2nd Annual Symposium of the Giovanni Armenise-Harvard Foundation for Advanced Scientific Research

The Foundation Mission

To establish multidisciplinary, basic science research that will support leading scientists at Harvard Medical School and at foremost institutions in Italy, in the pursuit of knowledge and scientific discovery for the benefit of humankind, in the fields of medicine and agriculture.

About the Symposium

In the summer of 1997, nearly 100 researchers representing basic science departments that had received Foundation grants met together for the first time in Erba, on the shore of Lake Como in Italy. This new tradition was repeated in June 1998, when 110 scientists gathered in Chatham, on Massachusetts' Cape Cod, for their second annual symposium. Many have worked together during the intervening year, a great deal of progress has been made, and "the Foundation's dreams are being realized," said Dr. Daniel C. Tosteson, president and CEO of the Foundation and former dean of the Harvard Medical School.

Dr. Tosteson called the symposium's 20 formal lectures "the tip of the iceberg." Equally important, he said, would be scientific information exchanged in over 40 poster presentations and during around-the-clock networking among the participants, one-third of them from Italy and the rest from Harvard Medical School and affiliated hospitals. Oral presentations were grouped into five categories:

- Cellular and Molecular Neurobiology
- Membrane Traffic/Macromolecular Entry
- Signaling/Cell-Cycle
- Plant Defense/Pathogenesis
- Transcription/Regulation

This report is organized in the same manner, and each section begins with introductory remarks that provide context for individual presentations.

The Foundation has been awarding scientific grants since January 1997, and today it sponsors collaborative investigations at Harvard and five Italian institutions. At Harvard, the Armenise-Harvard science centers are in cell signal transduction, structural biology, neurobiology, human cancer viruses, and plant biology. In Italy, biomedical research is ongoing at 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, and DIBIT, the basic biomedical research branch of the San Raffaele Scientific Institute of Milano. Agricultural studies are underway at the Dipartimento di Biologia Vegetale at the Universita' di Roma La Sapienza.

Cellular and Molecular Neurobiology

Overview

Modern neuroscience has become so broad that it stretches from the biophysics of ion channels on one horizon to studies of human and animal awareness on the other, Dr. Gerald Fischbach said as he introduced the symposium's opening session. Techniques ranging from X-ray crystallography to new versions of classic behavioral experiments are being used to prove – and disprove -- theories that were once impossible to test. Neuroscience is in a state of unprecedented excitement, and the time is ripe for international collaborations, said Dr. Fischbach, chairman of neurobiology at Harvard Medical School. For example, researchers in his department are working with scientists at DIBIT in Milano, thanks to support from the Armenise-Harvard Foundation.

The topics covered in this session clustered at the molecular end of the neuroscience spectrum. The first presentation focused on dramatic events at the synapse, where messages are transmitted nearly instantaneously from one neuron to another. The next speaker considered how synapses are formed in the first place. The final papers addressed the molecular underpinnings of two very different, larger-scale phenomena: headaches and normal circadian rhythms.

Presentations

Protein targeting in neurons and endocrine cells

Kathleen Buckley, Associate Professor
Department of Neurobiology, Harvard Medical School

Speed is of the essence for an excited neuron, which must quickly transmit a chemical message across the synapse to the next nerve cell in line. Speedy transmission occurs as wave after wave of vesicles loaded with neurotransmitters fuse rapidly with the membrane and release their cargo. The neuron can signal continuously because it has a reserve pool of vesicles lined up behind the ones currently releasing neurotransmitters. But even these reinforcements would not be fast enough, were it not for the nerve terminal's ability to pluck used vesicle membrane and protein from the synaptic cleft and immediately recycle it into new vesicles. This research seeks to understand how neurons recognize, sort, and repackage these vital materials.

Dr. Buckley's experiments used various deletion mutants of the transferrin receptor (TfR) and chimeras of a synaptic vesicle protein (synaptobrevin) and the TfR in primary neurons to determine which organelles and molecular mechanisms channeled vesicle proteins to their proper destinations in both dendrites and axons. The proteins enter the cell via clathrin-mediated endocytosis, and then travel a recycling pathway where about half a dozen different targeting signals incorporate them into a new, functional vesicle. Synaptobrevin, which is essential for fusion, is one of the best known actors in this process. This work shows that at least two independent signals, originating in the cytoplasm, are required to target synaptobrevin to synaptic vesicles. The researchers are now exploring the possibility that the recycling pathway may have other functions as well.

