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ARMENISE-HARVARD SYMPOSIUM 2005

STRUCTURAL, BIOCHEMICAL, AND CELLULAR ASPECTS OF MICROBIAL PATHOGENESIS

9th Annual Symposium
Jul 14-17, 2005, Grand Hotel Villa Tuscolana, Frascati, Italy

About the Symposium

More than 20 years after AIDS served notice that the era of infectious disease was not at an end, microbial pathogens still account for more human misery and death than any other cause. This is immensely frustrating for biomedical researchers who hoped that revolutions in molecular biology and genomics would lead to vaccines and treatments that could defeat HIV, malaria, and other major global scourges. Today, it appears that obtaining sharper, more dynamic pictures of the deadly tango danced by pathogens and host cells may be they key to forging these maddeningly elusive weapons against disease.

Detailing the intricate, intimate relations between pathogens and their hosts was the goal of the 9th Annual Symposium of the Giovanni Armenise-Harvard Foundation, entitled "Structural, Biochemical, and Cellular Aspects of Microbial Pathogenesis." Eighteen invited speakers and 14 poster presenters demonstrated how important insights into these complex interactions can be obtained with tools as disparate as x-ray crystallography, small molecule screening, and specially reared mice. The Symposium was organized by John Mekalanos, director of the Armenise-Harvard Center for Microbial Pathogenesis and the Host Response, and Steven Harrison, director of the Armenise-Harvard Center for Structural Biology, both at HMS.

In his elegant summary of the two and one-half day symposium, Harrison said that six major themes stood out. Nearly half the presentations explored how viruses or bacteria invade host cells, some as whole organisms and others by injecting secretions into the cell. The second most prevalent topic was immune modulation: the relatively new idea that microbes not only elicit an immune response but also shape development and alter functioning of the host's own immune system. Other presentations examined a related theme – how disease-causing organisms harness and modify structures in host cells and tissues. A fourth set of talks focused on "non-classical" modification of gene expression in pathogen and host. The remaining speakers addressed

translational research, describing how more precise structural and biochemical information can be used to advance development of new vaccines or drugs.

As the Symposium drew to a close, Harrison said participants could not help but feel that "the study of bacteria and viruses and other microbial pathogens is even more exciting, revealing and important than we had imagined." This year some 80 scientific participants traveled to Frascati, one of the five "Roman Castle" towns that cling to a volcanic ridge southeast of Rome. The Symposium took place at the Grand Hotel Villa Tuscolana, originally constructed in 1578 as a country home with a panoramic view toward the city. Although the vista is eternal, the villa itself has been expanded into a stately hotel surrounded by gardens constructed 100 years ago. The Symposium drew representatives from Harvard Medical School, one HMS-affiliated center, and one of the medical school's teaching hospitals. European Union participants came from three Italian universities, two pharmaceutical companies and the Pasteur Institute in France.

Also on hand were twelve Italian science journalists, current and previous winners of the Armenise-Harvard Foundation Italian Science Writer Fellowships awarded over the past six years. This novel initiative seeks to improve the flow of scientific news to Italian readers, viewers, and listeners by providing two reporters the opportunity to participate in the annual Symposium and come to HMS, where they interview dozens of experts. At a special half-day workshop in Frascati, speakers from the scientific sessions briefed the journalists on topics including state-of-the-art microscopy and translation of basic science into drugs and vaccines. This year's fellows are Paloa Mariano of the Italian press agency, ANSA, and Nicola Nosengo, who writes for the on-line science magazine Galileo. Both contribute often to other Italian publications as well. Over the years, the science writing fellows have generated scores of articles in the Italian press about Armenise-supported research.

In his closing remarks, Harrison announced awards made each year to promising young researchers at Harvard Medical School and Italians studying away from home. Career Development Awards enable young Italian scientists who have been training abroad to return home and open their own laboratories. Neuroscientist Alberto Bacci, this year's winner, is wrapping up his work as a staff research associate in the Department of Neurology and Neurological Sciences at Stanford University Medical Center in California. The award supports his move to the European Brain Research Institute (ESRI) in Rome, where he will continue his investigations of different inhibitory mechanisms operating in fast-spiking and low-threshold-spiking interneurons. These mechanisms are involved in the formation of complex networks that underlie various behaviors.

The Junior Faculty Grants recognize outstanding young researchers at HMS. Antoine Van Oijen calls his project "The Unwinding Mystery of the Eukaryotic Replicative Helicase: A Single-Molecule Study." He'll carry out this work in the Department of Biological Chemistry & Molecular Pharmacology on the quadrangle. The other grant went to Rachel Wilson of the Department of Neurobiology, whose research focuses on early events in the processing of taste information in Drosophila.

Invasion, Rupture and Inflammatory Destruction of the Intestinal Barrier by Shigella: Clues to Understand the Homeostasy of Intestinal Inflammation

Philippe J. Sansonetti

Unité de Pathogénie Microbienne Moléculaire,Institut Pasteur, Paris

psanson@pasteur.fr

Speaking at the Symposium's opening night dinner, Dr. Sansonetti sounded the opening notes of what would prove to be this year's major theme: new insights into how pathogenic organisms invade their hosts.

While some bacteria act only on the cell surface, Shigella flexneri tricks cells lining the human gut into engulfing it. Once inside, the bacterium hijacks some of the cell's own machinery to gain intracellular mobility and then to spread from cell to cell. It touches off a cascade of pro-inflammatory signals that ultimately results in the mucosal abcesses and ulcers characteristic of shigellosis.

