1ST ANNUAL SYMPOSIUM
GIOVANNI ARMENISE-HARVARD FOUNDATION FOR ADVANCED SCIENTIFIC RESEARCH

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ABOUT THE SYMPOSIUM

A new scientific tradition was inaugurated in Erba, Italy, in June 1997. The first annual symposium of the Giovanni Armenise-Harvard Foundation for Advanced Scientific Research brought together nearly 100 biomedical researchers from Harvard Medical School, Harvard-affiliated Massachusetts General Hospital, and four leading Italian scientific institutions. Attendance was limited to researchers who had received grants from the Foundation, which gave participants an opportunity to present their latest findings and explore future collaborations in a collegial and private atmosphere.

The Foundation's philosophy is that multidisciplinary, basic science research will lead to practical advances in medicine and agriculture that will ultimately improve the lives and health of people around the world. In addition to supporting projects that use the tools of molecular biology to unlock the secrets of living cells, the Foundation actively encourages researchers on opposite sides of the Atlantic to pool their expertise and work together. This three-day symposium promoted the kinds of informal discussions that often give rise to new and fruitful collaborations.

Oral and poster presentations at the Foundation's 1st Annual Symposium were grouped under five headings:

* Cellular and molecular neurobiology
* Signal transduction
* Control of cell proliferation
* Development
* Structural biology and enzymology

This report is organized along the same lines. Each of the five sections begins with a general overview of the topic, followed by brief summaries of presentations made at the symposium.

The Foundation awarded its first scientific grants in January 1997, and today sponsors collaborative research at five Italian institutions. Biomedical centers are the European Institute of Oncology in Milan, 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 Milan. The first agriculture-related grant has been made to the Dipartimento di Biologia Vegetale at the Universita' di Roma La Sapienza.

1. CELLULAR AND MOLECULAR NEUROBIOLOGY

Overview

Neurons – the cells of the brain and nervous system – make it possible to see, hear and smell the world around us, to think about everything from Greek tragedy to grocery shopping, and to propel ourselves up a cliff or merely across the street. Not surprisingly, the study of these versatile cells is one of the fastest growing areas of biomedical research. When neurons fail to develop properly, when they lose their ability to function, or when they die prematurely, the impact on human health is tremendous. A young athlete's spinal cord is crushed on the playing field, a middle-aged woman inherits Huntington's disease, and millions of people live long enough to fall prey to late-life disorders such as Parkinson's or Alzheimer's disease.

Clearly there is an urgent need to know more about neurons, how they're constructed, and how they carry out their daily chores. As scientists unravel these mysteries, the hope is that their findings will be possible to treat or prevent diseases that have stymied medicine so far. Perhaps an injured spinal cord can be induced to repair itself, or the Huntington's mutation corrected, or aging brain cells fortified against deterioration. Although none of these clinical advances is going to happen tomorrow, the first steps are already being taken in basic science laboratories around the world.

The papers presented during this session describe some of those early steps. One of the most striking features of neurons is their ability to build up and release an electrical charge that rushes from the cell body to the end of its axon, where it triggers a spray of chemical messengers that communicates with the next nerve cell. The first two papers in this Symposium session concern the regulation and measurement of electrical excitation and the third focuses on how neurotransmitters are released at the end of the axon. The fourth describes biomechanical aspects of hearing that may be sabotaged by certain inherited forms of deafness, the final paper describes how genetically engineered mice can be used to study retinal networks.

Presentations

The molecular machinery of voltage-activated K^+ channel

Gary Yellen, Associate Professor
Department of Neurobiology, Harvard Medical School

Voltage-activated K^+ channels are one of several types of voltage-gated channels that generate the electrical excitability and rhythmic activity of neurons and other cells. These are narrow pores in the plasma membrane of certain cells, such as those of nerve and muscle, that selectively allow potassium ions to rush in or out. Genetic defects in the proteins that make up these passageways have been linked to human diseases called K^+ channelopathies. Episodic ataxia and Bartter's syndrome involve loss of muscle control, whereas long QT syndrome is a cardiac abnormality.

The flow of K+ ions across the membrane is regulated by a process called "gating." When the voltage across the membrane becomes more positive, the channels open so that K+ can exit

rapidly. When the membrane has recovered its equilibrium, they snap shut. Dr. Yellen's analysis reveals that instead of being a swinging door that opens in either direction, the K+ channel actually involves two distinct doorways made of different proteins. Scientists used site-directed mutagenesis of cloned K+ channels, in which cysteine residues are added to the proteins, to discern the separate movements of these gates.