Role of Rho family GTPases during neuritogenesis and neuronal maturation
Ivan de Curtis, Group Leader of the Cell Adhesion Unit
Cell Adhesion Unit, DIBIT - Institute San Raffaele

In the earliest days of embryonic development, how does the tentative tip of an axon know which direction to head and when to form a synaptic connection with a second neuron? One explanation is that the neuron's cell body generates positive signals that attract the growing tip of the axon or negative ones that tell it to stop, and that the axon tip is equipped with receptors that pick up these commands.

Dr. de Curtis and his colleagues have recently identified a gene called *cRac1B*, which is specifically expressed in the embryonic nervous system of the chicken. It belongs to the Rho family GTPases, which have been implicated in cytoskeletal reorganization during neuritogenesis. The new gene appears to have a distinct role during neural development. When cRac1B is overexpressed in primary retinal neurons it raises the number of neurites per neuron and dramatically increases their branching. In contrast, cRac1A GTPase – a closely related substance found in many different cells -- does not affect neuronal growth. Furthermore, expression of either an inactive or a constitutively active form of cRac1B strikingly inhibits neuritogenesis. The cRac1B GTPase stimulates growth only in neurons; in other cell types it has the same impact as cRac1A. Detailed analysis of cRac1B proteins indicates that the carboxyterminal portion is essential for increased neuritogenesis and neurite branching. The researchers are now identifying neural regulators and/or effectors implicated in Rac action during neural development.

Neuronal voltage-dependent calcium channels: single channel studies (and headaches)
Daniela Pietrobon, Associate Professor
Department of Biomedical Sciences, University of Padova

Genetic defects in the proteins that comprise voltage-gated channels have been linked to a number of human maladies, most of which are quite rare. For example, mutations in α_{1A}, the pore-forming subunit of neuronal P/Q-type calcium channels, is associated with three dominantly inherited human disorders: familial hemiplegic migraine (FHM), episodic ataxia type-2 and spinocerebellar ataxia 6. Dr. Pietrobon's lab studies four α_{1A} mutations that occur in about half of people with FHM, and seeks to understand how each alters calcium channels and what the clinical consequences of these changes might be.

She used HEK-293 cells, transiently transfected with cDNAs encoding either wild-type or mutant human α_{1A-2} and the regulatory human α_{2b}-δ and β_{2e} subunits. Mutations T666M and V714A, located in the pore-lining region of domain II, decreased the number of functional calcium channels in the membrane and reduced the influx of calcium. In a minority of patches, however, mutants did not change the number or function of channels. The main effect of the mutation I1815L, in IVS6 in a position similar to V714A, was a large decrease in the number of functional calcium channels in the membrane. The mutation V714A significantly increased the probability that a channel would be open; I1815L and R192Q had similar but less dramatic effects. The

mutation R192Q in IS4 increased the number of functional calcium channels in the membrane, without affecting their conductance. Future inquiries will focus on the location of mutated neurons in the brains of FHM patients, and on whether cells with fewer functional channels may die earlier than normal cells.

Molecular analysis of the mammalian circadian clock
Charles Weitz, Assistant Professor
Department of Neurobiology, Harvard Medical School

Endogenous, self-sustaining clocks that drive many physiologic activities and behaviors have been described in organisms ranging from fungi to humans. In mammals, the master circadian clock that drives the sleep-wake cycle and other behaviors is located in the super chiasmatic nucleus of the brain, and there are autonomous clocks in the retinas as well. Only last year the first mammalian circadian gene was identified and named *Clock*. Dr. Weitz suspected that the CLOCK protein would turn out to be a type of transcription factor that acts as a heterodimer, and that along with an unknown team-mate it would lead to expression of a classic circadian gene called *per*. About 20 possible partners were tested in his lab before one, BMAL1, was shown to be co-expressed with CLOCK and PER1 at known circadian clock sites in brain and retina. CLOCK-BMAL1 heterodimers activated transcription from E-box elements found adjacent to the mouse per1 gene and from an identical E-box that is associated with expression of the per gene Drosophila, suggesting a conserved regulatory mechanism. Mutant CLOCK from the dominant-negative Clock allele and BMAL1 formed heterodimers that bound DNA but failed to activate transcription. This is the first time that biochemical activity has been defined for a circadian clock component. Now that the researchers know that CLOCK and BMAL1 can turn on transcription of the per gene, the next question is what turns it off?

