antifungal activity *in vitro* and identifying amino acid residues that are critical for antifungal activity.

In addition, our lab has isolated genes encoding these peptides from *Medicago truncatula*, which is emerging as a model legume plant. The molecular, genetic, and biological analysis of these genes in *M. truncatula* will provide insight into the role of these peptides in plant defense and will lead to novel strategies for disease control in crops.

Design, production, and analysis of plant-based vaccines: Terry Woodford-Thomas, Ph.D., Domain Associate Member

The technology is now being developed to use plants for the delivery of edible vaccines designed to induce mucosal immunity against infectious pathogens or for oral immunotherapy. One aspect of the work is aimed at refining our knowledge of how modified, chimeric plant viruses such as TMV can be used as a platform for the display of immunodominant epitopes in the design of plant-based vaccines against human and animal diseases. Based upon structural and functional analyses, disease epitopes known to trigger an immune response are being incorporated into distinct regions in the viral coat protein for surface display. The prototype disease for studies on plant virus-based gene expression and vaccine production in plants is HIV/AIDS. Strategies are being examined that will allow the production of multivalent and combination vaccines to elicit both B and T cell immunity for protection, as well as to target the vaccine to the mucosal immune system for improved efficacy. A second aspect of the work focuses on the production of biopharmaceuticals in plants, including therapeutic antibodies.



Model of TMV, cross-section through the virion.

TMV-infected tobacco.

ILTAB aims to improve tropical crops through targeted research projects, to promote research capacity building in developing countries through training and technology transfer, and to help to coordinate global biotechnology research on tropical crops.

Claude Fauquet, Ph.D., Member and Principal Investigator

Our laboratory is dedicated to both research and training, specifically focusing its efforts on tropical agriculture. In keeping with this mission, the laboratory is designated the International Laboratory for Tropical Agricultural Biotechnology (ILTAB). The goals of ILTAB are to advance the application of molecular biology and biotechnology for tropical crop improvement through targeted research projects, to promote research capacity building in developing countries through training and technology transfer, and to help coordinate global biotechnology research on tropical crops.

In 2001, ILTAB moved into the new facilities of the Danforth Center. The Danforth Center includes excellent facilities for tissue culture and molecular biology in addition to cell biology and biochemistry. Within a few months, we had filled several greenhouses with transgenic cassava plants and begun studies of virus resistance.

The activities of ILTAB have been associated with the development of the Cassava Biotechnology Network (CBN). CBN was founded in 1988 and serves the worldwide research groups that work to improve cassava production. Despite the September 11 tragedy, we hosted the Fifth Scientific Meeting of the Cassava Biotechnology Network (CBN-V) on November 4-9, 2001. We had a very successful meeting with 175 participants coming from 35 countries. Electronic proceedings with all the recorded presentations are being published and distributed.

During the CBN-V meeting, scientists from the four organizing institutions, IITA (Ibadan, Nigeria), CIAT (Cali, Columbia), Embrapa (Brazil), and the Danforth Center, had the opportunity to meet and discuss the role of biotechnology for cassava improvement. This resulted in formulation of a concept note entitled the Global Cassava Plan. This is a plan that combines studies of biodiversity, biotechnology, breeding, and of the end-users and capacity building, with specific focus on a few traits. The plan has been endorsed by many individuals and institutions, and it was decided that the Global Cassava Plan will be hosted by the Global Cassava Development Strategy at FAO, in Rome.

Members of ILTAB continue to work on the biology of geminiviruses with emphasis on diseases of cassava. Several years ago, we reported that tobacco plants containing a transgene that encodes a ssDNA binding protein were resistant to infection by certain geminiviruses. Recent studies demonstrated that these plants are resistant to geminiviruses that infect tomato and cassava plants. This technology allows non-specific control of geminiviruses in any plant for any geminivirus, and it is now being transferred to cassava.

Transgenic cassava testing by Dr. Francis Ogbe from the Root and Tuber Crop Research Institute in Nigeria.

Recent Publications

Chatterji A, Beachy RN, Fauquet CM. 2001. Expression of the oligomerization domain of the replication-associated protein (Rep) of Tomato Leaf Curl New Delhi Virus interferes with DNA accumulation of heterologous geminiviruses. J Biol Chem 276:25631-25638.

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Masona MV, Taylor NJ, Robertson IA, Fauquet CM. 2001. Transferring a cassava (*Manihot esculenta* Crantz) genetic engineering capability to the African environment: Progress and prospects. Euphytica 120:43-48.

Pita J, Fondong VN, Sangaré A, Otim-Nape GW, Ogwal S, Fauquet CM. 2001. Recombination, pseudorecombination and synergism of geminiviruses are determinant keys to the epidemic of severe cassava mosaic disease in Uganda. J Gen Virol 82:655-665.

Taylor NJ, Masona MV, Carcamo R, Ho T, Schöpke C, Fauquet CM. 2001. Production of embryogenic tissues and regeneration of transgenic plants in cassava (*Manihot esculenta* Crantz). Euphytica 120:25-34.

Some unusual seed oils are found in non-crop plants and might have useful industrial applications. Our lab studies the metabolic pathways involved with regulating the composition of the oil in plant seeds.

Jan Jaworski, Ph.D., Member and Principal Investigator

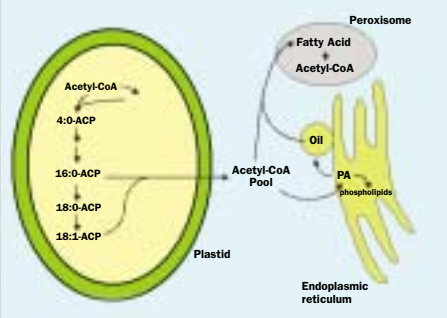
Oils from plant seeds have a broad assortment of compositions. The seed oils from agronomically significant plants are largely used for food; however, some unusual seed oils are found in non-crop plants and might have useful industrial applications. Our lab studies the metabolic pathways involved with regulating the composition of the oil in plant seeds.

Modification of seed oil composition

This research is part of a project funded by the Dow Chemical Company to solve basic problems associated with modification of oilseeds using biotechnology. My laboratory contributes two major components to this study. We are developing new techniques to evaluate the lipid metabolism of transgenic or mutant plants. These analyses will measure the levels of lipid metabolism intermediates such as acyl-ACPs and acyl-CoAs, as well as phospholipid and triacylglycerol pools. The second component of the research aims to discover genes to allow the production of unusual fatty acids in common crops such as soybean.