Disease or the use of certain drugs – including some used to treat cardiac arrhythmias or depression – can interfere with normal K+ gating and keep cells from functioning normally. A more detailed understanding of gating mechanisms could pave the way for new therapeutic agents.

Loose patch measurements of single quanta at individual hippocampal synapses in culture

Antonio Malgaroli, M.D.
Unit of Neurobiology of Learning, DIBIT, Scientific Institute San Raffaele

Signals are transmitted from one neuron to the next at specialized contact points called synapses: electrical excitation in the presynaptic (sending) cell releases neurotransmitters, which convey a chemical message across the narrow synaptic cleft to the postsynaptic (receiving) cell. This interaction is typically studied by recording electrical responses from the body of the postsynaptic cell. Although this aggregate measurement reflects the cell's response to the combined activity of many thousands of synapses, it tells scientists nothing about what is happening at an individual synapse. Dr. Malgaroli's laboratory has modified a familiar laboratory technique, patch-clamp recording, in order to record the activity of individual hippocampal synapses.

The researchers used a fluorescent dye to identify isolated presynaptic boutons, the tiny terminals of the sending cell. These were captured in a pipette that was loosely sealed on the corresponding patch of postsynaptic membrane. An elaborate recording system allowed scientists to compare miniature-excitatory postsynaptic currents (mEPSC's) – associated with release of neurotransmitters by the bouton in the pipette – with mEPSC's recorded simultaneously from the cell body. The average peak conductance at a single synapse is about 900 pS, corresponding to the opening of 90 AMPA-type glutamate receptor channels. The variability in this conductance was around 30%, which matches observations made for the junction between nerve and muscle cells. This variability is surprisingly large, and strongly suggests that it takes more than the contents of a single vesicle on the presynaptic cell to saturate a postsynaptic receptor.

Protein phosphorylation and the control of neurotransmitter release

Flavia Valtorta, M.D.
Department of Neurobiology, DIBIT, Scientific Institute San Raffaele

Cartoons of neurons in action typically show a nerve impulse, or action potential, racing down the axon and into the presynaptic terminal, where it sparks the release of neurotransmitters that carry chemical messages to a second neuron. But exactly what goes on in the axon terminal? When Dr. Valtorta's laboratory set out to answer this question, they uncovered a family of phosphoproteins that they call *synapsins*. Abundant in the nerve terminals, synapsins act as targets for multiple second messenger systems.

Changes in the levels of second messengers activate the phosphorylation of specific protein substrates by protein kinases, which in turn determines what quantity of neurotransmitter the presynaptic terminal will release. Synapsins appear to act as a kind of signal broker, determining how many vesicles are immediately available to release neurotransmitters.

Sensory transduction and adaptation in auditory hair cells: Biomechanics and deafness genes

David Corey, Professor
Department of Neurobiology, Harvard Medical School

For 20 years, Dr. Corey's research has explored the biomechanics of hearing. He focuses on how sound waves produced by mechanical forces – such as the movement of leaves or the clash of cymbals – are converted into nerve impulses. This process is called sound transduction, and scientists hope that by understanding it in greater detail they will be about to identify protein defects associated with some forms of inherited deafness. Genetic studies have identified more than 30 mutations that can cause hereditary deafness, and there may turn out to be even more.

Sound waves vibrate the eardrum and then the three tiny bones of the middle ear. The last of these wiggles a layer of tissue at the base of the cochlea, the shell-like tube in the inner ear that is essential for hearing. This movement stimulates the hair cells, which are carpeted in microscopic protrusions called stereocilia. The oscillation of the stereocilia opens transduction channels that allow K^+ to flow into the hair cells, which in turn leads to membrane depolarization. Instead of being open and closed by electrical charges (like the voltage-gated K+ channels described earlier by Dr. Yellen), these doorways appear to be mechanically pulled open by the stretching of fine filaments in the hair cells. Dr. Corey attributes this to a specific motor protein, myosin-Iß g, present in the tips of the cilia. It may be that this protein is damaged or missing in some types of heritable deafness.

A transgenic approach to retinal networks

Elio Raviola, Bullard Professor of Neurobiology
Department of Neurobiology, Harvard Medical School

Using a transgenic mouse model to study the functioning of retinal networks, this study focused on the dopaminergic amacrine cells (DA cells) of the inner plexiform layer of the retina. These cells, which coexist with several other types of neurons, turn out to be surprisingly versatile: it appears that they behave one way in the light and another in the dark.

The researchers were able to observe the activity of DA cells by using human placental alkaline phosphatase as a marker. Close observation revealed that DA cells are often connected with two very different types of neurons. One generates gamma amino butyric acid (GABA) and glycine, inhibitory neurotransmitters that can send a "don't fire" message to the postsynaptic DA cell; the other specializes in releasing glutamate, an excitatory neurotransmitter that tells cells to fire an action potential.