Membrane Traffic/Macromolecular Entry

Overview

Each cell is a miniature shipping terminal, busy around the clock as some materials enter and others go out. In endocytosis, essential materials come into the cell through an invagination in the plasma membrane; they are then packaged in vesicles and shuttled to their proper intracellular destinations. The trouble is that these normal endocytic traffic patterns can also be usurped by pathogens, observed Dr. Stephen Harrison, a professor of biochemistry and molecular biology at Harvard. Disease-producing organisms are able to accomplish this because they have evolved ways to breach the cell, escape detection, and make their way to the cytoplasm or the nucleus so they can carry out their harmful business.

The speakers in this session discussed how pathogens solve some of those problems. The first two considered the challenges faced by viruses and bacteria that must get past the cell's plasma

membrane without having membranes of their own to fuse with it. The third paper zeroes in on the details of endocytic traffic, and the final talk considers how host cells react to invasion.

Presentations

Springing the trap - conformational changes associated with poliovirus cell entry
James Hogle, Professor
Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School

Although poliovirus is an extremely well-studied pathogen, a persistent mystery has been how this uncoated virus enters target cells to replicate. Somehow the virus remains stable in stressful environments (such as the highly acid stomach), is stubbornly inert in the face of non-target cells, yet is poised to fuse with the membrane of the right cell.

The secret appears to be a maturation cleavage of a capsid protein precursor (VP0), which occurs in the final stages of virion assembly and locks the virus into a metastable state. This stability enables the virus to survive in the extracellular environment as it travels from cell to cell or host to host. When it encounters the right receptor, poliovirus turns itself inside out, revealing VP4 and the amino terminus of VP1, the two proteins that enable it to attach to membrane. In Dr. Hogle's lab, this transition – as well as a subsequent transition in which RNA is released from the altered particle – were induced in the absence of receptor by gently heating the virus. The idea of a metastable state that can be reversed by contact with the right receptor grew out of structural and genetic studies of the virus, structural studies of assembly and cell entry intermediates, and thermodynamic and kinetic studies of the in vitro conversions. This model parallels emerging ideas about the maturation and cell entry of more complex enveloped viruses such as influenza and HIV.

***Helicobacter pylori* virulence factors in the pathogenesis of gastric diseases**
Cesare Montecucco, Professor
Department of Biomedical Sciences, University of Padova

The ulcer-causing organism *Helicobacter pylori* exploits a normal endocytic pathway to enter to the cells of the gut. Once inside, a virulence factor called VacA throws the cell's normal membrane traffic patterns into chaos. This toxin is activated by low pH and can insert itself into the lipid bilayer. It forms vacuoles that crowd the cytosol and draws large quantities of urease into the cell, causing gridlock that makes it impossible for the cell to carry out its normal functions. Vacuolated cells recycle transferrin normally but are defective in degradation of external ligands such as EGF and in processing of cathepsin precursors, which are secreted in the medium. This may damage the stomach mucosa because acid hydrolases are particularly active in this environment. VacA also inhibits antigen processing, thereby depressing the response of T cells that would ordinarily detect *H. pylori* antigens on the surface of infected cells and destroy them. VacA also recruits inflammatory cells that erode the mucosal layer. These effects help the pathogen evade destruction so that it can establish a chronic infection. *H. pylori* is not only

extremely well adapted to its niche, but also capable of modifying the environment so that the stomach becomes even more hospitable to the organism. In the future, scientists may be able to use *H. pylori* to learn more about the physiology of the gut itself.

Protein interactions in clathrin-coated vesicles
Tomas Kirchhausen, Associate Professor
Department of Cell Biology, Center for Blood Research, Harvard Medical School

HIV and some other viruses sneak into the cell via a doorway that is ordinarily used to bring in key nutrients and other essential proteins. By understanding more about the molecular mechanisms that move membrane proteins within eukaryotic cells, Dr. Kirchhausen and his colleagues hope to pave the way for new antiviral treatments. Clathrin coated pits and coated vesicles, formed from clathrin and associated "adaptor" proteins, recruit proteins from the plasma membrane to the endosomal compartment and also convey proteins from the trans-Golgi network (TGN) to the endosome. Entry of important nutrients (e.g., iron and cholesterol), down-regulation of growth-factor receptors, intracellular transport of MHC molecules, membrane recycling, and delivery of degradative enzymes to lysosomes all rely on clathrin-dependent pathways.