Condensing enzymes of fatty acid biosynthesis

We are carrying out a structure-function analysis of condensing enzymes involved in both fatty acid synthesis and elongation. This important class of enzymes, the 3-ketoacyl synthases, initiates the series of reactions that lead to the two carbon extension of fatty acids. Most plants have three 3-ketoacyl-ACP synthase isozymes. We study 3-ketoacyl-ACP synthase III (KAS III) from spinach, a soluble condensing enzyme that catalyzes the intial condensation reaction of fatty acid synthesis.



Fatty acid and oil biosynthesis in seeds is a complex process taking place in several parts of the cell.

We were first to characterize, purify, and clone a plant KAS III. An *E. coli* homolog exists, and its crystal structure was reported from two different labs. Although the plant and *E. coli* enzymes appear to be very similar based on sequence analysis, site-directed mutagenesis of some active-site residues suggests that there may be significant mechanistic differences. We also study the *Arabidopsis* FAE1 KCS, a 3-ketoacyl-CoA synthase anchored to the endoplasmic reticulum by two membrane-spanning domains near the N-terminus. Because it is membrane-bound, analysis of the elongase condensing enzyme has been much more challenging, and we have no structural data available to guide our studies. To our knowledge, our characterization of the elongase condensing enzyme constitutes the first molecular study of any membrane-bound condensing enzyme. To date, we have engineered an N-terminal His-tag onto the enzyme and developed a yeast expression system that generates a fully active FAE1 KCS. We can solubilize the FAE1 KCS and isolate it on a Ni^{2+} affinity column with excellent recovery of activity. Assays we are developing for the partial reactions of substrate acylation of the active site and malonyl-CoA decarboxylation will put us in an excellent position to study structure-function relationships.

Recent Publications

Ghanevati M, Jaworski JG. 2001. Active-site residues of a plant membrane-bound fatty acid elongase β-ketoacyl-CoA synthase, FAE1 KCS. Biochim Biophys Acta 1530:77-85.

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Todd J, Post-Beittenmiller D, Jaworski JG. 1999. KCS1 encodes a fatty acid elongase 3-ketoacyl-CoA synthase affecting wax biosynthesis in *Arabidopsis thaliana.* Plant J 17:119-130.

Ohlrogge J, Jaworski J. 1997. Regulation of plant fatty acid biosynthesis. Ann Rev Plant Phys and Plant Mol Biol 48:109-136.

Identification of molecules and principles that regulate organellar positioning during cellular differentiation will significantly contribute to the understanding of development in multicellular organisms.

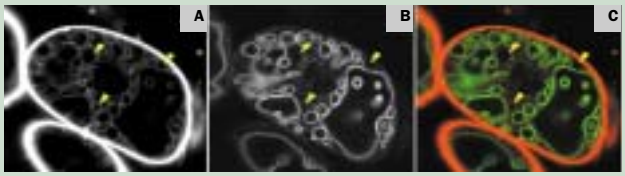
Erik Nielsen, Ph.D., Assistant Member and Principal Investigator

Plant vacuoles are multifunctional post-Golgi organelles that play critical roles in cellular growth, maintenance of ion homeostasis, and accumulation of storage compounds. Current models of plant vacuolar trafficking propose three vacuolar sorting signals and at least two different trafficking pathways. However, sorting and delivery mechanisms for different membranes and proteins, as well as organization and coordination of vacuolar membrane trafficking pathways during growth and development are incompletely understood.

Rab GTPases are a large family of Ras-like proteins that play key roles in membrane trafficking and specifically localize to the various organelles in eukaryotic cells. *Arabidopsis thaliana* has 57 Rab GTPase isoforms, but most have no assigned function in membrane trafficking. Phylogenetic comparison of *A. thaliana* Rab GTPases with those of *H. sapiens* and *S. cerevisae* demonstrates similarities that may suggest localization and function of plant Rab GTPases of unknown function.

We are characterizing the intracellular distribution and function of two *A. thaliana* Rab GTPases, AtRab7D and AtRab5A, that are predicted to be involved in biogenesis of plant vacuolar compartments. We are using a combination of strategies including time-lapse video microscopy, biochemistry, cell biology, and genetics.

To determine the roles of Rab GTPases in vacuolar membrane trafficking, GFP-Rab fusion proteins are being used in conjunction with fluorescence microscopy to examine the distribution of these Rab GTPases versus previously characterized marker proteins.



EYFP-AtRab7D labels a subset of vacuoles in Tobacco BY cells. BY-2 cells were labeled with the styryl dye FM 4-64 (5 mM; 10 minutes on ice) and then incubated at room temperature for 45 minutes. Confocal images of FM 4-64 (A) and EYFP-AtRab7D labeled vacuoles (B) were collected successively with appropriate excitation and emission filtersets and overlayed (C). In the overlay EYFP-AtRab7D fluorescence is false colored green and FM 4-64 signal is displayed in red. Arrows indicate vacuolar structures that are not labeled with EYFP-AtRab7D.

The respective function(s) of these Rab GTPases in vacuolar biogenesis will be assessed by expressing active and inactive Rab mutants in tobacco BY-2 suspension culture cells and by identification of Rab-interacting proteins by affinity chromatography and/or yeast two-hybrid screening.

This research will provide important insight into Rab GTPase functions in membrane trafficking pathways to plant vacuoles, as well as mechanisms linking membrane dynamics with cellular differentiation. Identification of molecules and principles that regulate organellar positioning during cellular differentiation will significantly contribute to understanding development in multicellular organisms. Additionally, insight into mechanisms regulating vacuolar biogenesis and expansion may lead to observations with practical significance because the plant vacuole plays a key role in protein storage, defense against pathogens, and response to drought and high salt stresses.

Recent Publications

Nielsen E, Severin F, Hyman AA, Zerial M. *In vitro* reconstitution of endosome motility on microtubules. In: Vernos I, editor. Methods in Molecular Biology. vol 164. Totowa, New Jersey: Humana Press; 2001. p 135-146.