Pathogenesis begins when Shigella makes contact with CD44 receptors on the cell surface. This activates a Type III secretory Mxi/Spa apparatus that has been assembled and poised for use. This bacterial syringe injects various "invasins" – such as Ipa proteins – into host epithelial cells. Dr. Sansonetti and his colleagues have identified a cascade of signals elicited by GTPases of the Rho family and by c-src that rearrange the cytoskeleton and cause the cell to internalize its attacker via macropinocytosis. A surface protein, IcsA, recruits N-WASP and Arp2/3 and sets about to assemble actin filaments that the bacterium uses to move about the cytoplasm. Infection activates pathways that open connexin-mediated hemi-channels and release ATP, which acts in a paracrine manner to cause calcium fluxes in adjacent cells and make them more permissive to invasion. Shigella spreads to neighboring cells via protrusions which are engulfed by a cadherin-dependent process.

Using in vitro and in vivo approaches, Dr. Sansonetti's team has shown how Shigella dupes infected epithelial cells into generating massive quantities of pro-inflammatory cytokines that destroy the lining of the gut. When proteins of the NOD/CARD family sense the presence of bacterial peptidoglycan, they activate the NF-KB and JNK pathways that trigger massive pro-inflammatory responses in infected cells. The researchers used the AFFYMETRIX system to identify a signature pattern of pro-inflammatory cytokine transcription dominated by massive expression of IL-8 mRNAs. The colonic epithelium sews the seeds of its own destruction, providing IL-8 that recruits massive numbers of polymorphonuclear leukocytes that destroy the lining of the gut as shigellosis progresses, Dr. Sansonetti reported.

More recently, his team has been studying plasmid encoded proteins, including Osps and IpaHs, that are made only when the bacterium's type III secretory system is activated. These appear to conspire in down-regulating pathways important for the host's innate immune response. Dr. Sansonetti is also working with collaborators in the U.S. and Bangaldesh to develop live attenuated and protein subunit vaccines to prevent Shigella infection.

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Presentations

Bacillus subtilis Spore Coat Components, their Assembly and Use for Surface Display of Heterologous Antigens

Ezio Ricca

Department of Structural and Functional Biology, University Federico II Napoli, Italy

ericca@unina.it

Beyond safety and efficacy, which are the essential requirements for any preventive vaccine, vaccinologists seek to create products that can be taken orally, shipped without refrigeration, and manufactured easily and inexpensively. A live-vector vaccine that uses Bacillus subtilis spores to deliver antigens could fulfill many of these requirements, according to Dr. Ricca.

B. subtilis spores are encased in a protein coat with a lamellar inner layer and a striated outer layer, and initially the researchers were more interested in how this coat is put together than in making a vaccine. Coat assembly is a complicated process involving the cot family of genes, whose expression is controlled by five transcriptional factors and also involves four morphogenetic proteins. Post-translational modifications including proteolytic processing, crosslinking and glycosylation are also needed for the coat to come together properly. The Ricca lab specializes in the regulatory role of CotH, a morphogenetic protein, in the assembly of two structural components of the outer coat, CotB and CotC.

In B. subtilis and other bacteria, Dr. Ricca's team and others have demonstrated that these two structural proteins can fuse heterologous antigens, present them on the spore surface, and elicit a protective response in mice. In one experiment, mice were given an oral dose of spores bearing the C fragment of the tenanus toxin (C. tetani) and challenged with injections of tetanus toxin 60 days later. All 8 animals survived a low-dose challenge and 7 of 8 were protected against a higher dose. None of the control animals survived.

B. subtilis spores have several potential advantages as vaccine delivery systems: i) they are impervious to heat; ii) they have proved extremely safe used as probiotics, mostly to help restore normal gut flora in antibiotic-treated patients; iii) industrial-scale production of commercial, spore-based products is well-established in Europe and in Japan. Encouraged by toxin-specific immune responses in the mouse model, Dr. Ricca is pursuing a more detailed understanding of CotB and CotC assembly in order to optimize display of heterologous molecules on the spore surface.

A Glimpse into the Molecular Basis for Symbiosis Between the Intestinal Microflora and the Mammalian Immune System.

Dennis L. Kasper

Departments of Medicine and Microbiology and Molecular Genetics, Harvard Medical School

<a href=""Dennis_kasper@hms.harvard.edu""
target=""_blank"">Dennis_kasper@hms.harvard.edu

An Equatorial rain forest looks as dull as a suburban lawn compared with the biodiversity of the human gut. In the gastrointestinal ecosystem of humans and other mammals, more than one thousand species live in homeostasis with their host, reaching a density of >1012 organisms per gram of colonic content. In this mutually beneficial relationship bacteria obtain a comfortable place to live and in exchange they aid normal digestion, resist colonization by harmful bacteria, and shape development of the neonatal gut and the host immune system. Although many publications attest to the symbiotic nature of this partnership, Dr. Kasper's team recently identified the first specific molecule that functions as a "symbiosis factor" linking bacteria and host.

IN 2004, Dr. Kasper and Sarkis Mazmanian isolated a bacterial carbohydrate that interacts with the host immune system in much the same way as a protein antigen. In Bacteroides fragilis they found a previously unknown antigen presentation pathway where a zwitterionic polysaccharide, called PSA, is processed and presented through the major histocompatibility complex class II pathway and then activates CD4+ T cells. In a more recent series of experiments, presented at the Symposium and published on the same day in Cell, Dr. Kasper and his colleagues have come to understand PSA's importance in development of normal Th-1 immune responses – cellular responses that must be acquired through experience – in mice.