In the absence of messages from other cells, DA cells are known to generate action potentials of their own accord. Dr. Raviola postulated that, in the dark, this inappropriate impulse is restrained by the action of GABA and glycine. In the light, neighboring cells release glutamate that allows DA cells to generate action potentials.

2. SIGNAL TRANSDUCTION

Overview

Although extracellular space teems with millions of messages, each type of cell is programmed to respond to only a select subset of them. Although a few of these messages enter the cell as tiny molecules that can diffuse across the membrane without help, most signals are actively picked up by receptor proteins on the cell surface. These receptors act as transducers: they convert the external binding event into a series of intracellular signals that determine how the cell will behave, whether it will divide or stay quiescent, whether it will live or die. A relay team consisting of enzymes, proteins, and other intracellular mediators (or second messengers) executes a series of handoffs that transports the message from the receptor to the nucleus of the cell. Once the message arrives it controls gene expression, which in turn determines what the cell will do next.

This portion of the symposium focused on various aspects of cell signaling in normal and cancer cells. Two papers concern a family of proto-oncogenes that code for enzyme-linked receptors needed for growth and development of cells in the epithelium and liver, one describes the actions of a versatile intracellular enzyme, and the final presentation examined a protein-protein interaction that exerts diverse effects on intracellular activity.

Presentations

The HGF receptor family

Paolo Comoglio, Professor and Chairman
Institute for Cancer Research, University of Torino School of Medicine

Hepatocyte growth factor receptors (HGFRs) are one branch of a sprawling family of receptor tyrosine kinases that has been extensively studied since the early 1980s. When HGFRs are activated by the binding of hepatocyte growth factor (HGF) or macrophage stimulating protein (MSP), they first change the shape of their own intracellular domain, then initiate a cascade of events that are crucial to the growth and differentiation of epithelial cells in normal and malignant tissues.

This presentation focused on three structurally related tyrosine kinases that belong to the HGFR family. These are encoded by three proto-oncogenes – *MET*, *RON* and *SEA* – that have been extensively studied at Dr. Comoglio's institution. (A proto-oncogene is the normal form of a gene that stimulates cell growth; abnormal changes can turn it into an oncogene that causes the runaway growth typical of cancer cells.) The researchers used transgenic mice to explore several aspects of proto-oncogene activity, including synthesis regulation and related phenotypes,

binding, and the interaction between proto-oncogenes and other proteins that act as signal transducers. By discovering how activated HGFRs can transmit such a wide range of biological signals, the researchers hope to learn more about the malignant transformation of normal cells.

Close and distant relatives of the *c-MET* gene.

Luca Tamagnone, M.D., Ph.D.
Institute for Cancer Research, University of Torino School of Medicine

This report focused on the recent discovery of genes that are related to the proto-oncogenes *MET, RON* and *SEA*. The prototype member of this new group has been named *SEX*, and related genes have been dubbed *SEP, OCT* and *NOV*. The genes are expressed in the early development of fetal brain and kidney and are critical to the normal development of neuronal tissue.

Like other members of the receptor tyrosine kinase clan, the newly identified genes code for large transmembrane proteins. These genes have cysteine-rich extracellular domains, and the DNA sequence of their cytoplasmic domains (the part of the protein that protrudes into the cell) is nearly identical in all the relatives of *MET*. Well-conserved sequences are thought to remain stable because they do something very important, and researchers are working to define what the function of this one might be. Future studies will also look for physical changes associated with elevated expression of these genes and to learn more about how they regulate development.

Signaling via PI (3)-Kinase

Lewis Cantley, Professor
Department of Cell Biology, Harvard Medical School

When a growth factor, hormone, or other stimulatory molecule binds to a receptor on the cell surface, a second messenger transduces the signal to the interior of the cell. Second messengers are formed when one of two enzymes – either phospholipase C or phosphoinositide 3-OH kinase (usually referred to as PI (3)-kinase) – alters the phospholipids found in the membrane itself. In different types of cells, the PI (3)-kinase signaling pathway is activated by different growth factor receptors or hormones. The types of proteins that PI (3)-kinase interacts with depends on the cell type, and the varied nature of these proteins may help explain how the PI (3) K enzymes regulate so many diverse functions. These include the survival, transformation and movement of cells, in addition to intracellular trafficking.