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Turner G, Gershenzon J, Nielsen EE, Froehlich JE, Croteau R. 1999. Limonene synthase, the enzyme responsible for monoterpene biosynthesis in peppermint, is localized to leucoplasts of oil gland secretory cells. Plant Physiol 120:879-886.

The goal of the research in my laboratory is to understand the regulation of shoot and floral meristem function using a variety of genetic, molecular, biochemical, and genomics/proteomics approaches.

Mark Running, Ph.D., Assistant Member and Principal Investigator

The vast diversity of plant form can be traced to the actions of meristems, small collections of cells from which all branches, stems, leaves and flowers arise. Meristems possess a complexity that belies their simple appearance: they must integrate a wide variety of environmental and genetic cues to produce and allocate cells to initiating organs and stems in specific patterns, while relying on internal signals to stably maintain a constant number of cells for future growth. Maintaining a group of undifferentiated cells throughout its life allows each plant to continually adapt its form to its environment, in contrast to mammalian development, in which the body plan is established early in the embryo and is fixed. The goal of the research in my laboratory is to understand the regulation of shoot and floral meristem function using a variety of genetic, molecular, biochemical, and genomics/proteomics approaches.

Our genetics approach involves development of a sensitized screen to readily identify mutants that promote or restrict meristem activity. We use plants that are misexpressing the *Arabidopsis KNAT1* gene in leaves, which results in leaf lobing and ectopic meristems.

Plants with abnormal meristems show patterning defects.
A. A wild type leaf compared to progressively more severe leaves that misexpress *KNAT1*.
B. A close up of a leaf misexpressing *KNAT1* and producing new leafy shoots, an indication of the presence of a meristem.
C. Wild type *Arabidopsis* flower.
D. A *pluripetala* mutant flower. Changes in meristem signaling lead to abnormal numbers and positions of floral organs.

The screen is rapid and can uncover a wider variety of mutants than traditional screens. We have identified several mutants that show an enhancement of ectopic meristem activity, indicating that the wild type function of these genes is to restrict meristem activity. We have also identified several suppressor mutants, which may act to promote meristem activity or may be involved directly downstream in mediating *KNAT1* functions.

Also, because plants overexpressing *KNAT1* have limited phenotypic effects outside of the leaf, it has been possible to identify meristem genes that act independently from *KNAT1*, such as those involved in flower meristem function. The characterization of these mutants should lead to increased knowledge of molecular mechanisms and pathways involved in meristem activity.

One mutant of particular interest, *pluripetala (plp),* was identified as an enhancer of ectopic meristem function. Single *plp* mutant plants have larger shoot and floral meristems and increased leaf, flower, and floral organ number, in addition to abnormalities in maturation time, organ development, and hormone responses. *PLP* encodes a key protein involved in prenylation, which is a post-translational lipid modification thought to play a role in signaling pathways and protein-protein interactions. Based on the mutant phenotype, *PLP* plays a role in meristem signaling events that are critical for maintaining correct cell number and establishing primordia initiation sites. Proteins with consensus target sequences for prenylation are readily identifiable using bioinformatics tools, allowing us to further characterize these signaling pathways using reverse genetics and proteomics approaches.

Recent Publications

Running MP. Nuclear staining for confocal microscopy. In: Weigel D, Glazebrook J, editors. *Arabidopsis*: a laboratory manual. Cold Spring Harbor: Cold Spring Harbor Laboratory Press; 2001. p 100-103.

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Jacobsen SE, Running MP, Meyerowitz EM. 1999. Disruption of an RNA Helicase/RNAse III homologue in *Arabidopsis* causes unregulated cell division in floral meristems. Development 126:5231-5243

My research is aimed at gaining a comprehensive understanding of the molecular mechanisms used by roots to obtain minerals and how these mechanisms aid in adaptation to problem soils.

Daniel Schachtman, Ph.D., Associate Member and Principal Investigator

Plant roots must grow through soils to acquire minerals and water and are the primary mineral acquisition systems for both plant and animal nutrition. My research is aimed at gaining a comprehensive understanding of the molecular mechanisms used by roots to obtain minerals and how these mechanisms aid in adaptation to problem soils. Roots must operate in soils that contain both deficient and toxic amounts of minerals. Therefore, an understanding of nutrient acquisition and the development of methods for changing transporter function will lead to novel approaches for increasing the sustainability of agricultural systems that operate on marginal lands.

Wild type *Arabidopsis* (left) and a mutant with a defective potassium transporter.

Salinity is a major soil problem worldwide. In saline soils, the growth of plants is limited by the excess uptake of sodium and chloride. A reduction in sodium, and sometimes chloride, uptake is associated with increased crop tolerance to salinity and increased yields. One goal of the research in my lab has been to identify and manipulate pathways for sodium and chloride uptake by plant roots. Several families of transporters in plants are involved in K^+ uptake and homeostasis, and it is likely that K^+ transporters provide a pathway for Na^+ uptake. We are studying the functional role of the *Arabidopsis* KUP transporters, a major family of K^+ transporters (13 members) that appear to be involved in K^+ uptake that drives cell expansion and plant development.

In order to begin to determine the roles of the genes encoding the KUP proteins in potassium uptake and in salinity stress responses, we have begun to study the patterns of spatial and temporal gene expression of each *KUP* gene. In a second project, we are modifying the ability of plants to compartmentalize Na^+ in the vacuole. Chosen genes will be overexpressed in lettuce to create transgenic material that will allow us to study how increased compartmentation alters plant growth and Na^+ uptake rates under saline conditions.

Another major worldwide problem is zinc deficiency, wherein the availability of zinc in soils is severely reduced by physio-chemical parameters. Decreased zinc availability reduces plant growth, yields, and ultimately the nutritional quality of foods. In order to increase plant uptake of zinc, we have been studying membrane transport mechanisms for zinc in cereals, which are among the most important world food crops. We have used information from the rice genome project (expressed sequence tags) and a yeast mutant deficient in zinc uptake. Several putative rice zinc transporters have been characterized. In addition, we are now testing transgenic barley overexpressing an *Arabidopsis* zinc transporter for changes in zinc uptake and seed zinc content. We will continue our work in this area with emphasis on how zinc uptake is controlled in roots and how zinc is loaded into seeds.

Recent Publications

Amtmann A, Fischer M, Marsh EL, Stefanovic A, Sanders D, Schachtman DP. 2001. The wheat cDNA LCT1 generates hypersensitivity to sodium in a salt-sensitive yeast strain. Plant Physiol 126:1061-1071.

