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ARMENISE-HARVARD SYMPOSIUM 2003

ENVIRONMENTAL SENSING AND THE CELLULAR RESPONSE

7th Annual Symposium
June 21-23, 2003, Trieste, Italy

About the Symposium

Trieste is an ancient city on the Adriatic Sea where Italian, Germanic, and Slavic cultures intersect. Before it became part of a united Italy 50 years ago, the city was at various times under the sway of Rome, Venice, Austria, Austria-Hungary, Italy, and Communist Yugoslavia. No wonder its people are adept at communicating in many languages, enjoying both strudel and gnocchi, and thriving in a complex and rapidly changing environment. According to speakers at the 7th Annual Symposium of the Giovanni Armenise-Harvard Foundation, living cells and biologic systems are every bit as adaptable as the host city's people.</p>

<p align=""left"">"Environmental Sensing and the Cellular Response" was the theme of the event, held in Trieste June 21-23. Marc W. Kirschner and Carla J. Shatz, who respectively head The Armenise Center for Integrative Biology and Physiology and The Armenise Center for Neurosciences at Harvard Medical School organized the Symposium. Many of the 15 invited lectures and 18 poster presentations offered molecular insights into the senses – including vision, hearing, and taste – that organisms use to explore and experience the outside world. Other speakers delved into the inner world of cells, exploring how they communicate with their own kind and with other cell types using biochemical, mechanical or other signals.</p>

<p align=""left"">Nearly 60 scientists accepted the Foundation's invitation to this year's Symposium, half from basic science departments at HMS and half representing Italian universities and research institutions in Milano, Padova, Pisa, Roma, Torino, and the host city of Trieste. This mix reflects the Foundation's commitment to stimulating knowledge exchange and collaboration between American and Italian scientists, a mission that echoes the history and goals of the International Centre for Theoretical Physics, which hosted the Symposium. The ICTP was founded in 1964 by Abdus Salam, a Pakistani-born physicist who spent most of his career in Italy and who shared the1979 Nobel Prize in Physics with two Harvard University professors.</p>

<p align=""left"">“Scientific thought is our common heritage,” Salam often said, and his motto serves equally well for the Armenise-Harvard Foundation. In his closing remarks, co-convener Marc Kirschner paid tribute to Count Giovanni Auletta-Armenise and former HMS Dean Daniel Tosteson for creating a powerful catalyst for collaborations among Italian and American scientists.</p>

<p align=""left"">Kirschner summarized the scientific sessions as “a perfect balance of information, in an environment where it can be appreciated, with the right number of participants.” At the closing session, he announced this year’s recipients of awards and grants sponsored by the Foundation. Career Development awards are intended to help young Italian researchers “jump start” laboratories in their home country, and this year’s winners are Sabrina Sabatini and Stefano Gustincich. Sabatini has been studying in Utrecht, Holland, and will pursue her research on growth hormones in plants at the University of Rome. Gustincich, who recently moved from Harvard to the International School for Advanced Studies (SISSA) in Trieste, worked with individual retinal neurons to devise methods useful for studying other neural networks as well.</p>

<p align=""left"">A second initiative, the Foundation’s HMS Junior Faculty Grants, helps underwrite research by promising young investigators at Harvard Medical School. This year’s grants went to Sean P. J. Whelan and Bernardo Sabatini, who described his research on synapse formation in a symposium presentation. Whelan works on reverse genetics of arenaviruses, microbes that cause animal and human diseases ranging from mild, flu-like illnesses to lethal hemorrhagic fevers.

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Sweet, Bitter and Umami: The Biology of Mammalian Taste

Charles Zuker

Professor of Neuroscience and Biology, University of California – San Diego

"How does the brain know what we just ate?" This is the complex question that Dr. Zuker posed more than four years ago, when he set out to analyze how taste information travels from the tongue to the brain and elicits responses ranging from delight to repulsion. Although how the brain knows remains mysterious, his lab has identified two families of cell surface receptors in the tongue that encode three basic mammalian tastes.</p>

<p align=""left"">Sweet and bitter, along with sour and salty, are instantly recognizable as the four classic categories for the vast array of gustatory sensations experienced by people and animals. Umami, a less-familiar taste modality studied in the Zuker laboratory at UC San Diego, has been recognized by scientists only since the mid-1980s. "This is the yummy, delicious taste that we often associate with Chinese food and headaches," Dr. Zuker said. Umami is the official name for the unique flavor imparted by monosodium L-glutamate and certain nucleotides, such as inosinate and guanylate.</p>

<p align=""left"">In a series of reports since 1999, Dr. Zuker and his team have reported on two families of taste receptors: one detects sweet and umami, flavors that attract mammals to high-calorie food sources, and a second, larger group perceives bitter, an aversive taste linked with toxic substances. These receptor families occur only in cells that constitute taste buds, and molecular analysis of their expression reveals that old-fashioned "taste maps," showing that sweet and bitter are picked up by mutually exclusive regions of the tongue, are "utter nonsense," Dr. Zuker said. No matter where a taste bud is, its cells are outfitted with at least some receptors for each of the five basic tastes. Receptor expression varies greatly from cell to cell, however, and this balance determines which taste an area of the tongue primarily detects.</p>