EH: a novel protein-protein interaction domain

Pier Paolo DiFiore, M.D., Ph.D.
Department of Experimental Oncology, Institute Europeo di Oncologia

Small protein modules that are made and folded independently often join together to form larger proteins that have important jobs to do. Dr. Di Fiore and his colleagues recently identified a new protein-protein interaction domain, located on two signal transducer proteins, *eps15* and *eps15R*, as well as on other yeast and nematode proteins. They call this protein-protein interaction site EH (for Eps15 homology).

In laboratory experiments, the researchers found that EH domains from *eps15* and *eps15R* bind to peptides containing the asparagine-proline-phenylalanine (NPF) motif. When they used EH domains to screen expression libraries, they found a number of putative EH interactors including the human homologue of NUMB, a developmentally regulated gene of Drosophila, and RAB, the cellular cofactor of ReV, a regulatory protein in HIV-1. Each of these interactors possessed the crucial NPF motif. Analysis of these findings suggests that that EH domains are involved in the transport and sorting of molecules within the cell, including cell trafficking, cytoskeleton organization, endocytosis and vesicle recycling.

3. CONTROL OF CELL PROLIFERATION

Overview

Because society imposes certain restraints on individual behavior, most people would not consider digging a swimming pool on someone else's property or moving their children into the neighbor's spare bedroom. In a similar fashion, the 30 trillion cells of a healthy human body can only live in harmony if they adhere to a complex system of rules. Normal cells, for example, divide only when other cells in their vicinity give them the go-ahead. This insures that one hand won't be noticeably larger than the other, and that the liver won't crowd the stomach out of its rightful place. This collaborative approach ensures that each tissue will attain a size and architecture appropriate to the body's needs. Cancer cells violate this scheme; they shut out external messages that tell them when to stop dividing, and single-mindedly proliferate according to a selfish agenda of their own.

Cancer cells are essentially good cells that have gone bad, and this session examined some of the wrong turns they can take. Speakers described two different ways in which the ubiquitin system, which normally marks cells for destruction when they have outlived their usefulness, can go awry. The ability of human papilloma virus or Epstein-Barr virus to disrupt normal constraints on growth were discussed, as well as the possible tumor-suppressing capacity of various proteins. Another intriguing presentation suggested that plants, like animals, have a mechanism for recognizing their potential enemies.

Presentations

Ubiquitin isopeptidases in growth control

Giulio Draetta, Division Director
Department of Experimental Oncology, Institute Europeo di Oncologia

Many proteins are essential during certain phases of the cell's reproductive cycle, but once they've done their job they must be swiftly eliminated. These proteins are marked for death by the attachment of a small protein called ubiquitin; once tagged, they will be hauled off and destroyed by the cell's internal garbage collectors. Key proteins regulated by ubiquitination include the tumor suppressor p53, the c-jun and c-fos transcription factors, the cyclin A and B

proteins, the NFkB transcription factor and its inhibitor IkB, and the p27 cyclin-dependent kinase inhibitor.

Now there is preliminary evidence that certain types of ubiquitin isopeptidases can sneak in and remove the polyubiquitin chain from some proteins that have been labeled for timely destruction – perhaps causing them to stay too long and cause abnormal growth. Although scientists have not directly observed this in animals, there is experimental evidence that certain isopeptidases are implicated in growth control. Because ubiquitination regulates the degradation of so many key proteins in humans, Dr. Draetta and his colleagues decided to search for human ubiquitin isopeptidases that might play a role in growth and cell cycle control.

They have identified and characterized a novel ubiquitin isopeptidase, called UBPY, that appears to play a critical role in controlling cell cycle progression. Now they are seeking to characterize others, and to figure out which proteins are hanging around too long due to their actions.

Structure and function studies on the E6AP family of ubiquitin protein ligases.

Peter Howley, Professor and Chairman of Pathology
Department of Pathology, Harvard Medical School

Just as ubiquitin isopeptidases may encourage runaway cell growth by removing the tag that rightly identifies proteins for timely destruction, ubiquitin protein ligases appear capable of the opposite effect. By prematurely attaching a "kill me" sign to proteins that are desperately needed for normal control, these ligases may set the stage for uncontrolled cell growth. This appears to happen when people are infected with one of the carcinogenic strains of human papillomavirus (HPV). This finding has important clinical implications, as these dangerous HPVs are responsible for 90% of all cervical cancer as well as with tumors of the vagina, vulva, penis, and perianal region.

Cancer-causing strains of HPV encode two viral genes that become integrated into the DNA of cervical cells: E6 produces a protein that teams up with a ubiquitin protein ligase that has been labeled E6AP (E6 associated protein). Together, E6 and E6AP target and destroy the tumor suppressor protein p53. With p53 out of the way, potentially carcinogenic mutations accumulate in the cell's DNA. Another HPV gene, E7, produces a protein that targets a second tumor suppressor, the retinoblastoma protein (pRB), thus permitting the cell to divide uncontrollably.