When mice were raised in a germ-free environment where there there was no chance to acquire normal gut flora, the animals' CD4+ T cell levels were deficient and spleen and thymus tissues were abnormal. TH1 immune responses were far below normal and as a result the animals' immune responses were unbalanced: they overproduced antibodies and had inadequate cellular responses. In comparison, animals exposed to wild-type B. fragilis had a normal TH1/ TH2 balance and normal development of lymphoid organs. The researchers identified PSA as the key actor in this symbiotic relationship by exposing mice to a PSA knockout mutant of B. fragilis and measuring systemic T-cell deficiencies and immunologic abnormalities. Additional studies showed that PSA acts by inducing specific TH1 cytokines, such as interferon-gamma and IL-12.

The discovery that a specific bacterial molecule is essential for normal immune development lends new weight to what is known as the “hygiene hypothesis.” This theory holds that overly fastidious parents, who isolate their children and douse them with products such as anti-bacterial soap, may be inadvertently setting them up for asthma, allergies, and auto-immune disease.

Anthrax Toxins and Their Role in Anthrax Pathogenesis

Cesare Montecucco

Dipartimento di Scienze Biomediche and Istituto CNR Neuroscienze, Università di Padova

Cesare.montecucco@uinpd.it

Continuing the theme of immune modulation introduced by Dr. Kasper, Dr. Montecucco described how a different invader alters the immune function of its host. For many years he has investigated pathogenic mechanisms of toxins, including those produced by anthrax, and his work on Bacillis anthracis accelerated after the bioterrorism attacks in late 2001. Now his lab has identified candidate compounds that may be able to improve prophylaxis in people exposed to anthrax spores.

Dr. Montecucco and his colleagues have a long-standing interest in two anthrax toxin subunits, edema factor (EF) and lethal factor (LF), that advance pathogenesis by undermining the ability of host cells to summon a protective T-cell response. EF is a calmodulin-activated adenylate cyclase that releases a flood of cyclic AMP and causes global disruption of cell signaling. LF is a zinc-dependent protease that acts more specifically by cleaving specific mitogen-activated protein kinase kinases (MAPKKs).

These researchers have found evidence that EF and LF disrupt functioning in macrophages and dendritic cells before symptoms are obvious, as a consequence inhibiting activation and proliferation of T lymphocytes and impairing the adaptive immune response. A series of experiments with fluorescently labeled substrates have made it possible to visualize the interactions of EF and LF with host cells over time, and to screen for small molecules that might interfere with their respective activities.

High-throughput screening showed that adefovir dipovoxil (bis-POM PMEA), an experimental agent already ruled out as a useful treatment for HIV but still being tested against Hepatitis-B, can interfere with EF's ability to flood the cell with cAMP. Assays developed in the Montecucco lab also made it possible to identify two compounds that inhibit reactions catalyzed by LF. One of these is (1)epigallocatechin-3-gallate (EGCG), a compound that inhibits MAPKK cleavage and – in some animal models – delays but does not prevent symptomatic disease. More research is needed to demonstrate that these candidate drugs are clinically useful in humans, but Dr. Montecucco says they may be useful adjuncts to antibiotic prophylaxis.

CagA of Helicobacter pylori induces ETM in MDCK Monolayers

Antonello Covacci

IRIS, Chiron Vaccines, Siena

antonello_covacci@chiron.com

Gastroenterology researchers have established that patients infected with strains of Helicobacter pylori carrying cytokine-associated gene A (cagA) are more likely to develop peptic ulcers and

gastric cancers than those infected with less virulent, non-cagA bacteria. Serotyping for cagA can also help stratify ulcer patients for treatment: clinical studies indicate that those who test positive for cagA are more likely to benefit from antibiotic therapy that those colonized by other strains.

Looking at an electron microscope image of the gut epithelium, Dr. Covacci noticed H. pylori in intercellular spaces and wondered what effects a cagA-positive strain would have on a monolayer of MDCK cells, a common epithelial cell line. The cag pathogenicity island encodes specialized Type IV secretion machinery that delivers virulence factors into host cells when it is activated during infection. After translocation, the effector protein CagA is phosphorylated on tyrosine residues in a specific sequence located in the C-terminal half of the protein. In the MDCK monolayer, tyrosine phosphorylation of cagA led to dramatic changes in cell morphology. Interaction of translocated CagA with host cell proteins, specifically ZO-1 and Jam, kept normal apical junctional complexes from forming. Transfected cells changed shape, lost normal cell-to-cell contacts and began to look like fibroblasts. Dramatic confocal microscopy images show them escaping the monolayer, penetrating the collagen gel and piling up in disheveled heaps.

Additional studies revealed that cagA has two distinct functional domains and that both are needed to induce abnormal cell differentiation, Dr. Covacci said. The kinds of changes that cagA brings about in epithelial cells -- altering polarity, turning order into chaos and inducing cells to migrate – are typical of both early and late stages of tumor formation. The mechanisms identified in the Covacci lab strengthen the link between cagA and elevated risks for gastric cancer and peptic ulcer disease.