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The Laboratory of Computational Genomics develops computational tools used for comparing and interpreting the sequence information generated by genome sequencing projects. The tools we create are algorithms for predicting protein function from sequence.

Jeffrey Skolnick, Ph.D., Member and Principal Investigator

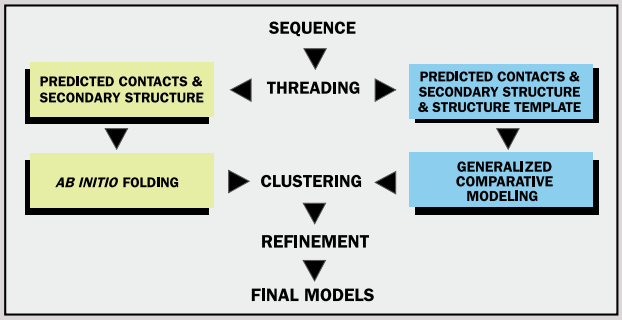
Overview

The Laboratory of Computational Genomics develops computational tools used for comparing and interpreting the sequence information generated by genome sequencing projects. The tools we create are algorithms for predicting protein function from sequence, including both *ab initio* folding and threading methods. Our *ab initio* folding approaches are capable of predicting low resolution structures for a substantial fraction of small, single domain proteins, and our threading algorithms can assign structures to at least half of the sequences in an average genome. We can use these predicted structures to assign biochemical function, to dock ligands to identify the binding site, to predict protein-protein interactions, and to assign the proteins to known pathways.

A unified approach to protein structure prediction

Our group has developed a unified methodology, TOUCHSTONE, to predict protein structure from sequences. The methodology uses a newly developed, iterative threading algorithm called PROSPECTOR. If there is no significant match to a template structure, the predicted consensus contacts and secondary structure extracted from the top 20 scoring structures are used as restraints in *ab initio* folding. On average, about one-third of the contacts are correctly predicted and 75 percent are correctly predicted within two residues. Application to a representative test set of 65 proteins gives the native state in one of the well-defined clusters in 51 cases.

We have also predicted the tertiary structure of all the small proteins in the *M. genitalium* genome and we estimate that the native structure has been successfully predicted in 51 of 85 cases. Conversely, if a global template is identified by PROSPECTOR, then a generalized comparative modeling approach called GeneComp uses the template alignment and predicted contacts and secondary structure (not necessarily from the template structure) as restraints in the *ab initio* folding algorithm. GeneComp has been demonstrated to build moderate resolution models even when the sequence identity to the template structure is ten percent. We have also extended PROSPECTOR to predict multimeric interactions and the resulting method seems to be quite robust, predicting over 2,100 dimeric complexes in the yeast genome, 500 of which have been experimentally observed. We have also developed a methodology to assign proteins to known pathways. For example, in the glycolysis pathway in *A. thaliana*, the number of assignments has doubled.



Unified approach to protein structure prediction.

Recent Publications

Fetrow F, Siew N, Yamout M, Dyson J, Wright P, Skolnick J. 2001. Genomic-scale comparison of sequence- and structure-based methods of function prediction: does structure provide additional insight. Protein Science 10:1005-1014.

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Crystallography is most effective when combined with biochemistry and other biophysical techniques. We have been engaged in several projects where crystallography can be used to answer larger questions.

Thomas Smith , Ph.D., Member and Principal Investigator

Crystallography is most effective when combined with biochemistry and other biophysical techniques. We have been engaged in several projects where crystallography can be used to answer larger questions.

We are examining the structure and function of glutamate dehydrogenase (GDH). GDH is found in nearly all organisms and catalyzes the reversible oxidative deamination of L-glutamate to 2-oxoglutarate. Its physiological role is modified by allosteric regulation. To better understand how enzymes are regulated via intra-subunit communication, we have determined the structure of GDH in the presence and absence of various active site and allosteric ligands. Children with defects in GDH regulation hypersecrete insulin, and when GDH is deleted in plants, their ability to accommodate higher nitrate containing soils is greatly affected. Understanding GDH regulation may not only permit modulation of insulin levels in mammals but may also lead to enhanced nitrogen assimilation in plants.

Our second project studies a series of naturally occurring antifungal agents. KP4 is produced by a virus that persistently infects *Ustilago maydis*, or corn smut. We have proven that KP4 blocks calcium channels in fungus and also exhibits cross-kingdom activity by blocking L-type calcium channels in mammalian cells. We are continuing these studies and collaborating with Dr. Dilip Shah on a naturally occurring alfalfa antifungal agent. These studies will identify novel targets for antifungal agents and may lead to fungal resistant plants.

We have been continuing our studies on insect transmission of viruses. Cucumber mosaic (CMV) is the type member of the genus Cucumovirus. CMV infects over 800 plant species and causes economically important diseases in many crops worldwide.

Structure of glutamate dehydrogenase in the 'open' conformation.

We have determined the structure of CMV and have mapped the residues involved in aphid transmission. We have developed monoclonal antibodies to the aphid transmission site and have determined the cryo-TEM structure of the Fab/CMV complex in collaboration with Timothy Baker's laboratory. While all 180 subunits of the virus capsid are chemically identical, only those about the 5-fold axes are recognized by the antibody. Modeling studies showed that these antibodies actually bridge two antigenic loops simultaneously. This result has implications in the display of antigens on plant viruses and also may serve as an important tool in examining viral assembly.

In collaboration with Himadri Pakrasi at Washington University, we have expressed, purified, and crystallized a zinc binding protein called ZnuA. ZnuA is the extracellular portion of an ABC transporter in cyanobacteria that imports zinc and, in other bacteria, is essential for the pathological effects. In plants, this is highly homologous to the manganese transporters that are essential for assembly of the photosynthetic complex.

Recent Publications

Broo K, Wei J, Marshall D, Brown F, Smith TJ, Johnson JE, Schneeman A, Siuzdak G. 2001. Viral capsid mobility: a dynamic conduit for inactivation. Proc Natl Acad Sci 98:2274-2277.

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Smith TJ. Antibody interactions with rhinovirus: lessons for mechanisms of neutralization and the role of immunity in viral evolution. In: Burton DR, editor. Current topics in microbiology and immunology: antibodies in viral infection. New York: Springer; 2001. p 1-28.

