

e_Connections



FEATURE

TECHNOLOGY

IN THE NEWS

FEATURE



Dr. R. John Collier. Photo credit: VBI Public Relations.

Collier reveals how protein toxins break and enter cells

Professor R. John Collier, Maude and Lillian Presley Professor of Microbiology and Molecular Genetics at Harvard Medical School, gave a talk entitled “How bacterial toxins cross membranes: The anthrax toxin example” at a recent lecture at the Virginia Bioinformatics Institute Conference Center. The talk, which took place on May 21, described research to dissect the molecular mechanisms that toxins use to break into cells and exert their often-lethal courses of action.

Collier is a pioneer in elucidating the structure and mode of action of bacterial protein toxins. Early in his career he discovered that diphtheria toxin inactivated mammalian elongation factor-2 by an NAD-dependent mechanism, which was later identified as ADP-ribosylation. This was the first demonstration that a protein toxin can penetrate to the cytosol of mammalian cells and directly modify a target substrate there. Since joining Harvard in 1984, Collier has focused his attention on the question of how protein toxins cross cell membranes. His studies on diphtheria and anthrax toxins have revealed much about the molecular mechanisms by which these toxins transport their enzymatic components into cells.

Said Collier: “For me personally, it has been a 40-year journey looking at the ways that bacterial toxins work. In the case of anthrax, we have perhaps

the first toxin system with a reasonable model for protein translocation across the cell membrane into the cytoplasm.”

Anthrax toxin has three protein subunits – edema factor, protective antigen, and lethal factor. Each subunit alone has little or no effect, but in different combinations they reveal the damaging properties of anthrax toxin. Collier’s studies on the protective antigen component of anthrax toxin – the vehicle for entry of the edema and lethal factors into the cell – have shown that this protein forms pores in the endosomal membrane. Endosomes are small intracellular membrane-bound compartments formed by “pinching off” parts of the cell plasma membrane. The vesicles migrate into the cell interior in this case carrying stowaway anthrax toxin subunits. The pores that are formed on the endosomal membrane ultimately provide a pathway for translocation of the enzymatic lethal and edema factors into the cytoplasm.

Over the years, Collier’s group has developed sensitive electrophysiological techniques that allow scientists to investigate and measure pore formation. Said Collier: “Protective antigen, when bound to its cell receptor, forms a prepore structure that can bind up to three molecules of anthrax toxin subunits. When this complex is delivered to the endosome, low pH leads to the formation of a pore

that spans the endosome membrane. Translocation of edema and lethal factor to the cytosol ensues after unfolding of the proteins.”

Collier added: “We now have evidence from different experimental approaches that favors a Brownian ratchet model for entry of the toxin proteins via the pore. Translocation across the membrane is driven by the pH difference across the membrane. The pH gradient gives a directional bias to the random movement of the proteins due to thermal motion.”

Once in the cell, the toxin subunits refold into harmful enzymes that bring about the lethal effects of anthrax toxin. Said Collier: “We now have a plausible model for how anthrax toxin breaks into and enters the cell. We hope to solve the complete crystal structure of the protective antigen pore to obtain a more detailed understanding of how the pore functions in translocation. It is already clear that the pore does not serve simply as an open, water-filled channel, but rather as an active transporter.”

Collier has received many honors, including the Eli Lilly Award in Microbiology and Immunology (1972), the Paul Ehrlich Prize (1990), the Selman Waksman Award (1999), The Bristol-Myers Squibb Award in Infectious Disease Research (2003), and the Abbott-ASM Lifetime Achievement Award (2006). He was elected to the National Academy of Sciences in 1991. ■

In This Issue

Collier reveals how protein toxins break and enter cells	1
VBI hosts Oomycete Bioinformatics Resources Training Workshop	2
VBI in the News	3
High school students get hands-on with DNA	4

FEATURE

TECHNOLOGY

IN THE NEWS

FEATURE



Oomycete Bioinformatics Resources Training Workshop.
Photo credit: VBI Public Relations.

VBI hosts Oomycete Bioinformatics Resources Training Workshop

The Virginia Bioinformatics Institute (VBI) at Virginia Tech recently hosted the “Oomycete Bioinformatics Resources Training Workshop” as part of the 2009 VBI Summer Institute. Forty participants, ranging from undergraduate students to professors, travelled from 12 different states in the U.S. and several countries, including Columbia, Sweden, the Netherlands and the United Kingdom, to visit the Institute on June 3-5 and learn more about the wide variety of resources and tools available for the annotation and analysis of oomycete genomic sequences.