A Phenylalanine Clamp Catalyzes Protein Translocation through the Anthrax Toxin Pore

R. John Collier

Department of Microbiology and Molecular Genetics, Harvard Medical School

jcollier@hms.harvard.edu

An earlier presentation by Dr. Montecucco described what happens in host cells after they've been invaded by two subunits of anthrax toxin, lethal factor (LF) and edema factor (EF). Dr. Collier's talk shifted the spotlight to an earlier part of this story, when anthrax toxins first enter host cells. The key player at this stage is a third Bacillus anthracis toxin, the so-called protective antigen (PA) component, which his lab has studied for many years.

In order to cause disease, a bacterium must translocate its proteins, which are both hydrophobic and hydrophilic in nature, across the cell's hydrophobic lipid bilayer. Anthrax, like several other bacterial pathogens, solves this problem by releasing multiple copies of proteins that assemble to form a homoheptameric pore in the endosomal membrane. This creates a narrow passageway that allows LF and EF, the enzymatic components of the toxin, to enter the cytosol and sabotage its normal function.

By using a "planar lipid bilayer" system that allows them to study PA's activities in a controlled fashion, Dr. Collier's team has been able to observe distinct stages in pore formation. PA first sets up a heptameric "pre-pore," which is converted into a pore as the seven phenylalanine-427 residues converge within the lumen, generating a radially-apposed heptad of solvent-exposed aromatic rings. This phenylalanine or "🚘-clamp" structure is required for protein translocation and Dr. Collier says it serves a chaperone-like function, interacting with hydrophobic sequences presented by the protein substrate as it unfolds during translocation.

The Collier lab has identified several point mutations that disable the 🚘clamp and block protein translocation and pore formation, and they regard this clamp as a potential target for drugs aimed at blocking anthrax infection.

Repression of Flagellar Motility Gene Expression by MogR is Required for Virulence of Listeria monocytogenes

Darren E. Higgins

Department of Microbiology and Molecular Genetics, Harvard Medical School

dhiggins@hms.harvard.edu

Out on the street, a kid with a skateboard probably won't get into trouble unless he bangs into someone. But if he takes that skateboard into the local shopping mall, the odds are that security guards will immediately throw him out. Inside cells, bacterial flagella are as provocative as a skateboard in a mall.

Listeria monocytogenes is a food-borne pathogen that is highly dangerous to newborns, elderly people, and others with less than robust immunity. Dr. Higgins studies how it gets around both outside and inside the cell, and he has recently described a switch that – in effect – allows the bacterium to stash its skateboard at the mall entrance.

In the extracellular environment, L. monocytogenes relies on flagella to propel itself from place to place, movement that is critical for chemotaxis and biofilm formation. The expression of flagellar motility genes varies with temperature, and earlier work in the Higgins lab demonstrated that MogR, a DNA-binding transcription factor, down-regulates expression of dozens of flagellar motility genes when the temperature exceeds 37˚C. MogR's regulatory importance is underlined by the discovery that when it is deleted, expression of flagellar motility genes soars regardless of temperature.

Once listeria invades host cells, however, the organism ceases using flagella and switches to actin-based motility by co-opting actin from the host cell. At this point, Dr. Higgins' work shows that MogR blocks expression of flaA (one of the genes that encode flagellin) regardless of temperature. This prevents cellular defense mechanisms from pouncing on this foreign protein. When the researchers deleted MogR, the resulting strain was 250 times less virulent than the wild type bacterium. Virulence was lost because flagellar motility genes were overexpressed, and the

resulting proteins were easily identified and dispatched by the intracellular equivalent of mall security guards. Support for this idea comes from additional studies that reveal more details about how MogR exercises control over flagellar motility genes.

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The X-Ray Structure of a Protein-Conducting Channel

William M. Clemons

Department of Cell Biology, Harvard Medical School

wclemons@hms.harvard.edu

In a January 2004 issue of Nature, Dr. Clemons published what some of his peers have called “no less than a breakthrough finding.” This was the first high-resolution study of Sec61/Sec Y, a highly conserved complex that forms a protein conducting channel in the inner membrane of bacterial cells and the endoplasmic reticulum membrane of eukaryotes. This channel allows newly made proteins to be released and shipped to the appropriate destination, and among other things illustrates how bacterial toxins are secreted.

Originally, Dr. Clemons and his colleagues determined the crystal structure of the complex from Methanococcus jannaschii at 3.2 Å resolution. This structure suggested that one copy of the heterotrimer serves as a functional translocation channel. The alpha-subunit has two linked halves, trans-membrane segments (TM) 1-5 and 6-10, clamped together by the gamma-subunit. A cytoplasmic funnel leading into the channel is plugged by a short helix and the plug probably swings aside when a protein is transported. The protein slithers through an “hourglass” shaped channel with a ring of hydrophobic residues at its narrowest point. This ring may form the seal around the translocating polypeptide, keeping other molecules from permeating the membrane.

More recently, Dr. Clemons has probed various positions in the structure with a single cysteine residue to arrive at a more detailed understanding of how the translocation pore works. By examining a series of binding events, the researchers have concluded that translocating polypeptides pass through the center of the SecY complex, making only the slightest contact with the channel, thereby minimizing friction and energy expenditure required to move the protein across the membrane. The structure also suggests mechanisms for signal sequence recognition and one of the team's most provocative findings is an apparent "side door" that may allow proteins destined for the membrane to exit laterally into the lipid bilayer. These observations are being pursued using biochemical means, three dimensional X-ray crystallography and cryo-electron microscopy.