Smith TJ, Peterson PE, Schmidt T, Fang J, Stanley CA. 2001. Structures of bovine glutamate dehydrogenase complexes elucidate the mechanism of purine regulation. J Mol Biol 307:707-720.

By studying the interactions of roots with the micro- and macro-organisms contained within the soil, we will better understand the interaction of plants with their environment and develop new strategies for sustainable methods of agricultural production.

Christopher Taylor, Ph.D., Assistant Member and Principal Investigator

Roots have developed complex relationships with the micro- and macro-organisms contained within the soil. These relationships range from mutualistic symbiosis (i.e., *Rhizobium* spp. and mycorrhizal communities) to those of parasitism and pathogenesis (nematodes, *Phytophthora, Pythium*). In response to these relationships, roots produce RNAs, proteins, and chemicals that promote or inhibit specific interactions. By studying these interactions, we will better understand the interaction of plants with their environment and develop new strategies for sustainable methods of agricultural production.

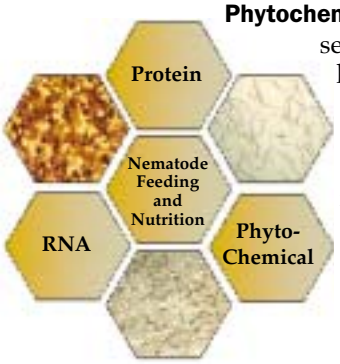
Plant-parasitic nematodes are among the most destructive plant pathogens, causing worldwide losses exceeding $77 billion annually. Current methods of nematode control, including crop rotations, application of nematicides, bio-control, or use of naturally resistant varieties of plants, have met with only limited success. Advances in molecular biology have made it possible to develop new strategies for nematode control. The research in my lab focuses on how nematodes feed on plants; by combining the study of nutrient transport during nematode parasitism with the identification of nematicidal RNAs, proteins, and phytochemicals, we aim to develop better means of nematode control.

Nematode Feeding and Nutrition: Virtually nothing is known about the molecular biology and biochemistry of the interaction between plants and parasitic nematodes. Studies have shown that a variety of plant genes, possibly modulated by secretions of nematodes, are up- or down-regulated during nematode infestation. Genes for membrane channels and transport proteins that aid in the movement of solutes and assimilates into and out of the nematode feeding site are highly up-regulated. My lab has begun to focus on characterizing the level of expression, location, function, and role in nematode feeding and nutrition of transport proteins found in nematode feeding sites. This research will complement ongoing research regarding the delivery of potential nematicidal RNAs, proteins, and phytochemicals.

RNAs: Double-stranded RNA has been harnessed as a highly specific inhibitor (RNAi) of translation. RNAi can inhibit expression of targets in a gene-specific manner. Currently, we are conducting research on whether RNAi expression in plants can also inhibit gene expression in obligate plant-parasitic nematodes.

Anti-Nematode Proteins: Using the established paradigm of controlling insects by using insecticidal proteins, we are looking for proteins and peptides that have nematicidal activity. Using a combination of small antimicrobial peptides and a diverse microbial screening bioassay, we are identifying novel proteins that control nematode growth and development.

Phytochemicals: We are looking for chemicals that are secreted by plant roots that influence nematode hatching, behavior, growth, and development. Important chemical pathways that influence nematodes will be identified and tested in crop plants for production of nematode resistant plants.



Adults, juveniles, and eggs of soybean cyst nematode.

Recent Publications

Opperman CH, Acedo GN, Skantar AM, Saravitz DM, Song W, Taylor CG, Conkling MA. Bioengineering resistance to sedentary endoparasitic nematodes. In: Bird DM, DiGiorgio C and Lamberti F, editors. Advances in molecular plant nematology. New York: Plenum; 1994. p 221-230.

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We are taking a combination of molecular genetic, biochemical, and functional genomics approaches to unravel the molecular events of the disease resistance signal transduction pathway.

Yiji Xia, Ph.D., Assistant Member and Principal Investigator

In addition to protecting themselves with constitutive physical and biochemical barriers, plants can develop induced responses to pathogen attacks. Plant cells can perceive signals from pathogens and relay, amplify, and integrate the signals through complex networks. This leads to coordinated expression of induced defenses to limit pathogen growth and prevent subsequent pathogen attacks.

***CDR1* plays an important role in the disease resistance in *Arabidopsis*. The *CDR1* antisense line exhibited enhanced susceptibility to infection by the virulent *Pseudomonas syringae* strain.**

We are taking a combination of molecular, genetic, biochemical, and functional genomics approaches to unravel the disease resistance signal transduction pathway. We are interested in identifying and characterizing the mobile signal generated at the site of infection and translocated at a distance to activate systemically acquired resistance (SAR). We have identified, through activation tagging, the *Arabidopsis CDR1* gene that encodes an apoplastic aspartic proteinase required for induction of disease resistance responses. Preliminary work has demonstrated that CDR1 functions through activation of a peptide signal system. The signal could be the mobile SAR signal that has to date eluded identification by plant biologists.

We are currently working to identify the elicitor signal and study its mode of action as well as to understand mechanisms underlying the activation of the signal. In addition, we are using GeneChip technology to elucidate the gene network regulated by CDR1 and identify downstream genes involved in the CDR1-mediated defense response.

A second research area involves a functional genomics approach to determine the biological functions of the members of the aspartic proteinase gene family in *Arabidopsis (AtASP)*. Aspartic proteinase is one of the five classes of endopeptidases and has been implicated in regulating a wide range of biological pathways, including processing of peptide prohormones, receptors, and other regulatory proteins. In yeast and animals, aspartic proteinases usually comprise a small gene family of eight to 14 members. In contrast, *Arabidopsis* has 66 putative aspartic proteinase genes. The disproportional expansion of this family in plants suggests that aspartic proteinases play important roles in a wide variety of developmental and physiological processes unique to plants. This notion has been supported by the identification of the *CDR1* gene and our preliminary phenotype characterization of over 20 *atasp* insertion mutants. Through analysis of the *atasp* knock-out lines, we have assigned several *AtASPs* to different biological pathways. Our long-term goal is to elucidate detailed cellular and physiological roles of the individual *AtASPs*. The study might provide novel insights into molecular mechanisms underlying many important biological processes in plants.

