ARMENISE-HARVARD SYMPOSIUM 1999

ARMENISE THIRD SYMPOSIUM

3rd Annual Symposium
June 28-July 1, 1999, Castelvecchio Pascoli, Lucca, Italy

About the Symposium

When the Armenise-Harvard Foundation convened its 3rd Annual Symposium at Il Ciocco, a conference center high in the Alpi Apuane, breathtaking views of tawny mountains and lush valleys reminded participating scientists that their work unlocks ""the impressive beauty of the biosphere,"" in the words of Foundation President and CEO Daniel C. Tosteson, former dean of the Harvard Medical School.

In his opening remarks to more than 100 researchers who gathered for the Symposium, HMS Dean Joseph Martin noted that it has been only three years since the Foundation began stimulating collaboration among Harvard's six basic science departments and between scientific centers in Italy and HMS. In his view, the Foundation has succeeded on both fronts. Today, its generosity supports not only the annual Symposium but also a broad range of joint ventures, including exchanges of technology and personnel and an increasing number of small seminars. Armenise-sponsored research is conducted at five Italian institutions: the European Institute of Oncology in Milano, the University of Padova, the Institute for Cancer Research and Treatment at the University of Torino School of Medicine, the Dipartmento di Ricerca Biologica e Tecnologica (DIBIT) at Scientific Institute San Raffaele in Milano, and Universita' Di Roma La Sapienza. At Harvard, the four Armenise centers are structural biology, neurobiology, cell signal transduction, and human cancer viruses. Representatives from all the Armenise programs participated in the 3rd Annual Symposium.

Impressed with the productivity of the Italian centers, the Foundation recently invited all of them to apply for a two-year extension of their original award. Dean Martin announced, during his opening remarks, that on the previous day the Foundation's board voted to extend funding for all five. ""This is very gratifying for the Italian part of the enterprise,"" Dr. Jacopo Meldolesi of DIBIT said in response to Dean Martin's news. The Armenise funding brings not only money, he said, but also scientific challenge and the opportunity to develop more mature, ongoing relationships with collaborators.

A considerable exchange of human talent is already underway. For example, Dean Martin cited the recent move of Armenise fellow Andrea Musacchio from HMS to the European Institute of Oncology, where he will lead a new structural biology department. In Dr. Daniela Pietrobon's closing remarks, the Padova researcher reminisced about three years she spent at HMS early in her career and credited the Foundation with making it possible for two young scientists from her group to follow in her footsteps. The Armenise grant has been invaluable for the support of young investigators and the strengthening of laboratory infrastructure, she said, and other scientists agreed.

The strength of the 3rd Annual Symposium's scientific program is one measure of the Foundation's success in fulfilling its mission. The event drew 112 basic scientists, slightly more than two-thirds of them from Italy. This year there were 46 poster presentations and 20 formal lectures grouped into five sessions:

- Neurobiology I
- Biomedical Research
- Plant Defense/Pathogenesis
- Neurobiology II
- Proteolysis/Apoptosis/Cell Cycle

Session 1: Neurobiology I

Overview

So many Armenise-sponsored investigators are engaged in exciting neurobiology research that this year's Symposium devoted two full sessions to this vital and fast-moving field. The conference's opening session introduced four issues that are fundamental for understanding neuronal and brain function, according to Dr. Jacopo Meldolesi, who moderated the program. As distinct as these areas of research are, all are in fast-moving areas where even more interesting news can be expected in the near future.

The first presentation explores how the human nervous system transduces smell from the binding of odorants to special receptors in the nose to the transmission of information to the brain. The second speaker homed in on a large family of receptors, the SEX/plexins, that in mammals appear to play essential roles in the development of nerve tissue. The focus shifted to the cell membrane for the third talk, which introduced a new process of membrane regulation in neurons that molds the excitability of those cells. The final paper dealt with a fascinating transcription factor that appears to exert itself during embryogenesis by suppressing neuronal fate determination.

Presentations

Deconstructing smell

Linda Buck, Associate Professor

Department of Neurobiology, Howard Hughes Medical Institute, Harvard Medical School

Email: Linda Buck

Smells have shapes. But instead of having one receptor dedicated to smelling bananas and another to pine trees, Dr. Buck's research demonstrates that individual odorant receptors (ORs) recognize specific parts of an odorant's structure, rather than the whole molecule. The olfactory system combines data from numerous ORs to determine what it's smelling, which explains how humans and other mammals can discriminate an enormous variety of odors as well as pheromones (chemicals that elicit sexual or other innate behaviors). This combinatorial coding scheme relies on about 1,000 ORs expressed by the millions of sensory neurons in the olfactory epithelium lining the nose. Each neuron expresses one OR gene and neurons expressing the same OR are scattered throughout one of four nasal zones, an arrangement that decentralizes information processing. When a volatile chemical enters the nose, different parts of its structure are recognized by scattered neurons equipped with various ORs. Axons from these neurons travel to the olfactory bulb, where input from neurons with the same OR-no matter where they may be located in the nasal epithelium-is channeled into specific glomeruli with fixed locations, much as scattered light is focused by a lens.

The vomeronasal organ, where the subconscious detection of pheromones begins, is equipped with two distinct families of candidate pheromone receptors, one of which was first identified by Dr. Buck's laboratory. Inputs from these receptors appear to be processed separately from ORs, ensuring that signals for primal behaviors such as mating and aggression won't be confused with commonplace smells.