The clathrin adaptor complex is a four-part structure that recognizes two basic motifs. When it encounters one of these signals, the clathrin complex sorts proteins into vesicles for transport within the cell. Dr. Kirchhausen's lab has found that this complex recognizes a peptide on the cytosolic tail of CD4, the coreceptor for HIV, and a motif found in the nef protein made by a major HIV virulence gene. In the presence of both CD4 and nef, the virus rapidly enters the cell via the clathrin coated pit. Once the cell is infected, experiments indicate that HIV with a functioning *nef* gene are able to downregulate the number of CD4 receptors on the cell surface. This may make it easier for formed virions to escape its orbit and proceed to infect other cells. Current investigations are focused on the high resolution X-ray structure of the interface between clathrin and associated adaptor proteins.

Host genetic factors affecting responses to polyoma virus
Thomas Benjamin, Professor
Department of Pathology, Harvard Medical School

There are two sides to every infection, and although a great deal of research focuses on the pathogen it is equally important to understand what happens in the host. When Dr. Benjamin and his colleagues inoculated newborn mice from more than thirty inbred strains with polyoma viruses known to cause widespread solid tumors, they found a tremendous variation in susceptibility and resistance to infection. One of the polyoma strains they tested is so virulent that it typically kills newborn mice before they have time to develop tumors; other strains produce tumors throughout the body. The researchers quickly saw that different mice responded differently to infection: a virus that produced tumors in 100% of one mouse strain might cause cancer in only 2% of another. Some of the mice resisted even the most virulent of the viruses.

The researchers discerned two patterns of susceptibility - one seen only in mice carrying the $H2^k$ haplotype and dependent on endogenous superantigen, and the other found in mice with different MHC types and apparently independent of superantigen activity. Mice with the dominant superantigen gene seem to eliminate T cells that might control tumor growth. Two distinct types of resistance have also been noted. Mice with immunologically based resistance develop tumors, instead of dying immediately, when exposed to the most virulent strains of polyoma. Animals with non-immunological resistance are able to block the spread of the virus from the inoculation site.

Signaling/Cell-Cycle

Overview

The life of the cell used to be seen mainly in terms of inputs and outputs. Cells received signals from the outside world – such as nutrients, hormones, or warning signs from a nearby pathogen. And they reacted with outputs – they secreted, divided, crawled, or drew back. It seemed simple enough. When scientists began to explore the connections between these inputs and outputs, however, the inner lives of cells came to seem infinitely more complex, said Marc Kirschner, head of cell biology at Harvard Medical School and chairman of this symposium session.

Today, the study of these connections, known broadly as "signal transduction," is a pervasive theme in biology research. Some of its revelations are surprising, including the realization that mechanisms for signal transduction are highly conserved across cell types and from one organism to another, Dr. Kirschner said. Tremendously diverse cellular responses are apparently being orchestrated by a relatively small cast of molecules. Just as biologists were once astonished by the versatility of the nucleotide bases in the genetic code, now they are surprised to discover that a small repertoire of signal transduction pathways can have such protean effects in living organisms.

The presentations in this session explored how various signal transduction pathways turn inputs into outputs at the cellular level. The first speaker considered how external signals are translated into particular types of growth in both normal and malignant cells. Three others focused on the roles that different kinases play in regulating cell proliferation.

Presentations

Biological responses to native HGF, MSP and recombinant hybrid factors
Paolo Comoglio, Professor
Division of Molecular Oncology, University of Torino Medical School

Scatter factors control the complex genetic program for branched morphogenesis, in which cells dissociate, migrate in the extracellular matrix, become polarized and form tubules. Plasminogen-related growth factors cause dramatic branching of epithelial cells when added to a culture, and Dr. Comoglio's laboratory focuses on members of this family known as HGF and MSP. Branching is pivotal during the embryonic development of neural, epithelial, and some mesodermal-derived tissues, including muscles and bones. In the adult stage, HGF sustains cell survival and regeneration. The receptors for HGF and MSP are tyrosine kinases encoded by the homologous genes *MET* and *RON*. As members of the *MET/RON/SEA* oncogene family, they are connected with the *ras* pathway and subject to mutations that may lead to malignancy and metastasis. These researchers have identified a novel family of *MET*-related receptors, the SEX-Plexin family, expressed mainly during development, which appear to play a role in scattering.