Recent Publications

Xia Y, Borevitz J, Blount J, Dixon R, Lamb C. 2002. Biopanning by activation tagging. Recent Adv Phytochem 36 (in press).

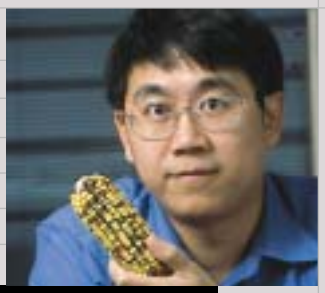
Borevitz J, Xia Y, Blount J, Dixon R, Lamb C. 2000. Activation tagging identifies a conserved MYB regulator of phenylpropanoid biosynthesis. Plant Cell 12:2383-2394.

Delledonne M, Xia Y, Dixon R, Lamb C. 1998. Nitric oxide functions as a signal in plant disease resistance. Nature 394:585-588.

Xia Y, Nikolau BJ, Schnable PS. 1997. Developmental and hormonal regulation of the *Arabidopsis CER2* gene which codes for a nuclear localized protein required for the normal accumulation of cuticular waxes. Plant Physiol 115:925-937.

Xia Y, Nikolau BJ, Schnable PS. 1996. Cloning and characterization of *CER2*, an *Arabidopsis* gene that affects cuticular wax accumulation. Plant Cell 8:1291-1304.

My lab focuses on the isoflavonoids, which play key roles in many legume-microbe interactions, inhibiting the growth of invading pathogens. These studies offer an unprecedented opportunity to understand an area about which little is known.



Oliver Yu, Ph.D., Assistant Member and Principal Investigator

Most of the 100,000 secondary metabolites produced by higher plants are synthesized for defense responses. My lab focuses on the isoflavonoids, which play key roles in many legume-microbe interactions, inhibiting the growth of invading pathogens. Isoflavones also serve as signal molecules and chemo-attractants for symbiotic rhizobia. Isoflavones have caught the public interest because of the health benefits associated with soy consumption, which range from reducing blood cholesterol to reducing the occurrence of breast and prostate cancers.

The isoflavonoids are synthesized from a branch of the phenylpropanoid pathway that exists in all higher plants. In legumes, isoflavone synthase (IFS) commits flavonoid substrates to isoflavones, which are further metabolized to pterocarpans and other phytoalexins. We have cloned IFS genes from a number of species. Under certain condtions, heterologous expression of IFS in non-legume plants led to the novel production of isoflavones in both monocots and dicots. These studies offer an unprecedented opportunity to understand regulation of isoflavonoid biosynthesis.

We are investigating the transcriptional regulation and coordinate expression of the isoflavonoid biosynthesis pathway. The promoter region of the IFS gene from soybean was ligated to a reporter gene and transformed into soybean and *Arabidopsis*. The IFS promoter demonstrated root-specific and defense-inducible expression patterns. The IFS1 promoter is strongly induced upon nematode and fungus infection and exposure to fungal elicitors and salicylic acid treatment.

The regulation of isoflavonoid biosynthesis is governed by interaction of the key enzymes. When a maize transcription factor that specifically activates the transcription of the maize phenylpropanoid pathway was ectopically expressed in soybean seed, the isoflavonoid profiles of the transgenic beans were altered significantly.

The regulation of isoflavonoid biosynthesis is governed by interaction of the key enzymes. When a maize transcription factor that specifically activates the transcription of the maize phenylpropanoid pathway was ectopically expressed in soybean seed, the isoflavonoid profiles of the transgenic beans were altered significantly.

One component of the isoflavones, daidzein, drastically increased while the other component, genistein, decreased to an undetectable level. Molecular analyses of the expression of pathway genes revealed that this change in isoflavone content was caused by specific activation of the flavonoid pathway but not the isoflavonoid pathway. Current research focuses on how the maize transcription factor affected the specific associations between key pathway genes.

The investigation of the regulation of isoflavonoid biosynthesis will not only reveal the mechanisms of plant responses to pathogenic and beneficial microbes at the transcriptional and enzymatic levels, but also broaden our understanding of general plant defense responses in an area not amenable to study using the model plant *Arabidopsis*. In addition to scientific advancement, direct and significant economic impacts are expected.

Knowledge of the regulation of the isoflavonoid pathway will allow further metabolic engineering of soybean and non-legume crops, facilitate the manipulation of isoflavone content, and lead to the production of novel phenylpropanoid compounds that will enhance pathogen resistance and increase the nutritional value of agronomically important crops.

F2 generation of isoflavone corn showing abnormal 6:7 red vs. yellow segregation.

Recent Publications

Jung W, Yu O, Lau SC, O'Keefe DP, Odell J, Fader G, McGonigle B. 2000. Identification and expression of isoflavone synthase, the key enzyme for biosynthesis of isoflavones in legumes. Nature Biotech 18:208-212.

Yu OX, Jung W, Shi J, Crose R, Fader G, McGonigle B, Odell J. 2000. Production of the isoflavones genistein and daidzein in non-legume dicot and monocot tissues. Isoflavone accumulation is related to activity of the phenylpropanoid pathway. Plant Physiol 124:781-793

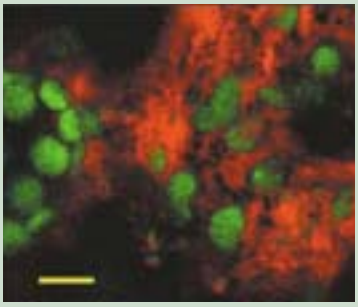
Jung W, Yu OX, Fader G, Odell J, McGonigle B. 1999. Nucleic acid fragments encoding isoflavone synthease. WO 00/44909, US Patent No. 60/117769, 60/144783, 60/156094.

Yu X, Sukumarian S, Marton L. 1998. Differential expression of the *Arabidopsis Nia1* and *Nia2* genes. Plant Physiol 116:1091-1096.

In 2002, we expect to be fully functional in high resolution imaging of plant cells by electron microscopy, and we are making every effort to ensure that live cell imaging in the facility will be state of the art.