In recent experiments, Dr. Buck and her colleagues have combined calcium imaging and single cell RT-PCR (a technique for amplifying parts of the genome that encode proteins) to identify ORs for odorants that people perceive quite differently even though they are structurally similar. The combination of receptors that codes for a floral aroma, they found, may overlap substantially with the combination that picks up a rancid smell-but the two will be channeled to different glomeruli in the olfactory bulb. These observations of single cells support three important conclusions: a single OR recognizes multiple odorants, a single odorant is recognized by different ORs, and most odorants can be detected by different combinations of ORs.

The human Sex/plexin gene family encodes surface receptors for semaphorins and controls cell repelling cues

Luca Tamagnone

Institute for Cancer Research, University of Torino Medical School

Email: Luca Tamagnone

Several years ago, when Dr. Tamagnone discovered an unusual family of receptor tyrosine kinase genes, he named the prototype SEX because it was located on the X chromosome. Additional research has expanded the universe of human SEX/plexins to include nine members grouped in four families. These genes were first identified in the nervous system, and they contain highly conserved sequences that are the same in nematodes as in humans. The SEX/plexins encode cell surface proteins homologous to the extracellular domain of the receptor tyrosine kinase that binds hepatocyte growth factor (HGF), a key growth factor in embryonic development. Early on, researchers determined that SEX/plexins appear to influence the migration of axon processes toward synapse formation. Now other roles are coming to light.

Dr. Tamagnone's team has observed that the extracellular domains of SEX/plexins (the parts that protrude from the cell surface) bear a strong resemblance to semaphorins, a large family of soluble and membrane-bound ligands. In Cell, they described an interaction between plexin-A and semaphorin-1 in Drosophila; since that report they have identified two semaphorins that bind to human SEX/plexin receptors. This is the first time that ligands for the SEX/plexins have been identified.

More recently, Dr. Tamagnone's laboratory has explored the possibility that the plexins are important in cell-to-cell signaling in epithelial and endothelial cells, in addition to their established role in neuronal development. When they used semaphorin protein as a probe, the researchers found that it bound to plexins that interact with neuropilins in cell signaling. Future experiments will explore whether semaphorin-binding plexins may also collaborate with neuropilins in signal transduction, acting via some novel cytoplasmic (inside the cell) structure.

Active chloride currents mold the electrical properties of the ""resting"" sympathetic neuron

Riccardo Fesce

DIBIT - Institute San Raffaele

Email: Riccardo Fesce

There's more to a resting neuron than meets the eye. Although the conventional view is that nothing much goes on when the membrane potential is below -60mV, Dr. Fesce and Oscar Sacchi, a biologist at the University of Ferrara, have detected a series of active conductances in the -60 to -80mV range. They now say that interactions between fluctuating currents of potassium and chloride mold the excitability of the cell and help determine how much synaptic input is required to trigger an action potential.

Working with the isolated, intact superior cervical ganglion of rats, Dr. Sacchi used a two-electrode, voltage-clamp system to characterize the conductances and electrical properties of these cells. Dr. Fesce used these experimental measurements to build a computational model of a complete sympathetic neuron, which revealed surprising interactions between a potassium current that acts just above the -60mV threshold and a subthreshhold chloride current. A potassium current called IA is activated at about -60mV and becomes fully activated at about -30/-40mV, causing transient depolarizations that rapidly relax to the initial value. During the upstroke of excitatory potential, Dr. Fesce said that this potassium current apparently gives rise to a counter current of chloride ions, which push toward repolarization. All this happens in a neuron that appears to be ""resting,"" with the result that a higher level of synaptic input will be required to trigger an action potential.

When a neuron is at rest, there is supposedly no net flow of ions across the plasma membrane. Nevertheless, Dr. Fesce found remarkable chloride currents that operate below -60mV. These currents respond to transient changes in membrane potential and return to a steady state after hundreds of seconds. Chloride conductance increases with depolarization, and at -60/-80mV the chloride equilibrium potential sustains a small inward current. Whatever happens to membrane

resting potential sparks changes in chloride redistribution and conductances, Dr. Fesce said, which challenges the notion that membrane potential reflects a simple balance between sodium and potassium.

Transcription factors in cell fate determination and differentiation

Yang Shi, Associate Professor

Department of Pathology, Harvard Medical School

Email: Yang Shi

Scientists have known for years that regulation of tissue-specific genes during embryonic development is controlled primarily at the level of transcription. Dr. Shi's experiments demonstrate that in Caenorhabditis elegans, certain enzymes are involved both in normal transcription and in abnormal cell growth. Nature has many strategies for releasing DNA from the compact chromatin packaging that enables it to fit into the cell nucleus. One such method is the attachment of an acetyl group to histones, which bind the chromatin package. Acetylation is determined by give-and-take between histone acetyltransferases (HATs), such as mammalian CBP and p300, and histone deacetylases (HDAs). Earlier work revealed that the adenovirus oncoprotein E1A cannot induce immature cells to divide endlessly so long as CBP/p300 are functioning, suggesting that these proteins are needed for normal cell growth and differentiation. More recently, Dr. Shi expanded on this idea by manipulating cbp-1, the gene encoding CBP-1, the C. elegans protein that corresponds to CBP/p300.