Structures of the Poliovirus 135S Particle and a Poliovirus-Receptor Liposome Complex

James M. Hogle

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School

jhogle@hms.harvard.edu

Alfred Steiglitz spent years circling Georgia O'Keefe with a camera, producing conventional nudes that over the years evolved into uncommonly revealing close-ups. An unforgettable image of O'Keefe's strong stained hands reflects changes in the model and in the photographer's understanding of her. In much the same way, Dr. Hogle has spent the past 20 years capturing higher and higher resolution structures of poliovirus in action. He describes this work as "peeling back the layers" to understand how this small, non-enveloped pathogen breaches the cell's defenses and delivers its genetic payload to a place where it can replicate.

This quest sheds light on poorly understood mechanisms that poliovirus and its non-enveloped relatives use to invade host cells without benefit of a lipid coat, which enveloped viruses simply fuse with the host cell membrane. Over the years, Dr. Hogle has employed biochemical, microscopic, and structural tools to arrive at increasingly detailed pictures of poliovirus entering host cells. For example, electron-cryomicroscopy has enabled his team to advance from a ~22Å-view of the virus-receptor complex to structures with a resolution of ~10Å.

Dr. Hogle and his colleagues have used these higher resolution pictures to propose working models of what happens after the 135S particle of the virus anchors to its receptor, known as Pvr or CD155, on the cell surface. For a long time they couldn't fathom how the virus released its RNA inside the cell, but higher resolution images allowed them to zoom in on a canyon on the cell surface where RNA is released. They were able to define the site at the deepest part of this trench where the N-terminus of VP1 exits the virion, and to recognize that the portion of VP1 that binds here is not the one they proposed in earlier models of RNA release.

In a very exciting development, Dr. Hogle's group recently solved the structure of a virus-receptor-liposome complex at ~30Å resolution. A soluble construct of poliovirus receptor (Pvr) was tethered to Ni-NTA containing liposomes via a C-terminal (membrane proximal) His tag. Receptor-decorated liposomes were then able to capture virus and form a stable and homogeneous sample for cryo-electron microscopy. The resulting reconstruction provides the first experimental evidence that receptor binding brings a fivefold axis of the virus into close proximity to the membrane. They have also identified a prominent crown-shaped density feature on the outer leaflet of the bilayer at the binding site, and future experiments seek to learn what role perturbation of the bilayer may play as the virus penetrates the host cell.

Structure of HSV-1 gD in the pre-receptor binding conformation reveals a mechanism for receptor-mediated initiation of virus entry

Andrea Carfí

IRBM P.Angeletti and Merck Research Laboratories, Rome

andrea_carfi@merck.com

Herpes Simplex Virus (HSV) is a common and clinically important pathogen, with 80% of adults testing seropositive for HSV1, which causes cold sores, and 20% testing positive for HSV2, the type responsible for recurrent genital lesions. As an enveloped virus, HSV enters host cells by fusing with the cell membrane. The first step toward fusion is binding between the viral envelope glycoprotein D (gD) and two cell surface receptors, nectin-1 (an Ig-like receptor) and HVEM (a TNFR-type structure).

Dr. Carfi's team uses x-ray crystallography and biochemical approaches to explore how HSV penetrates cells, capturing structural images of various portions of gD at different stages of recognition and fusion. Structures and biochemical studies of a C-terminally truncated form of gD, alone and in complex with the HVEM receptor, revealed that fifty C-terminal residues of the gD ectodomain are not involved in nectin-1 binding. Yet the same residues are essential for HSV cell entry.

More recently, the researchers have determined the structure of a disulfide-linked gD dimer that includes the gD C-terminal region. This structure revealed that the C-terminus is anchored near the N-terminus and folded in a way that masks residues needed to bind HVEM. To find out what would happen if gD was locked in this position, they generated a point mutation that kept the C- and N-termini from interacting normally. They also inserted a disulfide bond that locked the C-terminus in the position observed in the crystals. This "locked" gD molecule was recognized by conformation-dependent antibodies, including AP7, whose epitope is dependent on the conformation of both C- and N-termini.

Yet the immobilized version of gD could not bind fully to its receptors or enter the cell, indicating that controlled displacement of the gD C-terminus upon receptor binding is an essential feature of HSV entry, Dr. Carfi said. Unless this portion of gD is mobile, the virus cannot fuse with the membrane and infect the host cell.

Structural Rearrangements of a Non-Enveloped Virus Membrane Penetration Protein

Philip R. Dormitzer

Department of Pediatrics, Harvard Medical School, and the Laboratory of Molecular Medicine, Children's Hospital, Boston

dormitzer@crystal.harvard.edu

Rotavirus is the most important cause of dehydrating childhood gastroenteritis worldwide, killing approximately 500,000 children each year. A first-generation preventive vaccine was withdrawn from market after it caused complications and several deaths. Two live- attenuated rotavirus vaccines are nearing approval, but there is still room for a safe and effective protein-subunit product. Dr. Dormitzer believes that knowing more about how this non-enveloped, double-stranded RNA virus enters cells could be the key to optimizing antigens for use in a such a vaccine.

When rotavirus infects a cell, it goes through a series of conformational changes needed to translocate a 710 angstrom particle across the cell membrane. These shapes elicit various antibody responses, and Dr. Dormitzer's ultimate goal is to design a better vaccine based on discoveries about these structures and the antibodies they can elicit.