The role of the human papillomavirus E7 oncoprotein in cervical carcinogenesis

Karl Munger, Assistant Professor
Department of Pathology, Harvard Medical School

This presentation provided additional detail about the actions of the E7 ubiquitin protein ligase produced by carcinogenic strains of human papillomavirus (HPV). As mentioned in the previous talk by Dr. Howley, E7 appears to interfere with the tumor-suppressing activity of the retinoblastoma protein (pRB). In normal cells, this protein acts as a brake on the cell division cycle; in many human cancers pRB is inactivated and cells are able to divide non-stop as a result.

In the course of this study, healthy human cells were engineered to express normal HPV E7 or a mutant form incapable of marking pRB for premature destruction. The researchers found that pRB levels fell in the cells that expressed active HPV E7, but remained high in cells with the biologically inactive form. When the researchers measured the stability of pRB in cells expressing normal and mutant E7 proteins, they found that exposure to normal E7 quickly destabilized pRB. Once this tumor-suppressing protein is weakened by the action of E7, the researchers believe that other molecules move in for the kill. They are seeking to identify these assassins, and hope that eventually a clearer understanding of these mechanisms could lead to improvements in cervical cancer treatment.

How an Epstein-Barr virus oncogene alters cell growth

Elliot Kieff, Harriet Ryan Albee Professor
Department of Microbiology and Molecular Genetics & Medicine, Harvard Medical School

Human papillomavirus is not the only virus, of course, that that has been associated with human cancers. Since the 1960s, Epstein-Barr virus(EBV) has been linked to cancers found in Africa and Asia. In the industrialized world, EBV rarely caused more than a sore throat or mononucleosis – until the number of patients with compromised immune systems began rising due to AIDS to the use of drugs that prevent rejection of organ transplants.

Some years ago, Dr Kieff's team found that EBV makes a substance called latent membrane protein (LMP-1) which appears to play a part in triggering EBV-associated cancers such as lymphoproliferative disease, nasopharyngeal cancer, or Hodgkin's disease. The next step was to identify LMP1's cellular accomplice – a human protein that, when altered by LMP1, promotes abnormal cell growth. The researchers used genetic techniques to find a likely candidate, dubbed LAP1, which promotes cell growth when it acts in concert with LMP1.

The researchers then considered what else LAP1 might do in human cells. When they compared its DNA sequence with other known genes, they found that it resembles the mouse gene for a protein that helps transmit signals from a growth factor receptor (TNFR). When they tested this idea, LAP1 interacted with three types of TNFR receptors. One of these, called CD40, leads to cell proliferation when activated. It is typically found on malignant cells from patients with Hodgkin's disease or nasopharyngeal carcinoma.

As a result of these investigations, Dr. Kieff now regards LAP1 as a protein that contributes to cancerous cell proliferation in two distinct ways: it causes trouble by interacting with the viral protein LMP1 or, alternatively, it can stimulate the CD40 growth factor receptor. A clearer understanding of these pathways may advance the search for novel anti-cancer drugs.

Structure-function studies on PGIP, a plant LRR protein specialized for recognition of non-self molecules.

Giulia De Lorenzo, Associate Professor
Department of Biologia Vegetale, Universita di Roma "La Sapienza"

Unlike humans, plants do not have antibodies to protect them against disease-producing organisms. Yet plants are not totally at the mercy of their enemies, because they do have a sophisticated defense system that springs into action at the sites of an infection.

Proteins that encode leucine-rich repeats (LRRs) play a central role in the recognition of foreign invaders. Dr. De Lorenzo described how polygalacturonase-inhibiting protein (PGIP), a type of LRR, recognizes polygalacturonases – harmful enzymes that fungi use to damage plant cells. PGIP's ability recognize polygalacturonase secreted by an invader, and to oppose its activity, is a valuable model system for understanding how plants recognize non-self molecules.

Molecular genetics of acute promyelocytic leukemia

Pier Guiseppe Pelicci, Professor
Department of Experimental Oncology, Institute Europeo di Oncologia

Dr. Pelicci described the identification of a promyelocytic leukemia (PML) protein that appears to suppress tumor growth by inducing apoptosis (death) in malignant cells. Researchers studied PML's role in the context of a leukemia-specific fusion protein called RAR, a protein known to regulate cell differentiation. Experiments in transgenic mice revealed that 30% of the PML/RAR mice developed leukemia within 1 year of life.