C. elegans may be a simple organism with less than 1,000 cells, ""but it does all the things that we do, pretty much,"" Dr. Shi said of his favorite model. Many of its 20,000 protein-coding genes are homogolous with human genes. As in humans, cell fate is determined in part by transcription factors. When the researchers used a new technique called RNA-mediated Interference (RNAI) to inhibit expression of CBP-1, they saw undifferentiated embryos with no sign of normal morphology. At the stage when gut and muscle tissue are apparent, no such

differentiation had occurred. As expected, C. elegans genes that correspond to mammalian histone deacetylase appear to repress somatic differentiation. In a kind of tug of war, CPB-1 appears to promote endoderm differentiation by antagonizing the repressive effects of HDA. These experiments are the first to show how a homolog of the human proteins CPB and p300 functions in a live animal. These results also provide critical in vivo evidence that the histone acetylase activity of CBP-1 may be important for its biological activity. In addition to confirming Dr. Shi's hypothesis, these experiments yielded an unexpected result as well. When monoclonal antibodies were used to study the seemingly undifferentiated cells in worms without CPB-1 activity, those cells appeared to be neurons Ð suggesting that neuronal differentiation may be a kind of default setting for C. elegans cells in the absence of CBP-1. Similar observations have been made recently in Xenopus laevis, suggesting that HATs may be a highly conserved, essential player in the differentiation of non-neural tissue.

Session 2: Biomedical Research

Overview

As chairman of the Foundation's Scientific Advisory Committee, one of Dr. Peter Howley's responsibilities is to shape the program for the annual symposium. As he organized individual abstracts to create this year's sessions, Dr. Howley discovered that some of the cutting-edge investigations sponsored by the Foundation aren't easy to categorize. He gathered four of them together for this session, which he dubbed Biomedical Research. The opening presentation dealt with viral vectors for gene therapy, followed by a tour of the enzymatic assembly lines that microbes use to grow antibiotics. The third speaker introduced the first mammalian mutation gene that appears to increase stress resistance and extend lifespan, and the fourth took his listeners inside a ""chamber of doom"" where proteins are destroyed. As disparate as these topics appear, Dr. Howley said that ""they have a similar theme: all are in areas of research that interest me.""

Presentations

New generations of lentiviral vectors for experimental and human gene transfer

Luigi Naldini

Laboratory for Gene Transfer & Therapy, Institute for Cancer Research, University of Torino Medical School

Email: Luigi Naldini

Penetrating cells is a do-or-die proposition for viruses, which can't replicate until they've gotten inside host cells. Researchers who are mindful of this special viral skill are seeking to use them as vectors for gene therapy or vaccines. Many viruses, however, aren't well suited to this task because they can only enter dividing cells, they don't penetrate very many cells, or they don't express the transfected gene at high enough levels. To get around these limitations, Dr. Naldini and his colleagues have designed hybrid lentiviral vectors capable of transferring and expressing genes in several rodent tissues in vivo, and in primitive human hematopoietic stem cells ex vivo. They have accomplished this by combining core elements of HIV-1, the pathogen that causes AIDS, with the envelope of a less harmful lentivirus called vesicular stomatitis virus (VSV). The safety profile for these vectors has improved as the researchers cut back on the amount of HIV genetic material they use, they've increased transgene expression through selective use of HIV ltr (long terminal repeat) and packaging signals, and VSV elements permit entry into a variety of cell types. Dr. Naldini's latest and safest vectors inactivate upon transduction and include only a minimal set of HIV genes.

In earlier experiments, his group demonstrated efficient delivery and sustained expression of marker genes by these vectors, both in vitro with human donor lymphocytes and in vivo when injected into the brains of adult rats. More recently, Dr. Naldini has been working with a mouse model for metachromatic leukodystrophy (MLD), an inherited liposomal storage disorder that in infants causes rapid, dramatic death as lipids accumulate in the central nervous system and other major organs. His team designed a hybrid lentivirus vector carrying the gene for ASA, the enzyme that is lacking in this condition, which they are testing in two ways. When the vector is injected directly into the brains of MLD mice, there is preliminary evidence that the transgene appears to express well and in a stable fashion. A second set of experiments involves ex vivo reconstitution of hematopoetic stem cells with the transgene, which the

researchers speculate will repopulate and replace the missing enzyme in animals. Early results appear promising, and additional research is underway.

Assembly line enzymology: the biosynthesis of polyketide and nonribosomal peptide antibiotics

Christopher T. Walsh, Professor

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School

Email: Luca Tamagnone

Because they live in a bug-eat-bug world, funguses and other microbes have evolved thousands of antibiotics that they use to disable their enemies and competitors. Because many of these substances disrupt only the functions of procaryotic cells, leaving eucaryotic cells alone, doctors use them as invaluable weapons against microbes that cause human disease. All are made by a process that Dr. Walsh calls ""assembly line enzymology."" His laboratory seeks to understand the molecular logic of antibiotic assembly and ""the flip side, by which bacteria produce nonribosomal peptide virulence factors, which allow them to infect you and me."" Ultimately, the goal is to use combinatorial elaboration to assemble new antibiotics, block virulence factors, and fight growing problems of antibiotic resistance.

At a gestalt level, polyketide antibiotics such as erythromycin and tetracycline and peptides like penicillins and vancomycin look nothing alike. Yet all are templated natural products where assembly instructions come from the domain order of giant megasynthases. These molecules have ""way stations,"" modules that enzymes, such as polyketide or nonribosomal peptide synthetases, use to initiate, elongate, and terminate the natural product chains. At some of these way stations, Dr. Walsh and his colleagues have been able to substitute one module for another, an accomplishment that could lead the way to combinatorial biosynthesis of new antibiotics. If investigators can build a parts list for the antibiotic assembly line, Dr. Walsh predicts that it will be

possible to swap modules around and create antibiotics that have not yet been made in nature, but which might be powerful weapons against resistant bacteria.