A specific role for cdk6 in cancer cells?
Philip Hinds, Assistant Professor
Department of Pathology, Harvard Medical School

Dr. Hinds and his colleagues are interested in the retinoblastoma pathway's role in cell cycle control in cancer, particularly the G1 to S phase transition. In human tumor cells, several distinct disruptions of the RB pathway have been described. The retinoblastoma protein (pRB) itself can be inactivated, or the activity of its regulatory kinase, cyclin D/cdk4(6) can be elevated through subunit overexpression or because the inhibitor p16INK4a is lost. Although the end result of these events is the same, different elements of this pathway are activated or inactivated in different tumors, suggesting unique additional functions for these molecules. Dr. Hinds' experiments indicate that cdk6 does not behave the same as cdk4, a related regulatory kinase. Although expression of either can promote tumor growth, FACS analysis of breast tumor cells shows that only cdk6 significantly shortens the G1 phase and dramatically increases the number of S phase cells.

This effect of cdk6 is dependent on kinase activity, since a kinase-inactive mutant cannot shorten G1. Nor can a variant of cdk6 that cannot bind p16 (R31C) promote S phase entry. This is unexpected because the R31 domain's only known function is to interact with the (inhibitory) INK4 protein family. Localization may explain these effects, since wild-type cdk6 is partly nuclear, but the R31C is largely cytoplasmic in late G1. These data suggest that the ink4 binding region of cdk6 may be involved in its nuclear localization. In addition, the inaction of cdk4 in this assay suggests that regulation or function of these kinases may be distinct within the same cell.

eps15 in RTK-mediated signaling
Pier Paolo Di Fiore, Professor
Department of Experimental Oncology, European Institute of Oncology

Signals emanating from receptor tyrosine kinases (RTKs) regulate cell proliferation, differentiation, motility and cyto-architecture. These signals are initially propagated by an array of intracellular proteins, which are substrates for the kinase activity of RTKs. Dr. Di Fiore's laboratory developed a strategy for direct cloning of these molecules. He is especially interested in eps15, a highly conserved protein that has been identified in yeast, *C. elegans*, and mammals.

Experiments comparing the effects of mutants and wild-type proteins have allowed these researchers to make several important observations about eps15. To begin with, they have shown that it is a substrate for the EGF receptor and several other RTKs. They have also found that eps15 is found primarily in endocytic organelles, and that it is an essential component of the endocytic machinery. It also appears to play a role in cancer: certain cell lines become malignant if eps15 is overexpressed, and it is also rearranged in the t(1:11)(p32,q23) translocation associated with certain acute myocytic leukemias. The researchers also characterized the binding preferences and structure of a novel protein:protein interaction domain that is contained within eps15.

The more these researchers learn about eps15, the more versatile it appears to be. In addition to its importance for endocytosis, it helps organize actin in the cytoskeleton, recycles synaptic vesicles, and affects cell proliferation. Eps15 might also be involved in protein and RNA sorting within the cell and with neurogenesis.

Regulation of p70-S6 protein kinase by growth factors and oncogenes
John Blenis, Professor
Department of Cell Biology, Harvard Medical School

The protein p70-S6 kinase was one of the first oncogene- and growth factor-regulated serine kinases to be identified, and there is ample evidence that this enzyme plays an important role in the regulation of cell proliferation. In addition, p70S6k is activated in the absence of added growth factors by the expression of dbl, TIAM-1 and Akt, oncogenes responsible for various hematopoietic malignancies. Despite p70S6k's importance in cell growth, little was known about the signaling pathways that regulate it. Dr. Blenis and his colleagues found that many upstream signals are needed to activate this kinase, leading them to ask how p70S6k can be triggered as rapidly as they know it to be. By manipulating products of the *Src* oncogene, they found evidence that activation factors for p70S6k exist not individually but in large signaling complexes consisting of several protein kinases, phosphatidylinositol 3-kinase and Rho family G proteins. In effect, these complexes appear to reach a consensus about whether p70S6k should be activated before they transmit a signal.