<p align=""left"">When Dr. Zuker's group first identified the T1R family of G protein-coupled receptors they didn't know which – if any – specific tastes these structures encoded. To find out, they devised a screening assay in which cells expressing family members T1R1, T1R2, and T1R3 can be challenged with various sweet and umami tastes while their calcium responses were measured. Cells that co-express T1R2 and T1R3 responded to every natural sweetener, artificial sweetener, and D amino acid they were exposed to, but did not react to any non-sweet substances. In contrast, cells bearing only one of these receptors were indifferent to sweetness.</p>

<p align=""left"">Other researchers had shown that umami-flavored amino acids were attractive to mice, and Dr. Zuker suspected that there would be specific mammalian receptors for this modality as well. In his laboratory's screening assay, cells co-expressing T1R1 and T1R3 responded to MSG and to all 20 natural L amino acids but not to any sweet substances. Results from the cell-based screening assays were confirmed by a series of experiments with knockout mice. In a typical experiment, for example, mice that had avidly consumed sugar water while shunning plain water lost the ability to discriminate when the T1R2 gene was knocked out. Parallel results were obtained for umami receptors.</p>

<p align=""left"">Turning to noxious tastes, Dr. Zuker and his colleagues have identified a family of some 30 G protein-coupled receptors with a high affinity for bitter flavors. In evolutionary terms, it makes sense to have a large repertoire of highly sensitive receptors for harmful substances, because "if you eat it, you die," Dr. Zuker said. In mouse and human taste buds, these T2R receptors appear to be expressed in every cell. Acute sensitivity to bitter tastes makes normal mice adept at avoiding poisons such as cycloheximide, but when the researchers knocked out the receptor for this bitter substance, altered mice drank tainted water and died.</p>

<p align=""left"">Additional experiments in mice revealed that receptors for sweet, umami, and bitter rarely occur in the same cells, although cells bearing various receptors are bundled together in taste buds. Although the three modalities are functionally segregated, downstream the T1R and T2R receptor families share at least two signaling molecules. Knocking out either one of these yields mice that can't taste sweet, umami, or bitter – yet detect salty and sour stimuli with ease.</p>

<p align=""left"">At this stage of the research, Dr. Zuker said that he has a "good feel" for how the tongue knows what we have just eaten. "If we are lucky over the next few years, we will be able to draw a connectivity map between the tongue and the brain," he concluded.</p>

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<p align=""left"">Other Presentations</p>

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<p align=""left"">Immune Genes and Brain Waves in Brain Wiring

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<p align=""left"">No one would dream of working on a computer with the power turned on. Yet this is exactly how connections are formed in the developing brain. This biological process is “much more marvelous than a computer, because the wiring is done while the switch is on and the actual functioning of the brain is required for the wiring,” Dr. Shatz said, introducing her lab’s most recent discoveries about the genetic control of visual system development.</p>

<p align=""left"">Dr. Shatz was the first to describe how brain activity sorts a messy tangle of connections into the precise and orderly arrangement found in the adult visual system. Long before rods and cones are present, ganglion cells fire spontaneously and synchronously, generating ""waves"" of activity that sweep across domains of the retina. The growing tips of these cells use molecular cues from the environment to grow into the lateral geniculate nucleus (LGN), where signals from left and right eyes are entwined in what resembles a tangle of wires.</p>

<p align=""left"">After birth, a rush of electrical impulses stimulated by light triggers synaptic remodeling that prunes and reshuffles these connections, so that axonal connections from ganglion cells in the left and right eyes are sorted into two mutually exclusive layers in the LGN. Earlier experiments in her lab established that blocking signals from ganglia interfered with activity-dependent layering. The rule of thumb is: “Cells that fire together, wire together. Out of sync, lose your link,” Dr. Shatz said, summarizing how frequency of use translates into enduring structural change.</p>

<p align=""left"">The next step was to search for genes involved in synaptic remodeling, which the researchers thought would be expressed when the power switch was turned on. Screens for mRNA of genes active during LGN development confirmed that they are expressed when endogenous activity is normal, but not if it is blocked. The most surprising find was a family of genes for class I major histocompatibility complex (MHC I), a key player in the mammalian immune system that had not previously been detected in the brain. Not only is MHC I present, but Dr. Shatz’s team showed that these genes are expressed by CNS neurons at times and regions of activity-dependent axonal rearrangements. A series of experiments in cats and rodents suggests that MHC I genes play a novel role in CNS development and plasticity.</p>

<p align=""left"">Next, the investigators asked whether MHC I is essential for synaptic remodeling. To find out, they used knock-out mice missing class I MHC or CD3 zeta, a required signaling component for many receptors that recognize class MHC I. In these mice, activity-dependent refinement of the retinogeniculate projection did not develop into the neat and focal bundle of conductivity seen in normal mice, but instead was large and fuzzy in appearance. MHC I is also expressed and regulated by activity in the hippocampus; in adult mice lacking either cell surface MHC I or CD3 zeta, long term potentiation is supranormal and long term depression is absent. Together, these results suggest that normal activity-dependent synaptic remodeling cannot take place, either developmentally or acutely, unless class I MHC and CD3 zeta-containing receptors are present. Future experiments in the Shatz lab will focus on mice that overexpress the MHC I gene.</p>

<p align=""left"">So far, these investigations have demonstrated that nerve cell function is essential for specific activity-dependent gene expression and for the initial structural remodeling that wires the adult visual system. Since spontaneous neural activity is common in the developing CNS, similar activity-dependent mechanisms and molecules are probably used elsewhere to organize early connections into precise adult patterns, Dr. Shatz said.</p>