The p66^{shc} adaptor protein controls oxidative stress response and lifespan in mammals

Enrica Migliaccio

European Institute of Oncology

Email: Enrica Migliaccio

Although gene mutations that extend lifespan and enhance resistance to environmental stresses such as ultraviolet (UV) light or reactive oxygen species have been identified in C.elegans and other invertebrates, no such genes are known in mammals. In this presentation, Dr. Migliaccio announced that she and her colleagues have found a mutation in the mouse p66^{shc} gene that appears to have such properties. This gene is a splice variation of p52^{shc}/p46^{shc}, a cytoplasmic signal transducer involved in the transmission of mitogenic signals from tyrosine kinases to the Ras oncogene. Unlike p52^{shc}/p46^{shc}, which are known to cause malignant cell changes, p66^{shc} fortunately does not transform fibroblasts.

The researchers created a p66^{shc} knockout mouse that retained p52^{shc}/p46^{shc}, then in vitro subjected cells from that mouse to UV stress. After four days, wild-type cells were all dead, whereas the cells with the deletion were alive. Additional in vivo experiments showed the knockout mice to be more resistant to paraquat-induced oxidative stress than wild-type mice. The researchers suspect that p66^{shc} is part of a signal transduction pathway that regulates oxidative stress response, and that hypothesize that disrupting this pathway will protect against this well-known cause of aging. Further support comes from an observational study, in which a group of mice with a

double p66^{shc} deletion outlived those who were missing one copy and those with two normal copies of the gene.

Mechanisms of Protein Degradation within Eukaryotic Proteasomes

Alfred L. Goldberg, Professor

Department of Cell Biology, Harvard Medical School

Email: Alfred L. Goldberg

For cells, an important part of viability is the prompt and appropriate degradation of intracellular proteins. In mammalian cells, large structures called proteasomes degrade proteins that have been marked for destruction by ubiquitin. Some of the fragments that emerge from this process are converted to amino acids; others are antigenic peptides that trigger cytolytic T-cell activity after presentation on MHC-class 1 molecules.

Unlike typical proteases, mammalian 20S and 26S proteasomes degrade proteins in a highly processive fashion that Dr. Goldberg describes as a ""bite-chew"" model. Once a protein substrate has been labeled by ubiquitin, an ATPase shepherds it into the proteasome's central ""chamber of doom,"" where it will be unfolded and methodically chopped into small pieces. Each molecule is completely chopped up before the proteasome moves on to the next. Dr. Goldberg and his colleagues were surprised to find that all the products of this process are the same size, whether they started out as small polypeptides or big proteins, which they see as an indication that proteolysis continues until the products are small enough to diffuse out of the proteasome, then stops. About 99% of these fragments are smaller than 25 residues, with most in the 3-20 residue range. Only 10-15% of the products are 8-9 residues in length, the size required for MHC-class-1-presentation.

Eukaryotic 20S proteasomes contain active sites that cleave proteins in three distinct ways: two cut like chymotrypsin, two like trypsin, and two like caspase. The researchers were surprised to find that instead of acting independently, chymotripsin appears to ""bite"" the substrate first, which initiates ""chewing"" by the other processing sites. Caspase substrates signal chymotripsin when it's time to take the next bite. The resulting process is a highly efficient method for destroying abnormal proteins and for alerting the immune system to the presence of viruses and other undesirables. In the future, proteasome inhibitors may hold promise as treatments for cancer and other human diseases.

Session 3: Plant Defense/Pathogenesis

Overview

The more that scientists learn about how plants protect themselves against disease, the more parallels they see between plant and mammalian defense systems. Much of what is known about how plants respond to pathogen attack has come from studies of Arabadopsis thaliana or rice. This type of research will get a boost in 2000, when the genomes of these model systems are expected to be fully sequenced, Dr. Brian Staskawicz predicted in his introduction to this session.

Plants have many enemies, including bacteria, fungi, viruses, and nematodes. Agriculturists have been developing disease-resistant plants since the turn of the century, relying almost entirely on classical breeding, hybridization, and recurrent selection for desirable traits. Long before genes could be isolated, it was obvious that a single resistance gene could make the difference between a bountiful harvest and a failed crop, Dr. Staskawicz noted. After genetic mapping and map-based cloning became available, scientists began cloning specific genes for disease resistance. Many plant resistance genes have leucine-rich repeat(LRR) domains, which are important for recognizing non-self proteins in the environment and setting in motion a signal transduction pathway that ultimately leads to defensive action. The machinery for recognition and response closely resembles the mammalian immune system.

In recent years, studies of bacteria that prey on plants have revealed that they share many characteristics with human pathogens, including similarities in effector proteins known as

virulence factors. Now that both host plants and their pathogens can be genetically manipulated, scientists are going to find better ways to give plants an edge over their enemies. ""The field of plant pathogenesis and defense is exploding right now,"" Dr. Staskawicz said.