Promyelocytic leukemia arises from a chromosomal translocation that gives patients with this disease a better prognosis than those with many similar leukemias. The genetic abnormality in PML makes cells sensitive to large doses of retinoic acid, and this treatment often results in remission. Researchers are now seeking to learn more about how retinoic acid induces differentiation of leukemic cells and exactly what role the PML and RAR proteins might play.

Control of invasiveness by *MET* and *RON*

Silvia Giordano, M.D., Ph.D.
Institute for Cancer Research, University of Torino School of Medicine

Previous speakers described three structurally similar receptor tyrosine kinases, members of the hepatocyte growth factor receptor (HGFR) family, that are encoded by the proto-oncogenes *MET, RON* and *SEA.* In this study, the researchers set out to determine whether *MET*, acting on its own, could induce changes leading to a metastatic type of cancer cell – one that can migrate from the primary tumor site and cause malignant growth elsewhere in the body.

The investigators deliberately induced a mutation in the multifunctional docking site of the *Met* protein, which is known to bind any of several intracellular transducers. The study showed that while a single point mutation affecting signal transduction promoted malignant transformation, it resulted in cancer cells with no metastatic potential. These results led the researchers to conclude that *MET* can't generate metastatic cells on its own, and that this probably requires concomitant activation of one or more other signaling pathways. The protein encoded by *RON* may or may not be a player in this process.

4. DEVELOPMENT

Overview

One of the most absorbing quests in all of biology is the effort to understand how a creature as complex as a human being can arise from the cloning of a few cells, all of them containing identical genetic information. The study of development – how cells differentiate and organize themselves into patterns during embryogenesis – has been revolutionized by the rise of molecular biology. In addition to observing the myriad ways in which cells change and rearrange themselves, scientists are increasingly able to identify genes that choreograph individual steps in this dazzling performance.

As the presentations in this session suggest, scientists often rely on animal models to shed light on the complexities of human development. The topics covered here include genetic determination of cell fate and morphogenesis, formation of neural maps in the embryonic brain, gene dosage and myelination in the peripheral nervous system, and diversification of fiber types during the development of skeletal and cardiac muscle.

Presentations

Identification of new regulatory components in mesoderm and neuronal patterning

Marc W. Kirschner, Chair, Carl W. Walter Professor of Cell Biology
Department of Cell Biology, Harvard Medical School

The *Xenopus* frog is one of the classic models for studying how the three germ layers of the early embryo give rise to the organs and systems of the adult. Dr. Kirschner discussed the identification of signals that regulate cell fate and how these signals promote morphogenesis. This study focused on regulatory factors that arise in the mesoderm, but then act on adjacent ectoderm to encourage the formation of the neural tube – the precursor of the nervous system.

So far, the researchers have cloned numerous genes in their studies of the structure and function of regulatory components. In this presentation, Dr. Kirschner concentrated on two: XOMBI is a gene that is involved in morphogenesis, and CYRANO determines the competence of embryonic ectoderm to form the neural plate.

Eph ligands and receptors as guidance labels in the development of neural maps

John Flanagan, Associate Professor
Department of Cell Biology, Harvard Medical School

The connections between the eye and brain are so specific that if they are experimentally rearranged in an animal, the creature will no longer be able to make sense of what it sees. These tight connections between retinal axons and the optic tectum (the part of the brain that interprets visual signals) are thought to be governed by neural maps established early in development. Over the years there have been various hypotheses about how these maps are made. In this presentation, Dr. Flanagan described a new family of receptors and ligands that appear capable of guiding the proper wiring of neuronal connections.

In the 1960s, the leading theory was that each axonal neuron carried a specific molecular address on its surface, that these were readable by corresponding molecules on individual brain cells, and that this led each cell to make the right connection. Unfortunately, the number of unique addresses needed for this system far exceeds the number of genes available to code for them. This realization gave rise to the idea that instead of having unique molecular tags, incoming optic neurons had varying quantities of the same tag. The idea was that address information was probably spread across the incoming fibers in a concentration gradient, which was mirrored by a reverse gradient of identifying information spread across cells of the optic tectum. This would provide spatial coordinates that would enable each incoming neuron to find its place in the brain.

It is this hypothesis that Dr. Flanagan's work supports. In the optic tectum, his group found a molecule that they call ELF-1. They subsequently discovered that ELF-1 binds to a receptor, now called Mek 4, found on retinal neurons that map to the tectum. Even more importantly, ELF-1 is distributed over the tectum in a gradient as is Mek 4 in the retina. These gradients are complementary, and are present during the phase of embryonic development when the eye and brain make their connections.