A key player in penetration of the host cell membrane is the rotavirus spike protein, VP4. Dr. Dormitzer and his colleagues noticed that rotavirus, like some enveloped viruses, is primed to enter the cell when a protease cleaves VP4 into two parts, VP8* and VP5*. High-resolution x-ray crystal structures revealed that these closely resemble fusion proteins used by enveloped viruses, an

observation that led Dr. Dormitzer to ask whether VP4 might also undergo a "jack-knifing" rearrangement to penetrate the membrane – a common feature of fusion proteins.

When the researchers combined crystallography data with electron cryomicroscopy image reconstructions of the virus, they could see that VP4 was flexible until trypsin priming; immediately afterwards, two of the three VP4 molecules in each cluster join together to form a rigid spike tipped with a receptor-binding domain. Next, an unknown trigger causes all three molecules to stick together and form an umbrella-shaped trimer. This reorganization is accompanied by a jack-knifing conformational change that flips a hydrophobic region from one end of the protein to the other. This mimics the sort of move that enveloped viruses use to penetrate cell membranes, but at present Dr. Dormitzer admits that his lab knows more about rotavirus structures than about their functions. His research is now focused on functional studies, with an eye toward developing immunogens that will elicit a protective response against rotavirus.

Exploring the Pectin Network to Identify Determinants of Plant Resistance to Pathogens

Giulia De Lorenzo

Dip. Biologia Vegetale, Università La Sapienza, Roma

giulia.delorenzo@uniroma1.it

Two decades of studying microbial invasion from the plant's point of view, have given Dr. De Lorenzo unusual insights into similarities between plant and animal defense mechanisms. For example, a host organism must recognize an attacker as dangerous in order to begin defending itself. In Drosophila the key recognition molecule is the Toll receptor; in humans and other

mammals the alarm is sounded when Toll-like receptors detect signature proteins such as flagellin, which is a sure sign that bacteria are attacking the cell.

In plants, leucine-rich repeat proteins serve the same purpose as Toll receptors, and Dr. De Lorenzo's team was the first to determine the structure for such LLR domains. Once the attacking microbe is recognized, events in the cell wall shape the outcome of the battle between invader and host. Pathogens use polygalacturonases (PGs) to cleave pectins and breach the cell wall, while plants fight back by releasing proteinaceous inhibitors of PG activity, or PGIPs. These substances activate homogalacturonan (HGA) of long-chain oligogalacturonides (OGs) that induce additional defense responses. Recent genomic analysis in the De Lorenzo lab reveals that the expression profile of Arabidopsis responding to OGs overlaps extensively with the expression profile of plants attacked by bacterial flagellin or inoculated with the fungal pathogen Botrytis cinerea. Many of the induced genes are activated independently of classic self-defense signalling pathways mediated by salicylic acid, ethylene or jasmonic acid.

Additional experiments indicate that Arabidopsis plants pre-treated with OGs can successfully fight off B. cinerea infection. Dr. De Lorenzo and her colleagues have also boosted disease resistance in Arabidopsis and tobacco plants by introducing a gene of Aspergillus niger encoding a PG (AnPGII). Arabidopsis and tobacco plants expressing AnPGII generate high levels of hydrogen peroxide and apoplastic peroxidase, widely used weapons against pathogens of all sorts, and are less susceptible to infection by B. cinerea or Pseudomonas syringae, a bacterial pathogen. When Botrytis confront the modified cell wall in these transgenic plants, it appears to throttle back production of PGs that would ordinarily carve a path through the cell wall.

Growth Control of the Arabidopsis Root Meristem by Cytokinin

Sabrina Sabatini

Department of Genetics and Molecular Biology and Laboratories of Functional Genomics and Proteomics of Model Organisms ""La Sapienza"" University, Roma

sabrina.sabatini@uniroma1.it

In plants, as in animals, embryonic development and maturation of the adult are under tight genetic control. In plants, the hormones auxin and cytokinin have long been recognized as essential signaling molecules. They are thought to act synergistically or antagonistically to control fundamental developmental processes, such as cell division and cell differentiation, which ultimately lead to shoot and root organogenesis. Although molecular, genetic and biochemical studies have shed considerable light on auxin's role in controlling cell specification, cell division and cell polarity, cytokinin has been difficult to study and so its role is less clear. Dr. Sabatini realized that several recent discoveries – of a cytokinin receptor, transcription factor, and a signal transduction circuit – provided tools that she could use to clarify the role of cytokinin at the cellular and molecular level and to understand how this hormone interacts with auxin during plant development.

As an experimental system, her team focused on the root meristem of Arabidopsis thaliana. Its tissue is simple and organized in a well-defined way, which enabled the researchers to characterize developmental alterations following cytokinin manipulations – including bathing embryonic plants in exogenous cytokinin or knocking out the cytoninin gene's activity. In addition, they were able to type cells anatomically and by using a panel of cell- and tissue-specific markers. Phenotypic analysis of the root meristem, following mutation of various genes involved in the cytokinin-mediated transduction pathway, indicate that the hormone plays a role in balancing cell division and cell differentiation, Dr. Sabatini reported. The team substantiated these findings using a UAS/GAL4 trans-activation system to alter endogenous cytokinin levels in specific tissues.

Reflecting on Dr. Sabatini's presentation at the end of the symposium, co-organizer Steve Harrison said, "We've known for some time that what is true of E. coli is also true of elephants, and it's now clear that what is true of Arabidopsis is also true of elephants."