Presentations

Exploiting polygalacturonase-inhibiting proteins (PGIPs) to engineer novel plant receptors

Giulia De Lorenzo, Professor

Laboratory for Gene Transfer & Therapy, Institute for Cancer Research, Department of Plant Biology, Universita' di Roma La Sapienza

Email: Giulia De Lorenzo

Plants can be resistant to disease only when there is a match between a plant resistance gene and an avirulence (Avr) gene in the pathogen. Resistance genes are thought to code for receptors that recognize specific Avr products. With one exception, all the plant resistance genes that have been identified have leucine-rich repeats (LRR), which encode specific receptors for a wide variety of Avr proteins. Dr. De Lorenzo suspects that recognition of specific pathogens hinges on a hypervariable region in resistance genes that codes for variations in a specific region within LRR proteins. This is theoretical at present, however, because her team has not yet observed a direct interaction between LRR and Avr proteins. Polygalacturonase-inhibiting proteins (PGIPs), present in the cell wall of many plants, belong to the large family of LRR proteins and are structurally similar to other known products of resistance genes. PGIP recognizes endopolygalacturonases (PG), enzymes that disease-causing fungi use to breach the cell walls of plants. PGIPs and PGs offer a unique opportunity to analyze how LRR proteins recognize specific attackers. Dr. De Lorenzo's laboratory

has been using site-directed mutagenesis to explore how the recognition capacity of PGIPs can be manipulated. In one experiment, alteration of a single amino acid residue caused a PGIP to lose function, she reported. Now her team is working with mutations that may increase recognition, seeking to create chimeric proteins that will enable plants to identify and resist a wider range of pathogens.

Plant extracellular matrix and development: targeting pectins

Felice Cervone, Professor

Department of Plant Biology, Universita' di Roma La Sapienza

Email: Felice Cervone

Dr. Cervone's laboratory focuses on events in the plant cell wall, which is the organism's first line of defense against pathogenic invaders. For some years, they have pursued genetic mutations in the cell wall that may be important in both defense and normal development. When these proved elusive, the researchers decided to see if cell-wall mutations could be detected if they used well-characterized bacterial enzymes to manipulate extracellular matrix architecture. Dr. Cervone's team used Agrobacterium-mediated transformation to produce plants that overexpress polygalacturonase (PG) and polygalacturonase-inhibiting proteins (PGIPs). They were able to generate two kinds of transgenic plants: Arabidopsis and tobacco plants expressing PG from Aspergillus niger, and Arabidopsis, tobacco and tomato plants expressing PGIP from Phaseolus vulgaris. These alterations changed the pectins in the cells walls.

Although they expected that the morphogenesis of transformed plants would not be the same as normal ones, they were surprised when the altered plants grew much larger and more vigorously than the wild-type, a kind of plant version of super mouse. Another unexpected finding was that pectins from tomatoes with the pgip transgene exhibited a higher degree of methylation and acetylation than those isolated from non-transformed plants. This is consistent

with earlier findings that PGIP in vitro interacts with methylated pectins better than with non-methylated homogalacturonans, a perference that probably protects pectins from demethylation. Although much more research is needed, even at this early stage it is clear that plant transformation with PGs and PGIPs is a valuable tool for exploring how changes in pectin structure affect plant development, physiology, and defense.

Pseudomonas aeruginosa pathogenesis in diverse hosts

Laurence Rahme, Assistant Professor

Harvard Medical School and Massachusetts General Hospital

Email: Laurence Rahme

Dr. Rahme's laboratory studies the molecular mechanisms underlying Pseudomonas aeruginosa pathogenesis in mammals. This is a medically important opportunistic bacterium that infects burn and trauma patients, as well as other immunocompromised individuals, and it is the leading cause of death in people with cystic fibrosis.

She and her collaborators have shown that a novel strain of P. aeruginosa uses the same subset of virulence factors to cause disease both in plants and in a wide range of animals, including humans. This important finding gave them the opportunity to use plants as a screening system for bacterial virulence factors, which once found could point the way to the identification of new antibiotics. This screening algorithm markedly decreases the use of laboratory animals, yet it generates data relevant to pathogenesis in mammals. In plants, Dr. Rahme's group has identified several novel P. aeruginosa virulence-related factors, the majority of them affecting the persistence and severity of infection. When these factors were tested in a mouse model that involves infection after a non-lethal burn, they made infections worse and accelerated sepsis development.

More recently, Dr. Rahme has been using C. elegans to identify avirulence mutations that enable the worm to feed on pathogenic P. aeruginosa and survive. She has found several mutations that appear to increase the host's ability to limit disease development, not just in worms but in mammals as well.

Molecular genetics of plant bacterial disease resistance

Brian Staskawicz, Professor

Department of Plant and Microbial Biology, University of California, Berkeley

Email: Brian Staskawicz

Scientists have long anticipated a time when transgenic plants will be resistant to diseases that farmers now combat with chemical pesticides. Dr. Staskawicz and his coworkers will soon discover whether this moment has arrived for a type of bacterial spot disease caused by Xanthomonas campestris pv vesicatorio (XCV), which afflicts both peppers and tomatoes. A gene that produces durable resistance in peppers is being experimentally introduced into tomato plants, which have no natural resistance to this pathogen. It took Dr. Staskawicz' lab more than 8 years to nail down the function of this gene, isolate it, and prepare it for use as a transgene.

This work grew out of a broader inquiry into the molecular genetics of disease resistance, which showed that plants are protected against XCV-caused spot disease when the bacterium carries the avirulence gene (actually an effector protein) avrBs2 and the host plant has the resistance gene Bs2. A series of genetic and biochemical experiments demonstrated that the Bs2 gene product recognizes the business end of XCV-an effector protein that the pathogen needs to be at its most virulent. Having decided that Bs2 was a good candidate for insertion into the tomato genome, Dr. Staskawicz and his colleagues set out to map and clone the gene-no trivial task in a genome four times as large as the human genetic endowment. The next step is to show protection in field trials, which are expected to begin this year.