Plant Defense/Pathogenesis

Overview

The major challenge in modern plant biology is to understand plant resistance at the molecular level. This knowledge would help biologists devise rational strategies for protecting crops against pests, which could lead to a new agricultural revolution. These strategies will probably involve manipulating and potentiating natural plant defenses, predicted Dr. Giulia De Lorenzo as she introduced this session of the symposium.

When plants are attacked by any virus, fungus, or other pathogen, they almost always respond by initiating rapid programmed cell death at the invasion site and by synthesizing antimicrobial compounds that rush to the scene. The more that researchers learn about the specific pathways within this general response, the more complex and the more familiar this story appears. "The emerging picture is that the plant's cellular defense is analogous to the defense response in vertebrates," which suggests that self-protection is an ancient evolutionary mechanism , said Dr. De Lorenzo, of the Universita' di Roma La Sapienza.

Plant biologists now know that resistance occurs only when certain complementary genes are present in the plant and its attacker. It is easy to see how plants would benefit from a resistance gene, but less obvious why pathogens would carry avirulence genes that alert the watchful plant to their presence. These avirulence molecules probably have a separate function that confers a survival advantage, although that has yet to be demonstrated. The four papers in this session homed in on the details of plant-pathogen interactions, shedding light on the character of both the attacker and the attacked.

Presentations

***Pseudomonas aeruginosa*, a multi-host pathogen of mice, insects, nematodes and plants**
Fred Ausubel, Professor
Department of Molecular Biology, Harvard Medical School and Massachusetts General Hospital

In order to explore bacterial pathogenesis from an evolutionary point of view, Dr. Ausubel's lab developed a novel strain of the opportunistic human bacterial pathogen *Pseudomonas aeruginosa*. This versatile model, called PA14, is infectious in several mouse models and causes disease in the plant *Arabidopsis thaliana* and in the insects *Drosophilia melanogaster* and *Galleria mellonella*. Moreover, PA14 also kills the nematode *Caenorhabditis elegans*.

The researchers created an array of selective mutations, and screened them for virulence in *the Arabidopsis* and *C. elegans* models. This process identified 16 PA14 mutants that are not only less pathogenic in the *Arabidopsis* and/or *C. elegans* models, but also are generally less pathogenic in the insect and mouse models as well. While many of these had mutations in known virulence genes, 8 out of 10 identified in the *Arabidopsis* screen exhibited alterations to previously unknown genes.

On the host side of the equation, Dr. Ausubel sought genes that are important for fighting off the bacteria. He found that some *C. elegans* mutants, which experience high oxidative stress when exposed to a small molecule secreted by PA14, are especially likely to be killed quickly by the bacterium. A different set of *C. elegans* mutants, which appear less vulnerable to oxidative stress, are more resistant to the bacteria. These studies of PA14 have identified a cluster of novel pathogenesis genes that could be targets for anti-infective compounds, Dr. Ausubel said.

Recognition of non-self molecules in plants: the role of polygalacturonase-inhibiting proteins (PGIPs)

Felice Cervone, Professor
Department of Plant Biology, Universita' di Roma La Sapienza

Like an animal's skin, the cell wall of a leaf is the first battleground between pathogen and host. Here it may be decided whether the plant is going to resist disease or fall prey to it. And just as the human immune system is activated by recognition of a pathogenic antigen, plants resistance mechanisms are turned on when they recognize pathogenicity factors produced by enemy microbes. Fungi secrete *endo*polygalacturonases (PG), enzymes that not only macerate the plant cell wall so that fungi can enter but also elicit a defensive response from the plant. The plant's protective response is triggered by fragments of its own cell wall, called oligogalacturonides, that are released when PG interacts with polygalacturonase-inhibiting proteins (PGIPs), which are common in the cell walls of plants. PGIP recognizes fungal PG and modulates its enzymatic activity so that oligogalacturonides accumulate, thus sounding a loud alarm. Like most plant resistance genes, PGIP belongs to the superfamily of leucine-rich repeat (LRR) proteins. These proteins specialize in the recognition of non-self molecules, which is a key step in immunologic functioning.

Molecular basis of specificity in polygalacturonase-inhibiting proteins (PGIPs)

Giulia De Lorenzo, Professor
Department of Plant Biology, Universita' di Roma La Sapienza.