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<p align=""left"">Transduction and Adaptation in Auditory Hair Cells

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<p align=""left"">Dr. Corey’s lab wants to know how the brain makes sense of the sounds around us? Although this complicated process isn’t fully understood, an important first step is carried out by bundles of stereocilia on hair cells in the ear. These “hair bundles” look like sheaves of grain, swaying when they are buffeted by waves of sound. In the auditory and vestibular systems, they convert these movements into neural signals by means of mechanically-gated transduction channels located in the tips of the stereocilia, which open in response to tension in fine tip links connecting adjacent stereocilia. At rest, the hair cells maintain tension on these channels within a few tenths of a piconewton (pN), to keep them in their most sensitive range. At the same time, this

tension can change quickly in response to maintained bundle deflection, an important type of adaptation that is probably controlled by several independent mechanisms.</p>

<p align=""left"">Because the stereocilia have cores of actin filaments, researchers have long thought that some type of myosin is probably the motor that regulates tension at the hair cell tips. At least ten different myosins have been identified in the sensory epithelium of the inner ear, and mutations in five of these myosin genes have been linked with hereditary deafness. Dr. Corey's team found that only one of these myosins, known as myosin-1c, is enriched at the tips of the stereocilia. Immunogold electron microscopy studies found it concentrated within ~150 nm of tip link insertions, where the channels are most likely located.</p>

<p align=""left"">To determine the functional significance of this observation, Dr. Corey's lab and collaborators at Oregon Health Sciences University mutated tyrosine-61 of myosin-1c to glycine, which enlarged the ADP binding pocket so that it could be inhibited by oversized N6-modified ADP analogs. The investigators created transgenic mice that expressed the mutant myosin-1c in utricular hair cells, delivered an ADP analog through a whole-cell recording pipette, and found that the analog rapidly blocked adaptation in transgenic but not in wild-type hair cells. Movement of the wild-type myosin-1c was apparently blocked as well. "It's like you have a team of horses and half of them decide to lie down – the others aren't going anywhere," Dr. Corey said. Adaptation is inhibited and the system locks down, strongly suggesting that myosin-1c indeed regulates tension on transduction channels.</p>

<p align=""left"">Other teams of investigators suggested that a second mechanism is also involved. According to this alternative hypothesis, Ca2+ enters open transduction channels, then shuts them by binding to an intracellular site. Dr. Corey's lab followed up on this idea by applying forces to hair bundles with laser tweezers and measuring the movements evoked by Ca2+ entry. The results supported both the myosin and calcium models. Thus mechanical forces open channels in stereocilia bundles, and the influx of calcium both closes them directly and allows the myosin-controlled tension to relax faster. For both mechanisms, additional force can reopen channels. Dr. Corey's group was able to show that once Ca2+ closes channels by binding, an additional 0.7 pN of force is sufficient to pry them open.</p>

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<p align=""left"">How the Leech Interacts With the External World

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<p align=""left"">The desires of the leech are not so different from our own: these simple invertebrates are drawn to food and sex, they want to escape pain and injury, and sometimes they simply want to look around. The leech relies on swimming and crawling to move away from noxious stimuli and towards food and mating partners, mechanisms that have been described in detail by numerous researchers. The leech's reflexes for escaping danger, such as whole-body shortening and local bending, have also been well studied. Less is known about the inner workings of exploratory movements, in which the leech attaches itself to a surface using its tail sucker, and extends and turns its head and body.</p>

<p align=""left"">Although the leech's exploratory movements appear more complex and random than its approach or escape behaviors, all three are elicited and regulated by environmental cues, mediated by central mechanosensory neurons, and executed by motor neurons.</p>

<p align=""left"">Dr. Torre set out to understand how external signals are translated into exploratory behavior by quantifying the leech's behavior and the electrical activity underlying it. His team used superglue to attach tiny red, green, and blue beads to the rostral, mid-body, and caudal positions on the upper surface of the animals. A computerized visual analysis system tracked their behavior for as long as 24 hours at a stretch, using the beads' movements to determine what the leech was doing at any time. Some leeches reared up in the exploratory posture more often than others, but over long periods individual differences averaged out. The investigators observed that well-fed leeches ignored environmental irritants such as flashing lights or chemicals, and flinched only when they were physically prodded.</p>

<p align=""left"">Additional studies of escape reflexes in response to touch used a hemisection of a leech body wall flattened and pinned on a recording chamber, with the central segment kept innervated by its ganglion. When the leech skin was prodded, Dr. Torre's group used multi-electrode recordings to observe spike trains of mechanosensory and motor neurons, while simultaneously taking pictures of skin contractions.</p>

<p align=""left"">This approach uncovered differences between spike trains in mechanosensory neurons, which were highly reproducible, and those in motoneurons, which varied greatly. Despite this variability, the investigators found that motor output was reproducible and reliable. They identified two distinct biophysical mechanisms that translate variable spike trains into reproducible motor outputs. First, leech motor neurons contract slowly enough to smooth out the "jitter" of motoneuron spike trains, which would have a quite a different impact on rapidly contracting muscles. Second, the motor output is a distributed process, reflecting the simultaneous activation of many motoneurons, and the population's overall firing averages out differences in how individual motoneurons react.</p>

<p align=""left"">These experiments show that dependable motor reactions can arise from unstable spike trains, a finding that makes sense in evolutionary terms. Survival hinges on being able to escape from dangerous assaults, Dr. Torre observed. No wonder the nervous system is a able to turn unreliable components into reliable actions.</p>