R. Howard Berg, Ph.D., Director, Integrated Microscopy Facility

In the first quarter of 2002, we expect to be fully functional in high resolution imaging of plant cells by electron microscopy. The Major Research Instrumentation Program of the National Science Foundation awarded the Danforth Center $500,600 to purchase our transmision electron microscope (TEM) and Balzer's High Pressure Freezer. The freezer provides excellent specimen preservation without ice crystal formation, because tissues are frozen extremely rapidly in liquid nitrogen at high pressure (2,100 atmospheres—more pressure than produced by a .357 magnum). The Leo 912 AB energy filtering TEM has an in-column electron spectrometer, which provides a sophisticated way to optimize specimen contrast and extends the capabilities of our TEM in a number of ways.



Spectral imaging of chlorophyll (green, in chloroplasts) and ER-localized-DSRed in epidermal cells of tobacco leaf. Single confocal plane, Zeiss Meta detector. Marker= 5µm.

We are making every effort to ensure that live cell imaging in the facility will be the state of the art and are pleased that the Zeiss LSM 510 NLO confocal/multiphoton microscope that we have ordered will have Zeiss's new Meta detector. This detector uses an array of 32 PMTs to acquire spectra for each image pixel (sans emission filters), enabling computational separation of spectrally similar fluorescent molecules unencumbered by the limitations of glass filtration. We have successfully separated EGFP- and YFP-labeled proteins using this revolutionary technology. Multiphoton excitation for this system will be supplied by a tunable Ti-sapphire laser (Verdi 10 W-pumped Mira 900F, from Coherent) that we have acquired with the support of NASA funding.

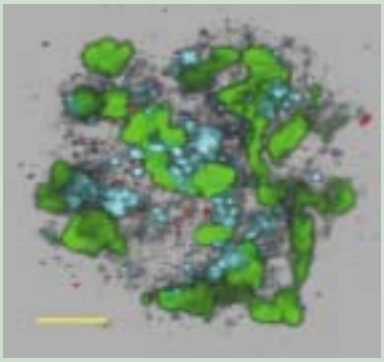
Our goal of quantitative analysis of living cells using the Zeiss system took a step forward with the establishment of a collaboration with Enrico Gratton's group in the Laboratory for Fluorescence Dynamics at the University of Illinois. The collaboration will build a fluorescence lifetime detector and a fluorescence correlation spectroscopy detector for the instrument.

We have established the Digital Image Lab with Bitplane image processing software running on an SGI Octane 2 and a PC workstation. The facility's darkroom is now functioning. The Nikon Eclipse 800 widefield fluorescence microscope is up and running and has been upgraded with dual Sutter 10-position filter wheels for analysis of a full range of fluorophores, FRET, and fluorescence time lapse imaging.

I have collaborated with several Danforth Center scientists, mainly doing widefield and confocal microscopy analysis of fluorescent proteins in plant cells. With scientists from Roger Beachy's lab, I have studied TMV development in tobacco BY2 cells using various recombinant forms of the virus. We have also investigated the role of actin filaments in virus infection. In work with Erik Nielsen, I have analyzed the structure and dynamics of compartments associated with various plant Rab GTPases in tobacco BY2 cells and in *Arabidopsis thaliana.*

This three-dimensional reconstruction shows the distribution of three TMV virus proteins within a single plant cell, imaged 16 hours after infection with the virus. The green-colored bodies are comprised of TMV movement protein, which is membrane bound (in part of the ER) and associated with another virus protein, TMV coat protein, shown in blue. The enzyme responsible for TMV replication (virus replicase) is shown in red. The 3-D animation made possible by this method of microscopy is essential for analyzing the co-distribution of the three virus proteins, which provides clues on how the virus completes its infection cycle in the plant cell. (virus proteins immunolabeled, marker = 5 µm). Go to the web site www.danforthcenter.org/imf to see the image in animation.

This image is the work of Dr. Sebastian Asurmendi and Dr. R. Howard Berg.



The Mass Spectrometry and Bioseparations Facility will provide high quality biomolecular separations and mass spectral resources for Danforth Center researchers and collaborators.

Julia Gross, Ph.D., Co-manager, Mass Spectrometry and Bioseparations Facility

The Mass Spectrometry and Bioseparations Facility will provide high quality biomolecular separations and mass spectral resources for Danforth Center researchers and collaborators.

The research-grade MALDI (Matrix-Assisted Laser Desorption/Ionization) instrument (Applied Biosystems Voyager-DE STR) is well suited for the analysis of large biomolecules such as proteins, DNA, and carbohydrates. The high resolving power and mass accuracy can be used to identify and/or characterize unknown analytes. Protein identification, profiling, characterization of posttranslational modifications, and partial sequencing are a few of the many applications being conducted at this time. High throughput MALDI analysis is possible using the automatic sample spotter (SymBiot, Applied Biosystems). This device speeds consistent sample preparation for subsequent analysis.

The Electrospray-Quadrupole-Time of Flight (Q-TOF) instrument can analyze large biomolecules as well as small molecules/metabolites using the superior resolving power and extremely high sensitivity of this instrument. In addition, the Q-TOF can perform exact mass measurements for molecular formula determination.

This photo shows the MALDI-MS (Matrix-Assisted Laser Desorption/Ionization-Mass Spectrometer). On the television screen is a picture of the analyzed sample preparation; the analyte is co-crystallized with a small organic compound, the matrix. The acquired mass spectrum can be seen on the computer monitor.

Coupling it with the liquid chromatography unit, we can perform on-line separations. The Q-TOF demonstrates enhanced capability for de-novo sequencing and posttranslational modification analysis.

A GC-MS (Gas Chromatograph–Mass Spectrometer, GCQ from ThermoFinnigan) is the instrument of choice for the analysis of small molecules, drug metabolites, and volatiles, especially those that do not ionize well by electrospray. The facility will continue to expand throughout 2002 to better serve the scientific community. Visit our homepage (www.danforthcenter.org/msb) to follow our progress and learn about our capabilities.

The Plant Cell Culture and Transformation Facility provides a common area where a wide range of plant transformation and culture systems can be done efficiently.

Nancy Mathis, Manager, Plant Cell Culture and Transformation Facility

The Plant Cell Culture and Transformation Facility provides a common area where a wide range of plant transformation and culture systems can be done efficiently. The 1,100 square foot suite in the lower level of the Danforth Center consists of the transformation room, media prep area, and kitchen. The main transformation room contains eight tissue culture hoods with work area for 11 researchers, including necessary equipment such as centrifuges, incubators, shakers, and supplies. Three large state-of-the art walk-in tissue culture rooms within the lab add 416 square feet of floor space and 650 square feet of growing area and include both platform shakers and shelf space, making it convenient to access plant cultures. Plant growth rooms are located directly outside, adjacent to the tissue culture facility for the next step of growing plants before they go to the greenhouses.