The post-transcriptional gene silencing machinery functions independently of DNA methylation to repress a LINE1-like retrotransposon in Neurospora crassa.

Carlo Cogoni

Dipartimento di Biotecnologie Cellulari ed Ematologia, Sezione di Genetica Molecolare,

Universita' di Roma La Sapienza, Roma

In the first of two talks about non-classical modification of gene expression, Dr. Cogoni described novel mechanisms for gene silencing that his team has identified in Neurospora crassa, a filamentous fungus that has been a mainstay in biology laboratories since the 1940s. When cells are menaced by rogue nucleic acids, such as transposons and viruses, defensive strategies for protecting genomic integrity include post-transcriptional gene silencing (PTGS) involving small interfering RNA (siRNA)-directed degradation of RNA transcripts and transcriptional silencing via DNA methylation. In addition, recent evidence from experiments with plants indicates that many transposons are silenced by a combination of the two mechanisms, and that siRNAs can direct methylation of Lys9 of histone H3 (Lys9H3) and DNA methylation of transposon sequences.

Dr. Cogoni and his colleagues investigated gene silencing mechanisms used by Neurospora to protect its genome during various life stages, with a particular focus on the contributions of DNA methylation and the PTGS pathway to transposon control. As the fungus prepares to shift from vegetative growth to reproduction, the researchers found that repression of the LINE1-like transposon, Tad, requires two proteins, QDE2 and Dicer. Both are needed for transgene-induced PTGS, which is also called "quelling" in N.crassa. In contrast, the RNA-dependent RNA polymerase QDE1 and the RecQ DNA helicase QDE3 – involved in silencing double-stranded RNA typical of viruses – played no apparent role in Tad control.

In contrast, Tad elements were not significantly methylated and the DIM2 DNA methyltransferase, responsible for all known DNA methylation in Neurospora, was irrelevant for Tad control. Thus, an RNAi-related transposon silencing mechanism operates during the vegetative phase of N.crassa that is independent of DNA methylation, indicating that specialized silencing pathways exist for

different kinds of repetitive elements. This highlights a major difference between this organism and other methylation-proficient species, Dr. Cogoni noted.

Herpes Simplex Virus versus the Host Immune Response

David M. Knipe

Harvard Medical School, Boston MA

david_knipe@hms.harvard.edu

As anyone who suffers from cold sores or genital herpes knows all too well, Herpes simplex virus keeps a low profile as a latent infection of sensory neurons then suddenly breaks out to cause unpleasant lesions. Like border patrols and illegal immigrants, HSV and the human immune systems have come to know each other well over the years, and each has evolved strategies for outwitting the other. This is the cat-and-mouse game that Dr. Knipe studies, and he and his team have identified numerous mechanisms that the virus uses to evade detection and the host immune system uses to fight back. The researchers' long-term goal is developing vaccines that could give the immune system a decisive win over the virus.

The virus's strategies include non-classical modifications of gene expression. For example, Dr. Knipe's group discovered that during latency, HSV makes itself a less obvious target by using latency-associated transcript (LAT), which promotes the assembly of heterochromatin on viral lytic promoters and down-regulates expression of these genes. This demonstrates that HSV has evolved ways to use histone modification mechanisms to regulate its own genes.

His lab has also shown that HSV activates Toll-like receptor 2 in host cells, resulting in chemokine and cytokine expression. Having set off this alarm the virus has at least two strategies for muffling its sound. In the nucleus of infected cells, the HSV immediate-early protein, ICP0, modulates TLR2 signaling – most likely by sequestering transcription factors from the cytokine genes. In addition, the researchers have recently found that while one HSV glycoprotein activates TLR2 another inhibits its response. This further illustrates how host and pathogen have evolved together.

The Knipe lab also confirmed an earlier report that HSV and HSV DNA activate TLR9, then went a step further and showed that TLR9 is essential for CD8+ T cell responses to HSV. Therefore, HSV activates both TLR2, which induces chemokine and cytokine expression, and TLR9 signaling, which induces type I interferon responses. Armed with this information, Dr. Knipe is developing replication-defective HSV strains as possible vaccines against genital herpes and for use as vectors for delivering immunogens for HIV/AIDS and other pathogens.

The Interactions Between the Subunits of Herpesvirus DNA Polymerases: Novel Targets for Drug Discovery

Arianna Loregian

Department of Histology, Microbiology and Medical Biotechnologies, University of Padova

arianna.loregian@unipd.it

While Dr. Knipe's group homes in on HSV, Dr. Loregian is using structure-based screening to search for drugs against not only HSV but also other members of the herpesvirus family. Better treatments are needed for many types of herpesvirus infections, because the usefulness of existing agents –

most of which target the polymerization activity of the viral DNA polymerases – is limited by pharmacokinetic issues, toxicity, or antiviral resistance.

Dr. Loregian's aim is to create more specific, less toxic drugs that interfere with protein-protein interactions among subunits of DNA polymerases. Interactions between specific viral proteins make attractive targets for drug discovery because they are essential to the pathogen, highly specific, and rarely affected by mutations that would render a drug ineffective.

Her group is using structure-based screening to identify peptides and small molecules which can inhibit specific interactions among subunits of DNA polymerases of Herpes simplex virus type 1 (HSV-1) and human cytomegalovirus (HCMV). This is a long-term effort that involves characterizing protein-protein interactions between enzyme subunits and solving the crystal structure of the accessory subunit of HSV-1 and of HCMV DNA polymerase bound to a peptide derived from the cognate catalytic subunit, as well as screening libraries of potential inhibitors. So far, the researchers have identified roughly 10 small molecules that inhibit HSV replication and five that interfere with HCMV replication, Dr. Loregian reported. More research will be needed to determine whether these compounds can be developed into safe and effective drugs.

