the giovanni ARMENISE HARVARD foundation

ARMENISE-HARVARD SYMPOSIUM 2006

CELEBRATING A DECADE OF EXTRAORDINARY SCIENCE

10th Annual Symposium
June 12-15, 2006, Grand Hotel Baia Verde, Catania, Italy

About the Symposium

The 10th Annual Symposium of the Giovanni Armenise-Harvard Foundation Symposium, entitled Celebrating a Decade of Extraordinary Science, demonstrates that grief can give rise to greatness.

Twelve years ago, Count Giovanni Auletta Armenise lost his beloved wife, Dianora Bertacchini, to a brain tumor that not even the best efforts of physicians at Massachusetts General Hospital could defeat. During her time at this Harvard Medical School teaching institution, the couple realized that improved treatments for this and other devastating diseases will be possible only if basic science research flourishes.

Following his wife's death, the Count did more than mourn her loss. He worked closely with Daniel Tosteson, then Dean of Harvard Medical School, to set up a Foundation that would promote investigation of profound questions about the workings of life itself. Not only that, but they devised plans for creating new connections between basic scientists in Italy and at HMS. These two men "set a path that we could follow, evolve and expand upon," current HMS Dean Joseph Martin said in his introductory remarks at this milestone symposium.

In the United States, the Giovanni Armenise-Harvard Foundation supports seven multidisciplinary research centers that involve more than 50 faculty members on the HMS quadrangle. These centers collaborate with Italian institutes and individual scientists, help train Italian postdoctoral fellows and junior scientists, participate in international research seminars and organize public conferences in Boston on a range of topics including neuroscience, systems biology, and aging.

Since 1997, 27 rising young researchers have received HMS Junior Faculty Awards underwritten by the Foundation. These two-year grants help recipients generate scientific publications and attract significant additional funding for their laboratories. Award-winners Azad Bonni, Tom Walz, David Rudner and Gahlit Lahav gave invited podium presentations at this year's Symposium.

At Massachusetts General Hospital, the Foundation has provided support to 13 researchers studying neuro-oncology and related disorders; on hand for the Symposium was two-time recipient Verne Caviness.

In Italy, the Foundation has invested more than $12 million in scientific research since its inception. During the first five years, 56 scientists at five Italian research institutes received support from Collaborative Research Grants. This program made it possible for researchers in Rome, Milan, Turin and Padua to pursue research questions with new collaborators on the HMS Quad. Armenise support also helped create a new structural biology facility in Milan. Three of the original principal investigators – Jacopo Meldolesi, Giulia De Lorenzo, and Tullio Pozzan – participated in the 10th Annual Symposium.

Today, one of the Foundation's proudest accomplishments is making it possible for young Italian scientists to establish laboratories and launch independent careers in their home country. Eight promising newcomers have received Career Development Program grants since 2001, and seven participated in the Symposium: Alberto Bacci, Stefano Casola, Davide Corona, Stefano Gustincich, Claudia Lodovichi, Carlo Sala and Rosella Visintin. All lead independent laboratories that are magnets for other young researchers, creating new opportunities for work and learning in Milan, Rome, Padua, Palermo and Trieste. Several of the career development awardees received graduate or postdoctoral training at HMS, and the Foundation presently underwrites two PhD candidates on the Quad. In all of Italy, this is one of only two initiatives aimed at helping this nation retain some of its best and brightest minds.

Dr. Martin emphasized that none of these programs would exist without the "incredible partnership" between Count Auletta Armenise and Dan Tosteson. Unfortunately, he told attendees, the former HMS Dean was injured in a fall on the eve of the Symposium and unable to participate. The Count ,however, was in the front row when his contributions to Italian science were formally recognized with the Targa della Presidenza della Repubblica, an award Italy's President bestows only on people or institutions of outstanding merit. This surprise presentation was made on the President's behalf by Tullio Pozzan, one of the original Armenise investigators in Italy. Pozzan is a professor at the University of Padova and the Venetian Institute for Molecular Medicine.

In addition to fueling research, the Foundation has been stimulating the flow of science news to the Italian public since 2000. The Science Writer Fellowship program has enabled 15 Italian journalists to attend annual symposia and travel to HMS, where they learn about science, make invaluable contacts and research articles while being hosted by the Office of Public Affairs. Fellows Daniela Cipolloni and Luca Sciortino participated in the 2006 Symposium; Antonio Carlo Larissa will join them at HMS.

Approximately 95 scientific participants traveled to the historic port city of Catania, on Silicy's northeast coast, to celebrate the Armenise-Harvard Foundation's first decade. Participants represented 15 centers, institutes and universities in Italy, three in the United States, and one in Switzerland. They gathered on the sunny terraces of the Grand Hotel Baia Verde in Catania, overlooking black lava cliffs and the blue Ionian Sea. This is one of three seas whose waves break on

Sicily's coasts, and the island's language and culture are marked by a succession of different rulers – Greeks, Romans, Byzantines, Arabs, Normans and Spaniards.