The event included presentations from VBI Professor Brett Tyler, VBI Plant Associate Microbe Gene Ontology (PAMGO) Coordinator Trudy Torto-Alalibo, and VBI Bioinformatics Team Lead Sucheta Tripathy. Tyler opened the workshop with a comprehensive overview of oomycete genomics, which included discussions of the oomycete genome sequences currently available, the challenges and benefits of new sequencing technologies and tools and databases for analysis, and the need for an undergraduate research focus in oomycete bioinformatics, as well as a common vision and voice to help mitigate the social, political, financial, and professional barriers facing the field of oomycete genomics. More specifically, Tyler used the miGenome Consortium as one example of how researchers can work together to overcome many of these challenges. The consortium is a community-

based initiative created to help catalyze the development of a customizable, common interface for accessing and analyzing the growing amounts of microbial genome sequence data with a goal of bringing users and developers together around a common vision and platform.

Additional lectures offered at the workshop highlighted other specific oomycete genomics and bioinformatics resources. Tyler also gave presentations detailing the resources and information available from the Phylogenetic Resources for the Interpretation of Genomes (PHRINGE) pipeline and the U.S. Department of Energy (DOE) Joint Genome Institute’s (JGI) genome portal. Tripathy presented information about the VBI Microbial Database, which contains genome sequence and annotation data for a range of plant pathogenic oomycetes, fungi, and bacteria. Tripathy also gave an overview of structural annotation, while Torto-Alalibo led a discussion of the Gene Ontology (GO) and functional annotation.

The lectures were followed by a two-day “mini-jamboree,” which consisted of a series of hands-on activities designed to expose participants to some of the available genomics and bioinformatics resources. For example, the participants’ first glimpse into the draft sequence of *Peronospora tabacina*, the blue mold tobacco pathogen, revealed a modest number of effectors, including homologs of the RXLR and Crinklers motifs, which were

originally discovered in *Phytophthora sojae* and *Phytophthora infestans*, respectively. There were also opportunities for in-depth comparisons of genes from the oomycete genome sequences currently available, which include *P. sojae* and *P. infestans*, as well as *Phytophthora ramorum*, *Hyaloperonospora arabidopsis*, and *Pythium ultimum*.

The event was sponsored by VBI and the National Science Foundation (NSF)-funded Molecular Genetics Research Collaboration Network (OMGN), which aims to facilitate the integration of investigators from a variety of backgrounds into the oomycete molecular genetics community, helping to provide a strong culture of collaboration and communication. According to Tyler, who serves as coordinator of the OMGN, “The goal of the workshop and the OMGN is to provide training opportunities for oomycete molecular genetics researchers, especially those from smaller institutions, in the use of bioinformatics and genomics resources. We want to encourage interest and participation in the field of oomycete genomics, as well as the sharing of new techniques and ideas to further strengthen the cooperative culture of this community.” ■

FEATURE

TECHNOLOGY

IN THE NEWS

FEATURE

Sobral named Virginia Tech outstanding advisor

VBI Professor Bruno Sobral was recognized as an Outstanding Advisor by the Virginia Tech Graduate School in March. Sobral served as co-advisor to Matt Dyer, graduate of the university's Genetics, Bioinformatics and Computational Biology (GBCB) Ph.D. program, along with T. M. Murali, assistant professor in Virginia Tech's Department of Computer Science. Dyer received the school's Outstanding Dissertation Award in Science, Mathematics and Engineering for his dissertation, "Pathosystems Biology: Computational Prediction and Analysis of Host-Pathogen Protein Interaction Networks."

Tyson elected to the Royal Academy of Belgium

John Tyson, VBI faculty fellow and University Distinguished Professor in Virginia Tech's Department of Biological Sciences, has been elected as an associate member of the Royal Academy of Belgium (Académie Royale de Belgique) in the Class of Sciences. The academy has a total membership of 300 members, divided into three sections: Sciences, Letters and Moral and Political Sciences, and Fine Arts. Each section has 50 members and 50 associate members. Tyson was one of six new associate members elected to the Class of Sciences.

Laubenbacher gives plenary address at international meeting

VBI Professor Reinhard Laubenbacher served as a plenary speaker for the 15th International Meeting on DNA Computing and Molecular Programming on June 8-11 at the University of Arkansas. In his talk, "Discrete models of gene regulation networks," Laubenbacher discussed various computational methods for the construction of Boolean models from experimental data, as well as the properties of these networks. The conference focuses on topics that merge mathematics, computation, biology, and nanotechnology and attracts top researchers in computer science, mathematics, chemistry, physics, molecular biology, and nanotechnology to foster interdisciplinary research in the molecular-scale manipulation of matter.

Supercomputer's model of human contact simulates swine flu – A group at Virginia Tech is working with the U.S. Department of Defense to tackle the H1N1 outbreak

BLACKSBURG—An extravagantly detailed computer model of the U.S. population is taking a crack at understanding the H1N1 "swine flu" outbreak. The model, built by researchers at Virginia Polytechnic Institute and State University, in Blacksburg, Va., is composed of realistic representations of the major ways that people in the United States come into contact with one another—in other words, real-world social networks. Last Thursday, the U.S. Department of Defense began using the model to provide recommendations to the Department of Health and Human Services, according to the Virginia Tech engineers.