Session 4: Neurobiology II

Overview

In this session, new scientific tools begin to pick apart some of neurobiology's venerable knots. For example, Dr. Elio Raviola said in his introduction, circadian biology used to mean watching how rats behaved when the light was switched on and off. The first paper described how molecular biology can pry open the mammalian circadian clock, so that some of its gears and springs can be spread out for inspection. The second presentation examined calcium pumps, certainly among the biggest and most important membrane transporters, and asked how they might be expressed during neuronal development. The next speaker tackled a classic problem, the ""inside-out"" migration of newly hatched neurons to their proper places in the cerebral cortex, and identified two major actors in this journey. Finally, the concluding speaker began to lift the veil on what could prove to be the master switch for neuronal exocytosis.

Presentations

Studies on the molecular mechanism of the vertebrate circadian clock

Charles J. Weitz, Assistant Professor

Laboratory for Gene Transfer & Therapy, Institute for Cancer Research, Department of Neurobiology, Harvard Medical School

Email: Charles J. Weitz

Circadian clocks are endogenous oscillators that drive daily rhythms and physiology, and as such they probably represent an ancient and fundamental mechanism. In mammals, the central clock is located in the super chiasmatic nucleus of the brain, with independent clocks in each retina. Two years ago, investigators at Northwestern University identified the first mammalian circadian gene, which they called Clock. Further research showed that this gene encodes a presumptive transcription factor that is closely related to a family of proteins that mediate dimerization and bind DNA.

These findings immediately reminded Dr. Weitz of what his lab had learned about the workings of the Drosophila clock, where per genes make proteins that dimerize with the product of tim(timeless). This protein pair is transported to the nucleus where it somehow shuts down the per and tim genes until the proteins disappear and the genes turn on again. These findings motivated him to look for a similar feedback loop in mice. The search for a partner for CLOCK protein turned up BMAL1, which is co-expressed with CLOCK and PER1 at known circadian clock sites in brain and retina.

Additional experiments showed that CLOCK-BMAL1 heterodimers activate transcription from E-box elements, a type of transcription factor binding site, located adjacent to the mouse per1 gene, and from an identical E-box known to be important for expression of per genes in Drosophila. If the CLOCK protein was mutated, however, it joined with BMAL1 to form heterodimers that bound DNA but failed to activate transcription. According to Dr. Weitz, CLOCK-BMAL1 heterodimers drive the positive component of per transcriptional oscillations, which appear to underlie circadian rhythmicity. This is the first time that binding has been proved to activate transcription.

More recently, transfection studies in mice have provided direct evidence that expression of the PER protein inhibits Per1 gene activation by CLOCK-BMAL1. Protein interaction experiments and further analysis suggest that PER binding sequesters the CLOCK-BMAL1 heterodimer in a manner that keeps the transcription factor from binding to its E-box target site.

the giovanni ARMENISE HARVARD foundation

Calcium controls the transcription of its own transporters in developing cerebellar neurons

Ernesto Carafoli, Professor

Department of Biochemistry, University of Padova

Email: Ernesto Carafoli

Eucaryotic cells maintain a low intracellular concentration of free Ca²⁺ mainly by relying on a membrane-bound ATPase, PMCA, that serves as a high-affinity pump. Calcium is also exported from cells by a low-affinity Na+/ Ca²⁺ exchanger (NCX). The PMCA protein has four isoforms that vary slightly in amino acid sequence, with PMCA4 being the best studied of these. In order to learn more about the others, Dr. CarafoliÕs laboratory made monoclonal antibodies to isoforms 1-3. Using these tools, they found PMCA2 and 3 only in brain tissue, whereas isoforms 1 and 4 appear ubiquitous.

In order to explore the role of PMCA isoforms 1-3 in neuron development, Dr. Carafoli and his colleagues used antibodies to track changes in these key carrier proteins in cultures of rat cerebellar granule cells (CGC). They found that PMCA isoforms 2 and 3, and a splicing variant of PMCA1 (designated as PMCA1CII) are strongly upregulated in the 3 to 5 days required for full maturation of the granule cells, whereas PMCA4 is much more rapidly downregulated. These effects occur at both the transcriptional and translational levels. Maturation of cerebellar granule cells requires the sustained influx of Ca²⁺ through L-type channels; when this was blocked with nifedipine the up-and-down regulation of PMCAs was abolished. At variance with the upregulation of PMCA 2, 3, and 1CII, the down-regulation of PMCA4 depends on increased levels of calcineurin, a relationship that was disrupted by immunosuppressive drugs. Dr. Carafoli also identified three isoforms of NCX at work in these cultured cells. NCX I and NCX III become slightly upregulated as the granule cells mature, whereas NCX II is strongly and rapidly down-regulated in a calcineurin-dependent way. The splicing variants of NCX1 also undergo a switch during maturation.

Expression of Ca²⁺ transporters may change because the cells need to gain better control over the increased Ca²⁺ influx required for the increased gene transcription that is integral to their maturation. Future research will explore the specific properties of the individual Ca²⁺ carrier proteins.

Function and dysfunction of cyclin-dependent kinase 5 in development and degeneration

Li-Huei Tsai, Assistant Professor

Department of Pathology, Harvard Medical School

Email: Li-Huei Tsai

Neurons are born close to the inner surface of the neural tube and migrate outward to form the layers of the mammalian cerebral cortex. In these layers, neurons are grouped according to morphology. During cortical development, successive generations of neurons migrant in an ""inside-out"" fashion, with the first-born brain cells settling closest to home and later cohorts traveling just past them to form the next layer. Although this pattern is well-known, the factors that guide newborn neurons into place have been a mystery.