Like Sicily itself, Celebrating a Decade of Extraordinary Science featured an exceptionally diverse and multicultural scientific program. Over the years, the Foundation has helped scientists explore topics in cancer biology, neuroscience, infectious disease, structural biology, genetics and genomics, systems biology, and integrative biology and physiology. The Symposium's 24 featured speakers and 18 poster presenters who gave participants at least a taste of all those topics and more. The scientific program was organized by Steven Harrison, Peter Howley, Elio Raviola and John Flanagan of HMS. Following the keynote address, lectures were grouped into four sessions:

- Membranes
- Cell Cycle and Gene Regulation
- Genes to Disease
- Signaling, Networks, and Cell-cell communication

This report is structured along the same lines, and provides a brief introduction to each session and summaries of individual presentations.

Fragile X Syndrome: Molecular mechanisms and Therapeutic Implications</p>

Stephen T. Warren

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The 1991 discovery of the gene responsible for Fragile X syndrome put Stephen Warren and his colleagues on the front page of the New York Times. This deserved headlines because defects in this gene, called FMR1, affect approximately one in 4,000 boys born worldwide – making Fragile X syndrome the most common heritable form of mental retardation in males. At the time, doctors were well aware that these boys' lives were often complicated by autism, hyperactivity and attention problems.

<p align=""left"">What physicians did not suspect was that abnormalities in FMR1 could cause premature menopause in 20% of women carriers, or that some grandfathers of affected boys would develop a neurological disorder easily mistaken for early-onset Parkinson's disease.</p>

<p align=""left"">Dr. Warren has spent 15 years analyzing the molecular basis for Fragile X syndrome, and he now knows that the dose makes the poison. There is a polymorphic CGG-repeat in the 5' untranslated portion of the X-linked FMR1 gene, and healthy people have alleles containing 7-55 copies of this triplet. Carriers who can pass along Fragile X syndrome have 55-200 copies, which the researchers call "pre-mutation" alleles. Patients with the syndrome have "full mutation alleles," typically containing far more than 200 copies. "It is remarkable how unstable this triplet is when transmitted," Dr. Warren said, "three sons of one mother can have drastically different numbers of CGG repeats." One might have Fragile X syndrome; the others no sign of it.</p>

<p align=""left"">Pre-mutation alleles deliver a moderate dose and are associated with RNA-mediated neurodegenerative disorders, found mostly in men. Women who receive the same 7-55 copies are at risk for passing Fragile X syndrome to their offspring and for premature ovarian failure. When more than 200 copies of the CGG triplet are present, the full mutation allele becomes heavily methylated and the chromatin shifts to a heterochromatic state. As a result FMR1 is silenced, unable to make messenger RNA needed to produce FMRP, its encoded protein.</p>

<p align=""left"">Researchers soon realized that the loss of FMRP is responsible for fragile X syndrome, but they “hammered away” for years before understanding how the protein works and why the number of CGG triplets makes such a difference. DNA microarrays, experiments with transgenic flies and mice, and a serendipitous misadventure with lab chow helped Dr. Warren’s team identify FMRP is a selective RNA-binding protein that shuttles between the nucleus and cytoplasm. FMRP binds with about 3% of mRNAs expressed in the brain, and appears to suppress translation of these targets.</p>

<p align=""left"">The study of FMRP gained considerable momentum when researchers found that it occurs mainly in association with ribosomes in dendritic spines. This is a location where local control of protein synthesis is critical in synaptic plasticity. After glutamate stimulation, phosphorylated FMRP appears to locally repress translation of associated mRNAs in a dynamic process that may also involve using microRNAs to put the brakes on protein synthesis. Individuals with Fragile X syndrome have essentially had their brake cables severed.</p>

<p align=""left"">Excessive translation of certain mRNAs, particularly following type I mGluR stimulation, probably accounts for the various cognitive, reproductive and neurological abnormalities associated with pre-mutation and full-mutation alleles. Experiments using mouse and Drosophila models of fragile X syndrome indicate that antagonists of mGluR signaling can reduce over-translation and rescue some affected animals, and a screen of some 2,000 small molecules yielded nine compounds that rescue the dFmr1-deficient phenotype in flies.</p>

<p align=""left"">Dr. Warren’s colleagues have devised a highly accurate screening test for pinpointing Fragile X syndrome mutations in newborn boys. The researchers expect to begin early clinical testing of candidate drugs about one year from now, and they are hopeful that early intervention might counteract the loss of FMRP.</p>

<p align=""center"">Session 1: Membranes</p>

<p align=""center"">At first glance the cell membrane appears to be a simple container for liquid cytosol and solid organelles, a slippery mix not unlike bubble tea. Look more closely and the bottle is made of a lipid bilayer that loves water on one surface and hates its on the other; zoom in again and the membrane is a teeming, dynamic marketplace where proteins engage in every imaginable commerce with the extracellular world. The opening session of this