Like the previous speaker, Dr. De Lorenzo studies polygalacturonase-inhibiting proteins (PGIPs). Her work centers on the role of leucine-rich repeats (LRR) in recognition and regulation of specific polygalacturonases (PG) produced by pathogenic fungi. Almost one hundred proteins with LRR have been identified in animals and plants. These are needed for molecular recognition in processes as diverse as cell adhesion, signal perception in cell development, resistance to pathogens, DNA repair, and RNA processing. Structural biologists are especially intrigued with the functional versatility of the LRR protein.

Just as the humoral immune system is able to form antibodies against myriad attackers, plants must recognize and resist a wide array of pathogens. It is the functional flexibility of the LRR proteins that enables them to protect against a range of phytopathogenic fungi, Dr. De Lorenzo hypothesized. In her laboratory, a series of experiments has explored the structure-function

relationships of PGIP1 and PGIP2, proteins that differ only by a few amino acids yet can recognize and regulate the activity of polygalacturonases (PGs) from a wide variety of fungi. Site-directed mutagenesis has been used to improve or disable recognition function and to change ligand binding. These experiments suggest that in the future it may be possible to create custom-made receptors on plant surfaces that could make them highly resistant to specific pests.

Signal transduction pathways specifying bacterial disease resistance in *Arabidopsis thaliana*
Brian Staskawicz, Professor
Department of Plant Biology, University of California

This presentation illustrates one of the concepts introduced by Dr. De Lorenzo when she opened this session: the right genes must be present in both pathogen and host for resistance to occur. Dr. Staskawicz has been studying *RPS2*, a gene that enables *Arabidopsis thaliana* to fight off strains of *Pseudomonas syringae* containing the corresponding avirulence gene, *avrRpt2*. In order to understand specific molecular events in pathogen recognition and subsequent signal transduction in this plant, his laboratory constructed an epitope-tagged *avrRpt2* avirulence gene and produced polyclonal antisera that detect the AvrRpt2 protein. With these tools, they have been able to identify AvrRpt2 protein in induced bacteria, inoculated plants and stable transgenic plants. These results suggest that this protein, which sets in motion a signal transduction cascade that leads to a protective response, is processed as the pathogen enters the plant cell or just after entry. Dr. Staskawicz and his colleagues are now trying to pin down the exact location of AvrRpt2 processing and to determine the sub-cellular location of the protein once it has penetrated the cell. They are also developing a screening technique, using transgenic *Arabidopsis* plants, that will enable them to screen for mutations in resistance genes that make plants especially vulnerable to pathogen attack.

Transcription/Regulation

Overview

This final set of presentations shifts the focus from the cell membrane to the nucleus, where DNA is transcribed into complementary RNA, which goes on to make the proteins that are essential for all forms of life. In this session, speakers explored the workings of transcription factors – which initiate or regulate DNA transcription – and regulatory sequences, whose presence on the DNA affects the rate of transcription initiation. Regulation is an especially complex topic because each gene has its own regulatory proteins and binding sites.

Dr. Pier Giuseppe Pelicci of the European Institute of Oncology introduced the four papers presented here. The first concerned a class of intermediary proteins that bridges a communication gap in a transcription pathway. The second described the role of a transcriptional repressor in human cancer, whereas the third took an in vivo look at transcription factors in normal development. The final talk focused mainly on signaling mechanisms of certain receptors in renal carcinoma, and dealt indirectly with transcription and regulation.

Presentations

CBP and P300: General co-activators for signal dependent transcription
Marc Montminy, Professor
Department of Cell Biology, Harvard Medical School and Joslin Diabetes Center

Numerous hormones and growth factors stimulate the expression of cellular genes by inducing the phosphorylation of specific transcription factors. Dr. Montminy's focus is on a new class of proteins called co-activators, particularly two substances called CBP and P300. In the pathway he studies, a signal received by a membrane receptor is amplified by the second messenger cAMP, which stimulates phosphorylation of the transcription factor CREB at Ser133. Instead of interacting directly with the basal transcription machinery, CREB uses intermediaries to create unique signals for regulating the expression of target genes. Dr. Montminy and his colleagues have shown that Ser phosphorylation of CREB recruits the general co-activators CBP and P300, which act together to stimulate transcription of specific genes. The CBP/P300 complex is able to recognize the target gene, bring it into contact with the appropriate promoter, and modify nucleosomes as needed. Unless CREB is properly phosphorylated, their experiments show that this process will be derailed.