A microscope room with a dissecting microscope, an inverted microscope plus monitor, and digital camera options for each is available close by. Special care has been put into providing the equipment and supplies needed to make the facility as useful as possible for researchers wishing to do their own tissue culture work. Gene delivery systems include two electroporators, a PDS/1000 Hepta Biolistics gun, and Agrobacterium-mediated transformation for use with protoplasts, calli, and whole plants. A hand-held helium gun and another biolistics gun are available for bombardment of whole plants. Plant species currently in use are *Arabidopsis,* cassava, rice, and tobacco with plans to add lettuce, *Medicago,* and soybean. Plant support functions such as plant production and maintenance of cultures for both inside researchers and outside contracts can be provided, as can training for visiting scientists and students.

Laboratory Staff

Dr. Beachy's Lab:
Sebastian Asurmendi, Ph.D., Postdoctoral Associate
Jennifer Bick, Student
Susan Blanke, Lab Technician II
Emily Brooks, Lab Attendant
Yajuan Cao, Ph.D., Postdoctoral Associate
Shunhong Dai, Ph.D., Postdoctoral Researcher
Masaaki Fujiki, Ph.D., Research Scientist
Tahzeeba Hossain, Ph.D., Research Associate
Shigeki Kawakami, Ph.D., Research Scientist
Ja Choon (Moses) Koo, Ph.D., Research Scientist
Bernadette Kurtz, Executive Assistant
Yi Liu, Ph.D., Postdoctoral Scientist
Kathleen Mackey, Administrative Assistant
Isabel Ordiz, Ph.D., Postdoctoral Fellow
Karel Schubert, Ph.D., Domain Member
Dilip Shah, Ph.D., Domain Member
Robert Spelbrink, Ph.D., Postdoctoral Associate
Terry Woodford-Thomas, Ph.D., Domain Associate Member
Zhihong Zhang, Ph.D., Postdoctoral Associate

Dr. Fauquet's Lab:
Adetunji Akano, Ph.D., Research Fellow
Javier Armisen, Graduate Student
Kwadwo Owusu Ayeh, Ph.D., Research Fellow
Patricia Cosgrove, Administrative Assistant
Bernadette Delannay, CBN-V Events Planner
Ben Fofana, Graduate Student
Bertrand Hankoua, Graduate Student
Nicole Kokora, Graduate Student
Karen Kraus, Summer Intern
Marunwa Makwarela, Ph.D., Visiting Scientist
Francis Ogbe, Ph.D., Research Scholar
Chellappan Padmanabhan, Graduate Student
Denise Peterson, Lab Technician
Justin Pita, Graduate Student
Vanitharani Ramachandran, Ph.D., Research Associate
Nigel Taylor, Ph.D., Domain Assistant Member
Jitender Yadav, Ph.D., Postdoctoral Fellow

Dr. Jaworski's Lab:
Lin Chen, Ph.D., Postdoctoral Associate
Jixiang Han, Ph.D., Postdoctoral Associate
Jia Li, Lab Technician
Janet Oriatti, Administrative Assistant

Dr. Nielsen's Lab:
Adamina (Adita) Akbani, Ph.D., Research Scientist
Christopher Happel, Lab Attendant
Michelle Liberton, Graduate Student
Jannie Serna, Lab Technician
Jessica Straatman, Lab Attendant

Dr. Schachtman's Lab:
Sung-Ju Ahn, Ph.D., Research Scientist
Cathy Kromer, Administrative Assistant
Ellen Marsh, Ph.D., Research Associate
Carolyn Neal, Lab Technician II

Dr. Skolnick's Lab:
Adrian Arakaki, Ph.D., Research Fellow
Marcos Betancourt, Ph.D., Domain Assistant Member
Eckart Bindewald, Ph.D., Postdoctoral Associate
Michal Boniecki, Ph.D. Visiting Postdoctoral Associate
Yury Bukhman, Ph.D., Postdoctoral Associate
Ting-Lan Chiu, Ph.D., Postdoctoral Associate
Turkan Haliloglu, Ph.D., Visiting Scientist
Julie Heger, Administrative Assistant
Daisuke Kihara, Ph.D., Senior Postdoctoral Associate
Piotr Klein, Graduate Student
Sebastian Kmiecik, Graduate Student
Andrzej Kolinski, Ph.D., Domain Professor
Wei Li, Ph.D., Postdoctoral Associate
Hui Lu, Ph.D., Postdoctoral Associate
Long Lu, Graduate Student
Piotr Pokarowski, Ph.D. Visiting Scientist
Weidong Tian, Ph.D., Graduate Student
Jorge Vinals, Ph.D., Visiting Scientist
Prince Xavier, Ph.D., Postdoctoral Associate
Yang Zhang, Ph.D., Postdoctoral Scientist

Dr. Smith's Lab:
Aron Allen, Lab Technician
Soojay Banerjee, Ph.D., Research Scientist
Umesh Katpally, Graduate Student
Joni Patton, Administrative Assistant
Steven Sarfaty, Lab Technician
Baoxian Wei, Ph.D., Research Associate

Dr. Taylor's Lab:
Byron Bertagnolli, Lab Technician
Ray Collier, Research Technician II
Lily Gavilano, Ph.D., Postdoctoral Assistant

Dr. Xia's Lab:
Charles Dietrich, Ph.D., Postdoctoral Associate
Jing Zhang, Ph.D., Research Associate

Dr. Yu's Lab:
Zhenhua (Gene) Guo, Ph.D., Postdoctoral Associate
Chris Menne, Ph.D., Research Assistant
Lyle Ralston, Ph.D., Postdoctoral Associate

"Within not too many years in the future, the work that you do here will touch every human being who lives on Earth..."

– Excerpted from an address by former U.S. President Jimmy Carter to Danforth Center staff on December 10, 2001.

975 North Warson Road | St. Louis, Missouri 63132 | WWW.DANFORTHCENTER.ORG | 314.587.1000