ARMENISE-HARVARD SYMPOSIUM 1997

ARMENISE FIRST SYMPOSIUM

1st Annual Symposium
June 18-20,1997, Erba, Italy

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.

Session 1: 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

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Protein targeting in neurons and endocrine cells

Kathleen Buckley, Associate Professor

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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 terminalâs 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

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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 neuronâs 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)

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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 ?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. Pietrobonâs lab studies four ?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 ?1A-2 and the regulatory human ?2b-? and ?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

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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?

Session 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

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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. Comoglioâs 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.

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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

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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

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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

Session 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

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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 M.D., Professor and Chairman of Pathology

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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

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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

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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 forrecognition of non-self molecules.

Giulia De Lorenzo, Associate Professor

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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

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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.

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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.

Session 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

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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

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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. Flanaganâs 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

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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

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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. Schiaffinoâs 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.

Session 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.

Presentations

Structure and regulation of human C-SRC tyrosine kinase

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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

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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 (a and/or aÎ) and two modulatory §-subunits. Close examination reveals that the structure of CK2? 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 §-subunits, demonstrate that they have both positive and negative regulatory properties.

Interactions among multiple transcription factors in eucaryotic

gene regulation

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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

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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

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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: Aurease, 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";"Symposium 1997: Erba, Italy";"";publish;closed;open;"";symposium-1997-erba-italy;"";"";2014-04-15 19:59:18;2014-04-15 19:59:18;"";0;http://www.armeniseharvard.org/?p=659;0;post;"";0