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<p align=""left"">Function and Molecular Diversity of the Hypervariable Receptor DSCAM

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<p align=""left"">Alternative splicing is a hot topic in biology these days, because this mechanism explains how a relatively small number of genes encodes the myriad proteins needed for life. Dr. Schmucker's studies of the Dscam gene are noteworthy because he has uncovered an extreme example of alternative splicing: one gene with the potential to generate more than 38,000 protein isoforms.</p>

<p align=""left"">Dr. Schmucker's ongoing investigations focus on how neurons find their correct paths in the developing brain, so that millions of cells representing diverse cells types make

precisely the right connections. In the course of studying this problem in Drosophilia, he zeroed in on Dscam, a member of the immunoglobulin superfamily that is expressed in most post-mitotic neurons and localized in the tips of growing axons in the embryonic central nervous system. Dscam takes its name from the human protein Down syndrome cell adhesion molecule (DSCAM), first found on chromosome 21 of Down syndrome patients. (The clinical connection between DSCAM and Down's syndrome is unclear.)</p>

<p align=""left"">In flies, loss of function mutations in the Dscam gene result in early lethality and disrupt the formation of many nerve connections. The Drosophila Dscam gene is large (about 270 kiloDaltons) and highly complex, with 115 exons including 95 that are variable. Dr. Schmucker found that alternative splicing of constant and variable exons could gener-ate at least 38,000 receptor isoforms with a conserved architecture containing three variable Ig and two alternative transmembrane domains.</p>

<p align=""left"">His team is now exploring the functional significance of Dscam's hypervariability and how its protean nature helps form highly specific connections in complex neuronal circuits. Dr. Schmucker's hypothesis is that different nerve cells express different Dscam isoforms, providing a specific molecular recog-nition code that guides individual neurons to connect with the right target neurons in the developing brain.</p>

<p align=""left"">He set out to test this in Drosophila, where every bristle on the fly's body is linked to a specific somatosensory neuron, so that researchers can track signals down a specific path. So far, early findings indicate that when Dscam is weakened by an induced mutation, axon projections from mechanosensory neurons find their way to the right area in the CNS, but don't actually communicate with target cells because they lack the branches that should make this contact. Experiments are underway to learn more about how this affects specificity and brain development.</p>

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<p align=""left"">Presynaptic Calcium Stores Modulate Afferent Release in Vestibular Hair Cells

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<p align=""left"">Just as some bundles of hair cells translate the movement of sound waves into auditory information, others translate the lurch of a taxi into the sensation of movement. Even a tiny acceleration of the head causes vestibular fluid to deflect hair cell bundles, which the brain perceives as motion. Like acoustic hair cells, these tiny motion detectors are mechanoreceptors – presynaptic to primary afferent neurons of the eighth nerve – which excite neural activity by the release of glutamate.</p>

<p align=""left"">Dr. Mammano has been investigating the role of intracellular Ca2+ stores in afferent transmission in several ways. Working with an excised patch of vestibular epithelium, he studied presynaptic changes by monitoring intracellular Ca2+ concentration ([Ca2+]i) in hair cells; postsynaptic changes were assessed by recording from single posterior canal afferent fibers. When the researchers added 1-10 mM caffeine to the hair cells,Ca2+ responses evoked by depolarization at selected Ca2+ “hotspots” shot up briefly, accompanied by an increase in cell membrane capacitance (DCm). This signaled a burst of Ca2+ exocytosis, which is what the presynaptic end of a

stimulated hair cell should do. The greatest response to caffeine was observed in a region located about 10 mm from the base of the hair cell.</p>

<p align=""left"">When Dr. Mammano used electricity to depolarize the hair cells, localized [Ca2+]i increases were observed following depolarizations lasting for 500 milliseconds, but not with 50 ms bursts, suggesting calcium-induced calcium release (CICR) from intracellular stores. Both Ca2+ and DCm responses were inhibited in cells that had been incubated with 40 mM of ryanodine for 8-10 minutes. Consistent with these results, afferent transmission was potentiated by caffeine and inhibited by ryanodine both at the level of action potentials (APs) and of miniature excitatory postsynaptic potentials (mEPSPs). Neither caffeine nor ryanodine affected the shape and amplitude of mEPSPs, indicating that both drugs acted at the presynaptic level.</p>

<p align=""left"">Dr. Mammano and collaborators at Universita di Pavia and Universita di Ferrara also used a frog model to study the impact of Ca2+ and ryanodine on single fiber afferent nerve activity with and without external stimulation. Responses to caffiene and ryanodine were as predicted, and in addition they found that caffeine’s ability to increase firing of afferent nerves increased 3 to 4-fold when the animal was spun on a turntable. These results strongly suggest that endogenous modulators of the CICR process will affect afferent activity elicited by mechanical stimuli in the physiological frequency range.</p>

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<p align=""left"">Regulated Exocytosis other than Regulated Secretion: Roles of Enlargeosome, a Little Brand New Organelle

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<p align=""left"">A great deal of research has been done on regulated secretory pathways that culminate in exocytosis. Neurotransmitter release and uptake is one of the crucial physiologic processes that depends on regulated exocytosis, in which small amounts of membrane deliver proteins of great importance, such as glutamate receptors, to discrete sites on the cell surface. For the past several years, Dr. Meldolesi and collaborators at Harvard Medical School have been exploring a different type of exocytosis that is crucial for processes such as mitosis, phagocytosis, and wound healing.</p>