Normal peripheral nervous system myelination depends on precise dosage of the P0 glycoprotein gene

Lawrence Wrabetz, M.D.
Department of Myelin Biology, DIBIT, Scientific Institute San Raffaele

Myelin is the essential insulator that enables an action potential to travel swiftly and efficiently from a nerve cell to a muscle. Without myelin, this electrical message would slow down or be lost as it traveled the length of the axon. In the peripheral nervous system myelin is formed by Schwann cells; in the central nervous system it is the work of oligodendrocytes.

The expression of myelin-specific genes is carefully regulated since too much or too little of this essential substance leads to neurologic disorders such as multiple sclerosis or, less frequently, to rare herditary conditions such as Charcot-Marie-Tooth disease. Close control of these genes is also important because if Schwann cells detect an excess of one gene product, they appear to turn off other myelin-producing genes.

These researchers used transgenic mice to study dose response to the gene for P0 glycoprotein, a cell adhesion molecule, which accounts for at least 50% of the protein in myelin-forming Schwann cells in peripheral nerve. When extra copies of the P0 glycoprotein gene resulted in a 30% increase in P0 expression, peripheral nerve myelination became noticeably less efficient. Thus it appears that normal myelination depends on precise dosage, even of a protein as abundant as this one. Dr. Wrabetz and his colleagues have since engineered a new transgene, mP0TOTA, which they plan to use to create mouse models of Charcot-Marie-Tooth 1B, a neuropathy that results from mutations of the human P0 gene.

Tissue specific and activity-dependent gene regulation in skeletal and cardiac muscle

Stefano Schiaffino, Professor
Department of Biomedical Sciences, University of Padova

In the earliest stages of embryonic development, both skeletal and cardiac muscle start out as mesoderm. And although they remain similar in certain ways, such as being striated instead of smooth, they become progressively more different as development progresses. Dr. Schiaffino's interest is in identifying factors responsible for the diversification of fiber types during skeletal muscle development, and in determining how muscle genes are regulated by nerves and by activity.

The researchers are also studying how genetic factors shape the development of the chambers of the heart. Using transgenic mice in which the gene for troponin I can be manipulated, they are studying transcriptional regulation of genes involved in cardiac embryogenesis

5. STRUCTURAL BIOLOGY AND ENZYMOLOGY

Overview

Although an accomplished carpenter can look at a stack of building materials and guess whether the finished structure is more likely to be a gazebo or a garage, he can't say for sure. Similarly, a biologist who knows the amino acid sequence of a protein will be able to make certain predictions about its shape, but will not be able to say with certainty what its three-dimensional folded structure will be. And without knowing a protein's structure it is impossible to say exactly how it functions in the cell.

Since the 1930s, scientists have used X-ray crystallography to visualize the 3-D structure of enzymes and other proteins at the atomic level. Although this technique has been refined considerably over six decades, the basic idea has remained constant: when a narrow beam of X-rays is focused on a pure crystal of protein, the atoms will scatter the waves in the beam and create an X-ray diffraction pattern that reveals the relative position of atoms are in the molecule. A second approach, nuclear magnetic resonance (NMR) spectroscopy, has been used to study protein structure since the 1980s. Although it has the advantage of not requiring pure protein crystals (which are famously difficult to prepare), it can only be used to analyze very small proteins.

The presentations in this section of the symposium concern the three-dimensional structures of two enzymes important for cell growth and division, a transcription factor, enzymes essential for DNA repair, and a viral toxin that is important in the pathogenesis of stomach ulcers.

Structure and regulation of human C-SRC tyrosine kinase

Michael Eck, Assistant Professor
Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School

Normal Src is a receptor tyrosine kinase that receives extracellular growth signals and turns them into signals that can be read by intracellular messengers. SRC accomplishes this by phosphorylation, which involves tacking phosphate groups onto tyrosine amino acids in other proteins. Although Src normally switches on and off as needed, research has shown that oncogenic mutations can lock Src into the "on" position – which sends the cell into a growth-promoting frenzy. The exact mechanism for this is unclear, although researchers suspect that Src is a multiplex switch capable of turning on or off in response to diverse types of input.

Dr. Eck's laboratory has produced a 3-D structure of the oncogene Src that is the highest resolution image ever made of a protein of this class. Of the protein's four distinct lobes, two make up the kinase that phosphorylates other proteins and two others, dubbed SH2 and SH3, regulate the kinase and help Src establish its site of action within the cell. The researchers found that several mechanisms work simultaneously to keep Src idle, but once it is activated its four lobes curl tightly around the active site – making it inaccessible to an "off" signal. This information could prove useful to drug designers in the long run.