Bacterial Cell Wall Synthesis: Assembly of Peptidoglycan Shell

Suzanne Walker

Department of Chemistry and Chemical Biology, Harvard University

<a href=""suzanne_walker@hms.harvard.edu""
target=""_blank"">suzanne_walker@hms.harvard.edu

Bacteria are encased in a layer of crosslinked carbohydrate polymer, called peptidoglycan, which prevents the cell wall from tearing when osmotic pressure changes inside the cell. This indispensable polymer is produced by one of the most highly conserved metabolic pathways in the bacterial world. This makes an attractive target for drug developers because there is no such pathway in humans. One drug known to interfere with peptidoglycan synthesis is ramoplanin, and Dr. Walker investigates its mechanisms of action and uses it as a tool for exploring the synthesis pathway itself. She is especially interested in determining how building blocks made inside the cell are assembled into a 3-dimensional structure on its surface.

As many as 10 extracellular enzymes act in concert to assemble the peptidoglycan shell, and they are difficult to study because their substrates are often present only in minute quantities and can be hard to isolate. Ramoplanin is thought to interfere with the final intracellular step in biosynthesis, when the enzyme MurG transforms a substrate, Lipid I, into Lipid II. Dr. Walker's team synthesized these substrates and created fluorescent chemical probes that enabled them to monitor peptidoglycan biosynthesis in live bacterial cells.

Ramoplanin inhibits Lipid II with "pretty high affinity," Dr. Walker said, adding that it appears to recognize disulfide bonds and other specific features found only in a specific region of Lipid II. The antibiotic had the same impact on peptidoglycan synthesis in B. subtilis as in human cytomegalovirus, and future experiments will use fluorescent ramoplanins to gain additional insights into this very important pathway.

Genomics, Novel Virulence Factors, and Vaccines

Rino Rappuoli

Chiron Vaccines, Siena, Italy

rino_rappuoli@chiron.it

“My job is to make vaccines,” Dr. Rappuoli said as he began his presentation on the genomic revolution in vaccine research and development. In the past, immunogens were made by killing pathogens, attenuating them, or conjugating their proteins – none of which required knowing anything about the organism’s sequence data. Today, however, no one would think of designing a vaccine without a genome in hand. But is sequence data for one strain of bacteria or virus enough? Dr. Rappuoli thinks not.

In collaboration with researchers from HMS and The Institute for Genomic Research, Dr. Rappuoli and his colleagues at Chiron sequenced eight strains of Streptococcus agalactiae (commonly known as group B streptococcus or “strep B”), among them representatives of the five major disease-causing serotypes. They found that all isolates shared a core genome that accounted for about 80% of the sequence. The remaining 20% of the genes were absent at least in one of the strains and each genome contained between 13 and 61 strain-specific genes. In theory, the number of genes in the strep B “pan-genome” is limitless – suggesting that the more genomes vaccine and drug developers can sequence, the more effective their products will be.

Researchers at Chiron, HMS, and TIGR used a computer algorithm to pick out genes most likely to encode surface features of group B strep because these are what the host immune system recognizes and resists. In mice, a cocktail of immunogens protected 59% to 100% of mice against challenge by 12 different strains of S. agalactiae – raising hopes that a globally protective vaccine for humans may be within reach. A new biological finding also emerged from this experiment, Dr. Rappuoli said. Three of the four antigens that elicited the strongest protection are part of pilus-like structures that had apparently been on the surface of group B strep all along, but had never before been identified.

These findings demonstrate that “reverse vaccinology” incorporating sequence data from multiple strains holds tremendous potential for making vaccines that once might have been impossible to design.

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Discovering New Compounds in Nature

Jon Clardy

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School

Jon_clardy@hms.harvard.edu

Compounds discovered in natural sources — Taxol and morphine from plants, vancomycin and rapamycin from soil bacteria, saxitoxin and epibatidine from puffer fish and poison frogs, among many others — have provided therapeutically useful drugs and small molecule probes for biological processes. The mother lode of novel chemicals made by living creatures is nowhere near tapped-out, Dr. Clardy said. Bacterial diversity, for example, far exceeds variation in animals or plants, and bacteria are busy and creative makers of small molecules.

The classic approach of natural products chemists was to collect soil, culture bacteria from these samples, and isolate what they hoped would be new and potentially useful compounds. And although useful products were discovered this way, in the long run it proved too inefficient to sustain interest on the part of pharmaceutical companies: the same molecules were found over and over and the return on investment was low. These disappointments motivated many investigators to seek new ways for finding useful compounds in nature.

Dr. Clardy's lab is taking advantage of the fact that 99 percent of all soil bacteria cannot be cultured. His team has developed methods for capturing large pieces of bacterial DNA directly from the environment and expressing them in alternative hosts. The investigators have obtained good results with two approaches, a phenotypic antibiotic assay and a DNA sequence-based assay, to identify new compounds with antibiotic activity, learn more about small molecule biosynthesis, and gain new insights into the biological function of small molecules made by soil bacteria. Current experiments are examining the structure and function of compounds identified in this manner, some of which may turn out to be tomorrow's antimicrobial drugs.