Over the past several years, Dr. Tsai has not only figured out how neurons migrate but also has uncovered connections between this phenomenon and Alzheimer's disease. Her experiments suggest that normal ""inside-out"" migration requires that cyclin-dependent kinase 5 work closely with p35, a regulatory protein. When she and her colleagues knocked out the p35 gene in mice, and when another lab independently knocked out the gene for cdk5, each produced mice with neurons layered ""outside-in."" Dr. Tsai's mice were defective but viable; the animals without cdk5 were not viable. She has since found evidence suggesting that the p35/cdk5 kinase complex facilitates ""inside-out"" migration by regulating actin cytoskeleton dynamics and reducing cell-cell adhesion, making it easier for freshly minted neurons to slip past the ones that have already settled in their appropriate layers.

When ischemia, hydrogen peroxide, or other means were used to stress the brains of mice, the animals converted p35 to p25, a protein that Dr. Tsai believes deregulates cdk5 and has no normal developmental function. Her team found massive accumulations of p25 and cdk5 in post-mortem brain samples from Alzheimer's disease patients, especially in the neurofibrillary tangles that are one major hallmark of the disease. Abnormally phosphorylated tau protein also abounds in these tangles. Future research focuses on the role of p25/cdk5 in apoptosis, and the possibility that this might be used as a target for treating neurodegenerative disorders.

Regulated secretion expression competence and multiplicity in neurosecretory cells

Jacopo Meldolesi, Professor

Department of Neuroscience, DIBIT, San Raffaele Institute

Email: Jacopo Meldolesi

Neurosecretion is the process by which cells express and release by exocytosis both the small vesicles, which contain classical neurotransmitters, and the large dense vesicles containing mixtures of amines, ATP, proteins and peptides. Because neurons and endocrine cells acquire secretory capacity during development and retain it, this is generally regarded as a stable trait that can be lost only in case of cell de-differentiation. Some years back, Dr. Meldolesi's laboratory developed what he characterizes as ""a neurosecretory cell that is incompetent for secretion."" Defective clones of pheochromocytoma PC12 cells appear phenotypically normal except that they lack the dense granules typical of neurosecretory cells; functionally they have lost the ability to secrete. These rat cells lack not only secretion products but also vesicle membrane proteins, including the vSNARE VAMP2, the plasmalemma tSNAREs, which are necessary for exocytosis, and various soluble regulatory proteins. The mechanism(s) sustaining the defect appear(s) to be at least in part post-transcriptional. When defective cells are fused with normal rat PC12 or with secretory human cells, or when they are transfected with one or more normal genes, neurosecretion returns

to normal levels. This implies the existence of genetic controls for exocytosis, and the nature of these mechanisms is now being investigated

Session 5: Proteolysis/Apoptosis/Cell Cycle

Overview

The topics covered in this session-protein degradation, cell cycle regulation, and apoptosis-may hold the keys to the development of better cancer therapeutics, Dr. Giulio Draetta said in his opening remarks. Unlike treatments for infectious diseases, which can be directed against features of the pathogen that aren't found in the human host, most cancer treatments must aim at molecules that are normally present. And this, of course, explains why so many anti-cancer treatments are so toxic to patients. In the future, molecular oncologists hope to have treatments that selectively kill cancer cells while leaving normal ones alone. Someday it may be possible for physicians to obtain a molecular fingerprint of the patient's cancer, then to select inhibitors that will moderate responses of the individual patient and the specific tumor. The presentations in session focus on signaling cascades that could ultimately prove relevant to the ultimate goal of finding less toxic treatments for cancer, Dr. Draetta said. He predicted that the next step will be looking for cross-talk among signaling pathways, in addition to exploring individual pathways, and that this will lead to even more ideas for high-specificity, low-toxicity treatments.

Presentations

The regulatory particle of the proteasome

Daniel Finley, Associate Professor

Laboratory for Gene Transfer & Therapy, Institute for Cancer Research, Department of Cell Biology, Harvard Medical School

Email: Daniel Finley

In his presentation on the first day of the symposium, Harvard's Dr. Alfred Goldberg described how cells use the ubiquitin-proteasome pathway to degrade proteins that would cause trouble if they were allowed to accumulate. Dr. Finley's work sheds more light on this crucial pathway. He used a yeast model to understand the opening and closing of the doorway through which ubiquitin-protein conjugates enter the lumen of the gigantic proteasome's 28-subunit core particle (CP). Once inside this chamber, tagged proteins are reduced to confetti. Although it would be easy to view the proteasome as a monolith, it is actually formed by the association of the CP with the 19S regulatory particle (RP), which sits over the CP channel and selects ubiquitin-conjugates for degradation. In addition, Dr. Finley's research indicates that the RP is a complex structure that fully unfolds substrates so they will fit through the 13Å-wide opening that leads to the CP lumen.

The yeast RP contains 17 subunits, 6 of them ATPases, and when viewed with an electron microscope this structure looks like a set of jaws that open and close to admit selected proteins. The lid is an 8-subunit subcomplex which can be dissociated from yeast proteasomes in vitro. The base is also an 8-subunit complex but it cannot be separated from the CP. The base contains all 6 proteasomal ATPases, which may both join the RP to the CP and propel some of the proteasome's targets into the CP for destruction, Dr. Finley said. By experimenting with various RP mutations, he and his colleagues have determined that rpt2, one of the ATPases found in the base, is needed to open the gated channel into the CP. The base is sufficient to activate the CP for degradation of peptides, perhaps indicating that it is competent to open the channel into the CP. However, the proteasome needs the lid to recognize ubiquitin-conjugates.