Molecular enzymology of protein kinase CK2 (casein kinase 2)

Lorenzo Pinna, Professor
Department of Chimica Biologica, University of Padova

In another study of the relationship between structure and function, Dr. Pinna's group examined the atomic details of a protein kinase called CK2. This is an essential, ubiquitous, and pleiotropic enzyme that is known to act on more than 150 substrates. Most interesting to cancer researchers is that its overexpression correlates with neoplastic growth.

Also CK2 at first appears of be a single structure, in fact it is formed by the tight and stable association of two catalytic (α and/or α') and two modulatory ß-subunits. Close examination reveals that the structure of CK2α has features that give it properties that are unique among protein kinases: it binds both ATP and GTP, in their *syn* rather than *anti* conformation; it recognizes phosphoacceptor sites marked by multiple acidic residues; and it has high basal activity due to displacement of the "activation loop" which interacts in a stable fashion with the N-terminal segment. Additional studies, using mutants and synthetic fragments of the ß-subunits, demonstrate that they have both positive and negative regulatory properties.

Interactions among multiple transcription factors in eucaryotic gene regulation

Stephen Harrison, Professor
Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School

Transcription factors switch on genes, or groups of genes, so that they generate the messenger RNA that is needed to produce proteins that the cell needs for growth. Transcriptional control is a key step because it determines when and how often a given gene is transcribed. It appears that instead of always coming from a single source, transcriptional commands sometimes take the form of integrated signals that come from several pathways. This suggests that when genes are inappropriately transcribed, the fault may be a mutation in one transcription factor or a flawed interaction between two or more of these regulatory substances.

Dr. Harrison and his team have studied how synthesis of the T-cell cell growth factor interleukin-2 (IL-2) is regulated, and have learned that control comes not from a single factor, but rather from a complex of proteins working together. Transcription of the IL-2 gene is predominantly regulated by nuclear factor of activated T-cells (NF-AT), which is activated in the cytoplasm of T-cells after a receptor has been stimulated by calcineurin. It forms a transcriptional complex with an activator protein,AP-1, which is a heterodimer of *fos* and *jun* oncoproteins. The researchers have determined the three-dimensional structure of a quaternary complex of NF-AT, *fos, jun,* and DNA. The interactions among these components are striking and extensive.

Structures of enzymes that make or break DNA

Tom Ellenberger, Assistant Professor
Department of Biological Chemistry, Harvard Medical School

Chemical assailants constantly chip away at the integrity of DNA, increasing the chance that it will be transcribed into defective proteins that will harm the body. Cells respond to this threat by dispatching crews of repair enzymes, which mend damaged areas by cutting out bad parts, making a new copy of genetic information that was destroyed, and sealing gaps. Dr. Ellenberger and his colleagues have done 3-D structures that reveal some of the details of the repair process. X-ray structures of a base excision-repair enzyme (AlkA) and a DNA polymerase (T7 DNA polymerase) show how these enzymes go about repairing DNA.

DNA repair is almost always desirable, because without it harmful mutations would accumulate and pose a threat to life itself. The exception comes in cancer cells which are under therapeutic attack by chemotherapy agents, In this case, the ability of tumor cells to repair damaged DNA often limits the effectiveness of therapeutic agents. Knowing more about the 3-D structure of repair enzymes could prove especially relevant to cancer research.

Cellular effects of the vacuolating toxin VacA from Heliobacter pylori

Manuele Papini, Researcher
Department of Biomedical Sciences, University of Padova

Heliobacter pylori (HP) is a corkscrew-shaped bacterium that lives in the gastrointestinal tract of 50% of the population, but causes symptomatic illness in only 10%. For that unfortunate minority, it can cause a wide range of troubles including gastritis, stomach and duodenal ulcer, and sometimes even adenocarcinoma or mucosal-associated lymphoid tissue cancers. The fact that not everyone who is infected becomes ill raises the possibility that some HP strains may have virulence factors that make them more dangerous than others.

There is high degree of interest in creating vaccines that could block HP infection or mediate its effects, and in designing better treatments for the common diseases the bacterium causes. Rational design of such agents could be advanced by more detailed information about virulence factors and their impact on the cells of the human gut.

HP produces three main virulence factors: A*urease*, essential for its survival in the stomach; *VacA*, which induces intracellular vacuolation that causes host cells to collapse and die; and *CaqA*, about which less is known. *VacA* is a toxin that is associated with more severe symptoms. In an effort to clarify its action, Dr. Papini added purified *VacA* to established cell lines and observed the morphological and functional alterations it induced. Although many bacterial change the cytosol of target cells, *VacA* appears to disrupt the structure and function of the cell's endocytic pathway in unique ways.

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