Pathogenesis of acute promyelocytic leukemia
Pier Giuseppe Pelicci, Professor
Department of Experimental Oncology, European Institute of Oncology

Some patients with acute promyelocytic leukemia (APL) respond better to treatment with retinoic acid (RA) than others. This difference in clinical response can be traced to a translocation that puts the gene for the α-retinoic acid receptor (RARα) next to one of three different genes. Patients have fusion proteins of RARα and one of three different nuclear proteins (PML, PLZF, NPM). In vitro, the three fusion proteins have different biological effects on RA-mediated differentiation of promyelocytes, which puts the disease into remission by stopping uncontrolled proliferation. PML/RARα stimulates terminal differentiation, while the PLZF/RARα fusion protein inhibits it. PLZF/RARα appears to block differentiation by associating with histone deacetylases, and patients with this mutation do not respond well to RA treatment. RA is thought to act on a gene called *p21*, which regulates the cell cycle. Expression of this gene must be upregulated for cells to commit to differentiation, and this is what occurs in patients with PML/RARα. Dr. Pelicci noted that a transcriptional repressor that resembles PLZF/RARα has recently been detected in some patients with acute myeloid leukemia, suggesting that the APL scenario may be played out in other cancers as well.

Emx2 in the developing cerebral cortex

Edoardo Boncinelli, Professor
DIBIT - Institute San Raffaele

Dr. Boncinelli's laboratory explores the effects of various homeobox gene families in the development of the vertebrate nervous system. *Emx1* and *Emx2* are two vertebrate homeobox genes that are expressed in the rostral brain of mouse embryos and in the human brain. They are related to the fruit fly gene empty spiracles (*ems*) and to a similar gene in planeria. In the developing cerebral cortex, *Emx1* is expressed in most neuroblasts and neurons at all stages of development, whereas *Emx2* expression is restricted to actively proliferating neuroblasts of the ventricular zone.

Emx2 mutations have been reported in sporadic cases of human schizencephaly. In this rare congenital abnormality, a full-thickness cleft separates the cerebral hemispheres: narrow clefts may have few if any clinical consequences, medium clefts are often associated with seizures, and large clefts are probably incompatible with life. Large portions of the cerebral hemispheres may be absent and replaced by cerebrospinal fluid. Although mutations in schizencephaly patients cluster in one of *Emx2*'s three exons, Dr. Boncinelli cautioned that other, highly lethal mutations may go undetected in embryos that spontaneously abort. There is a good correlation between the extent of genetic mutation and the size of the defect in the patient. The presence of *Emx2* mutations in cases of schizencephaly indicates that *Emx2* gene products are needed for normal development of the human cerebral cortex, and the researchers suspect that they play a role in proliferation or migration of cortical neuroblasts.

Signaling by *MET* oncogenic mutants

Alberto Bardelli, Assistant Professor
Division of Molecular Oncology, University of Torino Medical School

The hypothesis that genes for tyrosine kinase receptors could be important in human cancers has recently been confirmed by the identification of oncogenic mutations in *RET, KIT* and *MET*. These discoveries raise the hope that new therapeutic strategies might arise from a molecular insights into how these genetic lesions bring about neoplastic transformation. As a rule these mutations deregulate kinase activity, although how this leads to oncogenic transformation of cells is unclear. A pattern is emerging, however, as the same residues mutated in the kinase domain of *RET* and *KIT* turn out to be altered in *MET* from patients with hereditary papillary renal carcinomas (HPRC). Scatter factors are among *MET*'s ligands, and in HPRC runaway branched morphogenesis causes abnormal tubular structures to arise in the kidney.

Dr. Bardelli and his colleagues have identified several consequences of *MET* mutations in HPRC patients. Kinase activity increases, substrate specificity changes, there is increased phosphorylation of a peptide mimicking the receptor tail, and constitutive coupling of the receptor to signal transducers is essential for oncogenic transformation. Dr. Bardelli predicts that *MET* mutations will prove to be important in more and more cancers, having already been identified in some primary tumors of the GI tract, prostate, and larynx.

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Amanda J. Pullen, Ph.D.
Administrator

Cheryl R. King
Administrative Assistant