In the model, called EpiSimdemics, real cities are represented as groups of artificial people whose demographic attributes match data from the last census and land-use databases. By seeding the model with a handful of infected individuals in a manner that mirrors the real cases—say, 45 teenagers in one part of New York City—the model can run hundreds of simulations to illustrate possible future infection patterns across a population of between 50 million and 60 million in nine regions, according to Madhav Marathe, a deputy director of Virginia Tech's Network Dynamics and Simulation Science Laboratory (NDSSL). In one experiment, for example, the model was asked to determine the impact of school closures on flu transmissions.

One question that the model may help answer in the upcoming months is whether to release an H1N1 vaccine, assuming one is developed while the flu is still active. "Suppose the current outbreak goes away in the summertime," says Stephen Eubank, a physicist on the project. "There are going to be a lot of questions about what to do with the vaccine if it's ready by the fall." The model can bring to light the full range of possible outcomes from a vaccine intervention, including changes in the flu's virulence and the number of people who might become infected under different scenarios.

Another use is to help understand the impact of antiviral medicines. The use of antivirals places a specific type of pressure on a virus, which could cause a more virulent strain to evolve. Public health officials may need to decide whether to try to aggressively snuff out the strain with antivirals. The alternative, if the H1N1 virus proves not to be particularly virulent, is to hope that the strain dies out on its own.

Source: IEEE Spectrum magazine, May 6, 2009.



FEATURE

TECHNOLOGY

IN THE NEWS

FEATURE



Students extract DNA from strawberries. Photo credit: VBI Public Relations.

High school students get hands-on with DNA

The usually quiet summer Virginia Tech campus got a burst of life on June 19, when 155 West Virginia high school students made a visit for a day full of activities, including a morning stop at the Virginia Bioinformatics Institute (VBI).

VBI's Education and Outreach group coordinated the visit with the Upward Bound Program from West Virginia State University, which is located near Charleston, W.Va. The program serves high school students from four counties in West Virginia, providing academic instruction in mathematics, laboratory sciences, composition, literature, and foreign languages. Tutoring, counseling, mentoring, cultural enrichment, and work-study programs are also provided in support of the program's overall goal of encouraging participants to pursue post-secondary education. The program has a solid success rate, with an average of 85% of its graduates attending a post-secondary educational

institution. The students visiting VBI represented two subgroups of Upward Bound – Upward Bound Classic and Upward Bound Math-Science.

The visit to VBI included a welcome from VBI Graduate and Post-Graduate Education Program Manager Kristy DiVittorio, who gave the students an overview of the institute and discussed what DNA is and why it is an important part of life and an integral component of the work at VBI. The students also received tours of the institute, which included highlights of many of VBI's ongoing research projects. To help the students develop a better understanding of DNA, the students got the opportunity to work in a VBI lab, extracting DNA from strawberries. Using a mixture of salt and detergent, the students isolated DNA from the strawberries. When the students layered rubbing alcohol over the strawberry extract, white strands of DNA began to form. Comments such as "awesome" and "cool"

were heard throughout the lab as the students watched the fibers of DNA form and collected them with a skewer in preparation for taking their strawberry DNA home with them in plastic bags.

"Our goal was to show the students that they could extract DNA using everyday household items such as salt and detergents," explained DiVittorio. "This activity gave us the opportunity to talk to the students about the link between DNA and our research at VBI and why the study of this genetic code helps us uncover important information about living things. We want to encourage kids' interest in science with the hope that they will consider a future career in a science-related field."

The Upward Bound Classic program gives students the opportunity to spend six weeks on a university campus in the summer. The students stay in residence halls and take classes in mathematics, science, literature, communication, computers, and drama. The program also provides students with year-round tutoring, academic instruction, and assistance with college admissions and financial information. The Upward Bound Math-Science program is designed to strengthen the math and science skills of participants. With the goals of helping students recognize and develop their potential to excel in math and science and to encourage the pursuit of postsecondary degrees in math and science, the program includes year-round counseling and advisement, computer training, interaction with university faculty members conducting research in mathematics and the sciences, and scientific research completed under the guidance of faculty members or graduate students.

Upward Bound is one of eight TRIO programs supported by the federal government. These initiatives are designed to support students from disadvantaged backgrounds in preparing for college entrance. The program provides opportunities for participants to succeed in their precollege performance and ultimately in their higher education pursuits. ■

VBI e_Connections is a quarterly publication of the Virginia Bioinformatics Institute produced by the Public Relations team. The newsletter includes feature articles, technology updates as well as interviews that may be of interest to VBI's audiences. Contributions are welcomed. Please direct submissions to newsletter-editor@vbi.vt.edu. Newsletter team: Susan Bland: Editor; Barry Whyte: Editor.

For further information, please contact: Barry Whyte at Tel: 540-231-1767, email: whyte@vbi.vt.edu. Website: www.vbi.vt.edu