To determine whether the base of this assembly unfolded proteins in addition to opening the door, the researchers used citrate synthase (CS) as a model substrate. As they predicted, base ATPases acted as molecular chaperones for CS. Only after it was unfolded could CS be bound by the base, Dr. Finley said, and this reaction was independent of ubiquitin tagging. These data suggest that ubiquitin-protein conjugates are initially tethered to the proteasome via specific recognition of

their ubiquitin chains, followed by a nonspecific interaction between the base and the target protein, which is coupled to unfolding and translocation of the target protein into the CP.

Mitochondria in cell death: the (w)hole story

Paolo Bernardi

Departments of Biomedical Sciences and Biological Chemistry, University of Padova

Email: Paolo Bernardi

Mitochondria are often referred to as the ""power plants"" of the cell because they specialize in synthesizing ATP. The most striking feature of these organelles is that they have a double membrane, which divides them into two compartments: the intermembrane space and the matrix space in the center of the structure. Mitochondria make few proteins in-house, and for the most part they import proteins from the cytosol which form complexes with mitochondria-made proteins. Since the early 1990s, mitochondria have been under close scrutiny as regulators of apoptosis, and as potential targets for therapeutic interventions directed at accidental or programmed cell death.

Some researchers suggest that the permeability transition pore (PTP) is a major player in mitochondrial apoptotic signalling. They postulate that when this high-conductance inner membrane channel expands to admit solutes, leading to tremendous swelling of the mitochondria, this may trigger release of intermembrane apoptosis-inducing factor and possibly of cytochrome c. In mechanistic terms, however, it is difficult to understand how this pore might be linked to the release of death factors by the organelle's inner membrane, Dr. Bernardi said. One barrier to understanding this process is that in vitro studies of cell-free mitochondria may not correspond well with in vivo events. Working with populations of mitochondria in suspension, Dr. Bernardi and his colleagues manipulated a variety of factors to try and mimic the in vivo opening and closing of the PTP. They found that depolarization always leads to opening of the pore, but that the reverse is not true. Further, they determined that a closed pore did not necessarily mean that the pump that drives ATP synthesis was also out of commission. More recently, the researchers devised a novel method for studying mitochondria in intact cells, which involves chemically blotting out background activity in the cell so that mitochondrial events stand

out. So far it appears that depolarization can result not only from opening of the PTP, but also from increased ATP demand or calcium influx. It also appears that GD3 ganglioside opens the pore and increases the likelihood of apoptosis, Dr. Bernardi said, and this is being studied further.

Structural communication in apoptotic pathways

Gerhard Wagner, Professor

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School

Email: Gerhard Wagner

In order for a cell to die or commit suicide, its mitochondrial ""power plants"" must be shut down. But which of the many proteins involved in intracellular apoptosis pathways delivers the fatal blow to these organelles? The answer appears to be BID, a new protein that Dr. Wagner and his colleagues have identified in the complicated Fas signal transduction pathway. BID appears to link intracellular death signals to the mitochondria, where it sets in motion a chain of events that culminates with the activation of fatal caspace enzymes.

When the researchers used NMR spectroscopy to determine the structure of BID, they were surprised to find that this pro-apoptotic protein looks much like Bcl-xL, a protein known to inhibit apoptosis. Models of BID and Bcl-_{xL} binding indicate that the two join easily in the presence of caspace-8, a death enzyme that loosens Bcl-_{xL}'s ordinarily tight structure. The complex of BID and Bcl-_{xL} may interfere with the anti-apoptotic effects of APAF-1, which ordinarily binds with Bcl-_{xL}. This sets the stage for lethal caspace activation that knocks out the mitochondria.

Regulation of cell cycle progression by the E2F transcription factors

Kristian Helin

Department of Experimental Oncology, European Institute of Oncology

Email: Kristian Helin

In mammalian cells, the retinoblastoma proteins (pRB) are key regulators of the cell cycle, serving as one of the main brakes on progress around the cell-division cycle. These proteins are essential for fundamental decisions about whether a cell should proliferate, differentiate, or undergo apoptosis. Of the numerous cellular proteins that interact with members of the pRB family, the best characterized are the E2F transcription factors. It is widely believed that the ability of the pRB family proteins to restrict cell proliferation depends on their ability to inhibit E2F transcriptional activity.

E2Fs are important for normal cell function, and dysregulation of these proteins has many consequences. Dr. Helin and his colleagues have generated cell lines expressing E2F-1, E2F-2, and E2F-3, each fused to the estrogen receptor ligand binding domain (ER), an innovation that makes it possible to manipulate ERE2F levels with hydroxy tamoxifen. Using this system, the researchers have found that activation of all three E2Fs can relieve the mitogen requirement for entry into S phase, and activation of the E2Fs leads to a shortening of the G0-G1 phase of the cell cycle by 6-7 hours. E2F can also induce apoptosis even in cells fed growth factors that would ordinarily sustain them, Dr. Helin reported. The researchers have also demonstrated that several genes containing E2F DNA binding sites are efficiently induced by the E2Fs in the absence of protein synthesis. More recently, Dr. Helin's laboratory has identified two novel targets for E2F transcription factors, both cell-division-cycle genes. One, cdc25A, is a tyrosine phosphatase essential for the activation of certain cyclin-dependent kinases and S-phase initiation; it is also overexpressed in many tumors. The second, cdc6, is not only needed for the cell cycle to advance but also is a very sensitive marker for cell proliferation; in some cancers it may be a marker for the aggressiveness of tumor cells. In the future, E2Fs can be used to scan the human genome for additional genes important for apoptosis, cell proliferation, or DNA replication, Dr. Helin said.