<p align=""left"">This second type of exocytosis rapidly enlarges the plasma membrane in response to signals from specific intracellular messengers triggered by outside stimuli. In Dr. Meldolesi's laboratory, patch clamp capacitance assays show that in less than one second, the surface of various cell types enlarges by 15-20% in response to [Ca2+]i increase. Exocytotic fusion of what appear to be many small organelles, each less than 0.1 mm in diameter, seems to account for this enlargement. The search for a marker that could be used to track this process led the researchers to a high molecular weight, non- transmembrane protein called Ahnak/Desmoyokin (dA) that concentrates in a subcellular compartment and to a lesser extent in plasma membrane. This protein appears on the cell surface only after an influx of [Ca2+]I , which suggested to Dr. Meldolesi that it would be useful for studying calcium-dependent exocytosis.</p>

<p align=""left"">The team developed a monoclonal antibody against dA, which they used to trace exocytosis carried out by this mystery organelle, which appears to have multiple functions. During differentiation, it rushes to the cell surface but is not co-located with secretory vesicles. When the

cell membrane is injured, the organelle rushes to the site and facilitates formation of new membrane to repair the breach. Although the conventional wisdom is that lysosomes alone are responsible for such repairs, Dr. Meldolesi's experiments show that the new organelle is also involved.</p>

<p align=""left"">No matter what calls these special vesicles into action, their arrival at the cell surface causes rapid membrane enlargement – an observation that led Dr. Meldolesi to call them "enlargeosomes." Monoclonal antibody studies indicate that enlargeosomes rest in the cytoplasmic layer adjacent to the plasmalemma; when stimulated, they rush to the cell surface and remain there for prolonged periods, apparently anchored to a specific binding protein. Dual labeling experiments demonstrated that enlargeosomes are distinct from other organelles, including the ER, GC, TGN endo- and lysosomes, Glut4 vesicles, and other vesicles of constitutive secretion. Dr. Meldolesi and his colleagues are continuing to explore the enlargeosome's role in cell differentiation and wound healing.</p>

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<p align=""left"">How do Mammalian Cells Sense Directionality?

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<p align=""left"">Among mammalian cells, the speed champion of chemotaxsis is the neutrophil: unlike cells that take hours to move toward or away from a diffusible chemical, neutrophils can turn on a dime once they detect a chemotactic signal. Movies made in Dr. Cantley's lab show neutrophils zigzagging in pursuit of pipette tainted with bacterial peptide like hockey players chasing a puck.</p>

<p align=""left"">This sort of directional motility, as well as directional growth of eukaryotic cells, involves complex temporal and spatial signaling. Shallow spatial gradients of stimulants in the external environment are amplified inside the cell to produce sharp internal gradients. These reorient the cytoskelton, which commits the cell to growth or movement in a specific direction. The central coordinator of this sort of rapid decision-making is the enzyme phosphoinositide 3-kinase (PI3K), which is activated by receptors that detect bacterial chemokines or endogenous growth factors.</p>

<p align=""left"">PI3K floats in the cytosol, then binds to the plasma membrane where it plays a critical role in directionality by generating a lipid, phosphatidylinositol-3,4,5-trisphosphate (PIP3), at the plasma membrane in response to growth factors and chemokines. PIP3 phosphorylates a host of different proteins involved in chemotactic response and cell migration, ultimately causing actin to concentrate at the leading edge of the moving cell. Given that receptors for chemotaxsis signals are studded all over the cell, how does the cell know which way to turn?</p>

<p align=""left"">Dr. Cantley's group is exploring this question by examining what happens inside neutrophils chasing a moving bacterial peptide signal. The researchers are using biochemical, cell biological, and genetic methods to define the signaling pathways that positively and negatively regulate PI3K. They have found that the cells are exquisitely sensitive to stimulant gradients in the environment, and automatically concentrate PIP3 manufacture on the edge of the cell nearest the stimulus. When the pipette tip is moved, PIP3 manufacture immediately shifts to the new leading

edge, while production of PTEN, which degrades PIP3, breaks down PIP3 at what was previously the cell's front end. By confining PIP3 production to the leading edge of migrating cells, this combination of local positive feedback loops and global negative feedback loops enables neutrophils to make sudden turns.</p>

<p align=""left"">More recently, Dr. Cantley's lab has been examining the role of the PI3K pathway in fibroblast response to growth factors, with the aim of learning more about how wounds heal. This process is neither as fast nor as directional as what happens in neutrophils, but knock-out experiments suggest that the PI3K pathway is necessary for proper wound repair. Additional investigations are underway.</p>

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<p align=""left"">Signaling Mechanisms that Regulate Axon Guidance in Drosophila

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<p align=""left"">The great anatomist Ramon y Cajal described the relationship between a neuron and its target as a great love story that begins with a quest, Dr. Van Vactor said at the beginning of his presentation. His investigations focus on a key player in this quest – the growth cone at the tip of the axon. He views this structure as “an exquisitely sensitive molecular compass” that translates environmental information into directional decisions that are crucial to normal development.</p>

<p align=""left"">Growth cone behaviors are regulated by phosphorylation-dependent events controlled by protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). In addition to receptor-class proteins that directly link PTK or PTP catalysis to conserved extracellular domains, several intracellular enzymes have been implicated in axon guidance decisions as signaling partners of separate receptor proteins. Dr. Van Vactor has been exploring this complex process by studying axon guidance decisions around the developmental midline of the fly. Here, the Abelson PTK (Abl) and the Slit protein (encoded by the gene called Robo) team up to push axons away from the midline divide and into parallel paths on either side. Lethal loss of function mutations in Slit or Abl cause axons to make incorrect decisions and grow across the midline, creating abnormal crossovers between the two sides.</p>

<p align=""left"">The next step in Dr. Van Vactor’s quest was to identify new proteins that interact with Abl and might influence interactions between different cytoskeletal networks. He and his team identified candidate genes by analyzing mutations that caused obvious abnormalities in the complex eye of the fly, reasoning that products of such genes would probably be actors in kinase signaling pathways. Several candidates from this screen contain protein motifs suggesting they might play a role in the Abl pathway, and also show expression in the developing nervous system. Future work in the Van Vactor lab will be focused on testing whether these new genes are vital players in the navigational machinery that collaborates with Abl to control key aspects of neuronal morphogenesis.</p>

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<p align=""left"">New Mechanisms for HGF Receptor Activation and Inactivation

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<p align=""left"">Invasive growth is a multistep morphogenic process in which the cells dissociate from their neighbors, leave their original environment and migrate into the surrounding territories. Once they settle into their new location, they increase in number and eventually undergo terminal differentiation. Scatter factor receptors, a family of tyrosine kinases including Met (the receptor for hepatocyte growth factor) and Ron (the macrophage stimulating protein receptor), are key molecules that orchestrate intercellular signals needed to carry out this process. Tightly regulated scatter factor activity is essential for normal development.</p>

<p align=""left"">Scatter factor receptors become problematic, however, when they escape close control of activation or inactivation – a situation that can lead to malignant transformation and acquisition of metastatic properties. Met, for example, is overexpressed in gastrointestinal tumors and those of mysenchymal origin, and amplified in metastasis.</p>

<p align=""left"">Dr. Giordano's laboratory investigates molecular mechanisms that down regulate Met. She has found that this process is normally controlled by a complex that involves endophilins (which initiate endocytosis), a ubiquitin-like protein called Cbl, and a protein Cbl interacts with called CIN85. In healthy cells, Cbl marks Met for destruction, which leads to its endocytosis – diminishing the receptor population and lowering HGF level. If formation of the endophilin/CIN85/Cbl complex is inhibited, HGF levels remain high, prolonging signal transduction that increases cell mobility and can lead to transformation.</p>

<p align=""left"">More recently, the researchers have shown that scatter factor receptors can be activated by mechanisms other than ligand binding, specifically by interaction with other cell surface receptors. HGF receptors physically associate with plexins, a family of transmembrane receptors for proteins called semaphorins, and the cross-talk between these molecules mediates the whole invasive program of cells, Dr. Giordano reported. When the Plexin B1 receptor associated with Met is stimulated by semaphorin 4D, both receptors are activated. Similarly, blocking Met also impedes semaphorin 4D responses. Met and Plexin B1 are found together in human tumor cells that exhibit invasive traits typically triggered by scatter factors: compared with normal cells they are more mobile and likely to migrate, more likely to branch, and more likely to proliferate without anchors.</p>

<p align=""left"">While most examples of cross talk come from pathways in the cytoplasm, these experiments clearly show that a similar conversation – or perhaps conspiracy – can start at the upstream end of the signaling path. More recent experiments have turned up additional interactions between Met and other receptors. The take-home message is that far from being loners, some of the many receptors on the cell surface join forces to manipulate specific kinds of cellular activity, Dr. Giordano said.</p>

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<p align=""left"">Environmental Signals for Cell Scattering: Oxygen Sensing

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<p align=""left"">If some malevolent authority began packing people into a tiny room, so densely that the air supply would soon be exhausted, any sane person would rush for the exit. And this is exactly what many tumor cells do if their environment becomes hypoxic, Dr. Michieli said. No wonder the risk of invasive growth and metastasis is higher, and the prognosis worse, for patients whose solid tumors are riddled with hypoxic regions.</p>

<p align=""left"">Better-known responses to low oxygen include the ability of cells to adjust their metabolic processes and to induce the growth of new, oxygen-carrying blood vessels. Hypoxia-induced angiogenesis has been a hot topic in recent years, generating many publications and spawning a race to develop drugs that can block this process. Less attention has been given to the mechanism that enables cells to “leave the room” and invade surrounding tissues where oxygen and nutrients abound. This is the phenomenon that Dr. Michieli has been exploring.</p>

<p align=""left"">He focuses on the same pathway that Dr. Giordano discussed in the preceding lecture, which is activated when hepatocyte growth factor (HGF) binds to the receptor encoded by the c-met proto-oncogene. Dr. Michieli's experiments have demonstrated that hypoxia induces expression of the Met receptor both in vitro and in vivo, clearly implicating it in the cellular response to oxygen deprivation. When his team grew various lines of tumor cell under normal (21% oxygen) and hypoxic (3% oxygen) conditions, they found that Met protein and mRNA levels increased by about three fold after several hours of low oxygen.</p>

<p align=""left"">In experimental tumors, Met protein levels are highly increased in coincidence with HIF-1-positive, hypoxic areas, forming an expression gradient that is inversely proportional to blood vessel proximity. When Dr. Michieli and his colleagues analyzed the human met promoter, they showed that this induction is transcriptional and is mediated by two HIF-1 binding sites (HBSs) and an AP-1 site. Hypoxia-induced Met overexpression causes cells to become highly sensitive to HGF, and along with low oxygen tension this pushes cancer cells to become more aggressive. Finally, using a gene transfer approach and RNA interference technology, the researchers showed that hypoxia-driven Met overexpression is necessary and sufficient to activate the invasive growth program. High levels of Met have previously been observed in cancer cells, and these experiments provide a molecular explanation for its purpose.</p>

<p align=""left"">This finding has important implications for tumor biology and for clinical care, Dr. Michieli said. The demonstration of an angiogenesis-independent, hypoxia-induced "invasive switch" provides a link between tumor hypoxia to increased malignancy. But clinical care may become more complex as a result: anti-angiogenic therapies may increase the metastatic potential of solid tumors by suffocating them, driving malignant cells to migrate in search of fresh air.</p>

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<p align=""left""> Astrocytes as Central Mediators of Neurovascular Coupling

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<p align=""left"">“There is no evidence that glia are directly involved in electrical signaling. Signaling is the function of neurons,” asserts the author of a classic neurology text. But if additional research continues to bolster discoveries made in Dr. Carmignoto’s lab, this passage may need to be revised before the next edition is published.</p>

<p align=""left"">There is growing evidence for the existence of a distinct signaling system that enables astrocytes (the most numerous glial cell type) to communicate with neurons. Indeed, astrocytes respond to the synaptic release of the neurotransmitter glutamate with repetitive elevations in their intracellular calcium concentration ([Ca2+]i). Parallels between these [Ca2+]i oscillations and fluctuations in glutamate release led Dr. Carmignoto to characterize astrocytes as “accurate detectors of synaptic activity which encode information on the status of neuronal activity into [Ca2+]i oscillations of distinct frequencies.” Furthermore, when astroglial cells are closely associated with both synapses and cerebral vessels, they may transfer signals from neurons to blood vessels via these [Ca2+]i oscillations. If so, then astrocytes are key players in neuronal control of microcirculation.</p>

<p align=""left"">In a series of in vitro and in vivo experiments, Dr. Carmignoto’s lab obtained results that support this hypothesis. When neurons were stimulated in rat cortical slices,

glutamate-mediated [Ca2+]i oscillations were seen in astrocyte processes touching cerebral microvessels, which dilated in response. When a glutamate receptor antagonist was introduced, Ca2+ responses were inhibited and neuronal activity-dependent vasodilation was impaired, while the selective activation by a patch pipette of single astrocytes in contact with arterioles triggered their relaxation. When [Ca2+]i oscillations were stimulated with the metabotropic glutamate receptor (mGluR) agonist t-ACPD, arteriole dilation resulted. The researchers also saw evidence that astrocyte-mediated control of arterioles relies mainly on a cyclooxygenase product. In cultured astrocytes, mGluR-mediated [Ca2+]i oscillations trigger a pulsatile release of prostaglandins, presumably the vasodilator prostaglandin E2. Working in a mouse model, Dr. Carmignoto and his colleagues found that blocking glutamate-mediated [Ca2+]i elevations in astrocytes reduced blood flow increase in the somatosensory cortex during contralateral forepaw stimulation.</p>

<p align=""left"">“By revealing the direct participation of astrocytes in the control of cerebral microcirculation, our data provide a mechanistic background for a distinct role of neuron-to-astrocyte signaling in the phenomenon of functional hyperemia,” Dr. Carmignoto concluded. These findings may provide new opportunities for treating degenerative diseases such as Alzheimer’s, in which cerebrovascular blood flow diminishes as disease progresses.</p>

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<p align=""left"">Role of NMDA-Type Glutamate Receptors in Formation and Maintenance of Dendritic Spines

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<p align=""left"">New approaches developed in Dr. Sabatini's lab are enabling his team to challenge the conventional wisdom about how neural connections and synaptic function are regulated. His lab is especially interested in dendritic spines, which are targets for many excitatory synapses in the mammalian brain. The growth of spines is highly regulated during development, with the majority appearing during periods of massive synaptogenesis. Once synapses are made, spine growth and remodeling is promoted by activity patterns that trigger long-term potentiation.</p>

<p align=""left"">Neither the functioning of dendritic spines nor the signaling cascades that lead from synaptic activity to spine growth is well understood, Dr. Sababini said. In his presentation, he described a series of experiments that cast doubt on earlier claims that spine formation is independent of NMDA-type glutamate receptor (NMDA-R) activation. This investigation was triggered by observations of spine growth and retraction in the brains of young rats, which caused Dr. Sabatini to suspect that far from being irrelevant, NMDA-R might be essential.</p>

<p align=""left"">In contrast to earlier work, which relied on pharmacological manipulations to study spine growth, Dr. Sabatini's team used RNA-inactivation (RNA-I) to knock out NR1, an obligatory subunit of the NMDA-R, in rat hippocampal slices. A low-efficiency gene gun transfected only about 10 pyramidal neurons in each slice, marking these with a fluorescent protein and enabling the researchers to perform patch-clamp experiments on labeled cells. The rest of the cells in the slice were normal, and the whole sample could be maintained and observed for weeks.</p>

<p align=""left"">When cells with normal NMDA-R were electrically stimulated, they depolarized and showed long signal decay. This response was lost, however, in RNA inactivated cells. In these

cells, Immunostaining confirmed that RNA-I directed against NR1 dramatically reduced NR1 protein levels and eliminated functional synaptic NMDA-Rs. Deprived of synaptic NMDA-Rs, the number of dendiritic spines declined gradually. Eight days into the experiment there was no significant difference between knockout and normal cells; after 18 days, there were virtually no spines in the altered cells while controls retained full dendritic trees, Dr. Sabatini said.</p>

<p align=""left"">Evidence that NMDA-Rs are necessary for both spine formation and maintenance motivated Dr. Sabatini's team to construct a "dual laser scanning microscope" for learning more about spine dynamics. Future experiments will also investigate genetic control of synaptic plasticity in human diseases such as Alzheimer's.</p>

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<p align=""left"">Molecular Mechanisms of Plasticity in the Visual Cortex

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<p align=""left"">Dr. Maffei's team has taken important steps toward restoring eyesight to the blind, at least in terms of restoring lost plasticity to visual cortex cells. Decades ago, Nobel Prize-winning work by Harvard's David Hubel and Torsten Wiesel proved that covering one eye during a critical period in development, even for only a few days, could cause permanent visual impairment. The duration of this critical period is proportional to the lifespan of a species, ranging from a few weeks in mice to several years in humans. During this time, the plasticity of the visual cortex depends on electrical activity and molecular signals involved in stabilization and remodelling of neural circuits. For example, his group has demonstrated that ERG pathway activation is necessary for experience-dependent plasticity (monocular deprivation) and for long-term potentiation of cortical synaptic transmission.</p>

<p align=""left"">After the critical period ends, plasticity disappears and depriving one eye of stimulation no longer causes a shift in ocular dominance. Recent work in Dr. Maffei's lab has sought to identify factors that close the critical period and limit adult plasticity. One series of experiments used a transgenic mouse that over-expresses brain-derived neurotrophic factor (BDNF), which mediates neurotransmitters including inhibitory gamma aminobutyric acid (GABA). In this mouse, which has elevated GABA levels, development of visual acuity was accelerated and the critical period closed early. The researchers asked whether plasticity could be restored by blocking GABA synthesis and uptake in adult rats, then covering one eye. The surprising answer was yes: there was a shift in ocular dominance typically found only during the critical period, indicating that the "window of plasticity had been reopened in adult rat brain," Dr. Maffei said.</p>

<p align=""left"">A second series of experiments explored the role of extracellular matrix factors in visual cortex plasticity. They focused on chondroitin sulphate proteoglycans (CSPGs), which inhibit axon sprouting and mature just as the critical period ends. At this time, CSPGs undergo a dramatic reorganization into perineural nets.In animals reared in the dark, however, CSPG maturation is delayed and these nets do not take shape. The researchers used injections of chondroitinase ABC to degrade CSPGs in the adult visual cortex, and found that ocular dominance plasticity could be restored without harming receptive field size or visual response properties, Dr. Maffei reported. Investigations into the relationship between extracellular matrix components and visual cortex plasticity continue in his laboratory.</p>

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<p align=""left"">What is the Relationship Between Physiologic and Evolutionary Adaptation?

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<p align=""left"">On the heels of a series of reports reports delving into the details of cellular sensing and response, Dr. Kirschner stepped back to examine these studies in relation to a much broader universe. He linked basic science findings both to physiologic adaptations at the individual level and to species-wide adaptations to the environment, drawing on concepts features in Genes, Embryos, and Evolution. This book, written by Dr. Kirschner and John Gerhart in 1997, calls on molecular biology and classic Darwinian theory to show how factors such as genetic variability and conservation shape both individuals and the enduring traits of a species. Even a physiologic adaptation that manifests only under certain conditions, such as hemoglobin's capacity to bind oxygen more tightly at high altitudes, can promote long-term hereditary change, Dr. Kirschner said.

If individuals with a trait survive those without it, a once marginal characteristic becomes mainstream.</p>

<p align=""left"">Biologic variability occurs at many levels, ranging from phenotypic hallmarks that distinguish horses from zebras to the hard-to-detect cellular and developmental variations described during this Symposium, Dr. Kirschner said. Many of these variations can be explained by principles of what he called a “neo-Darwinian synthesis”: Evolution depends on heritable variability; external environment does not generate heritable variants (children of skilled pianists don’t necessarily play well); large phenotypic changes occur gradually and accumulate; evolution is descent by modification. The “molecular addendum” to these tenets is that change occurs via random mutations, and that resulting alterations in proteins or regulation affect phenotype. What scientists can’t do quite yet, of course, is examine genetic sequence data and predict its results, he said.</p>

<p align=""left"">Dr. Kirschner closed by describing the paradoxical nature of “explanatory processes” such as microtubules, which are highly conserved yet versatile enough to provide rigidity to the skeleton or enable leukocytes to change direction in an instant. “Diversity itself is a selected property in biology,” he said, and “we wouldn’t be here if it wasn’t for the factors that generate diversity. Rather than thinking of conservation as the end result of why things can’t change, I think we should look at conservation as being preserved under continual selection because it provides the capacity to change in both evolution and physiology.”</p>";"Symposium 2003: Trieste, Italy";"";publish;open;open;"";symposium-2003-trieste-italy;"";"";2012-09-14 12:59:16;2012-09-14 12:59:16;"";0;http://armeniseharvard.sypdevelopment.com/?p=256;0;post;"";0