10th anniversary Symposium showcased the work of researchers who examine the inner lives of membranes, exploring how they expand, change shape, form junctions, transfer information and are altered by disease proteins.</p>

<p align=""left"">Regulated exocytosis: what's new about enlargeosomes</p>

<p align=""left"">Jacopo Meldolesi</p>

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<p align=""left"">At the 2003 Armenise-Harvard Symposium in Trieste, Dr. Meldolesi introduced what he described as "a little, brand-new organelle" that races to the plasma membrane in response to calcium stimulation. Unlike other vesicles controlled by regulated exocytosis, which serve to deliver specific payloads to the cell surface, these newly discovered structures were empty. He dubbed them "enlargeosomes," because their sole purpose seemed to be expanding the cell membrane.</p>

<p align=""left"">At the time, Dr. Meldolesi's presentation was cautiously received: these structures had not been reported in the scientific literature, and the classical view of regulated exocytosis was that it exists to shuttle discrete packets of neurotransmitters, hormones, enzymes and other substances into the extracellular space. Dr. Meldolesi insisted that this was not the whole story. After all, even exocytic organelles fuse with the plasma membrane after releasing their contents, expanding the surface area of the cell.</p>

<p align=""left"">A literature review showed that regulated exocytosis is mentioned in about one-tenth of all biomedical publications, mostly the secretory form. Non-secretory exocytoses that enlarge the plasma membrane have been described in reports concerning cell differentiation in

plants and animals, wound-healing, cyto-diuresis, phagocytosis and neurite growth. Dr. Meldolesi concluded that non-secretory exocytosis is just as important as the secretory form (see Chieregatti and Meldolesi, Nature Rev. Mol. Cell Biol., 2005).</p>

<p align=""left"">In his own laboratory, enlargeosomes were first identified in a secretion-defective clone of PC12 cells. The Meldolisi team developed a marker for pinpointing where enlargeosome membranes have fused following calcium stimulation. This marker, a high molecular weight, non-transmembrane protein called Ahnak/Desmoyokin (dA), enabled the researchers to document enlargeosome fusion in approximately 15 cell types following [Ca2+]i increase.</p>

<p align=""left"">Dr. Meldolesi and his colleagues used immunocytochemistry to determine the ultrastructure of these vesicles and track exocytosis in response to stimulation. Enlargeosomes are small, only about 60nm in diameter, and their membrane is coated on both sides. The inside is lined with a large protein, Ahnak; the cytosolic surface of the vesicle is coated with annexin2, a protein that binds to other membranes only in response to an influx of [Ca2+]i. The team's latest finding is that enlarged cells can shrink by "recycling" fused enlargeosome membranes, which they accomplish via a peculiar, non-clathrin-dependent process, Dr. Meldolesi reported. Enlargeosomes remain the chief focus of his laboratory's investigations, and he is confident that additional findings will continue to emerge. His listeners, more receptive now than in 2003, appeared to agree.</p>

<p align=""left"">Snake neurotoxins, membrane bending and exo-endocytosis.</p>

<p align=""left"">Ornella Rossetto</p>

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<p align=""left"">Some of the world's most dangerous snakes paralyze and kill their victims by catastrophically disrupting synapse function. Because venoms from kraits, taipans and similar

poisonous snakes are so powerful, these natural poisons are invaluable tools for studying how cell membranes behave, Dr. Rossetto said.</p>

<p align=""left"">Her laboratory makes extensive use of SPANs, or snake presynaptic phospholipase A2 neurotoxins, both in cell culture experiments and a more complex system using an ex vivo preparation of mouse hemi-diaphragm. As snake neurotoxins intoxicate and ultimately paralyze the neuromuscular junction (NMJ), abnormal bulges form on neuronal extensions and synaptic vesicles rapidly deplete. Dr. Rossetto hypothesizes that these changes come about because snake toxins accelerate exocytosis and inhibit endocytosis at the same time.</p>

<p align=""left"">Working with cultured primary neurons, her team observed that exposure to nanomolar concentrations of four different SPANs (beta-bungarotoxin, taipoxin, notexin and textilotoxin) induced dose-dependent formation of discrete bulges at various sites on neuronal projections. Using a variety of markers, the researchers could see neurotransmitter-carrying vesicles concentrate at the bulges; exocytosis was confirmed by the presence of the lumenal domain of synaptotagmin I, a protein detectable only when vesicles deliver their cargo to the cell surface. At the same time, the cultured neurons released glutamate and lost signals from FM 1-43 dye – a sure sign that vesicles are being recycled after delivering neurotransmitters. These findings support the idea that SPANs cause bulges by boosting exocytosis and slowing endocytic retrieval of emptied vesicles.</p>

<p align=""left"">There is a great deal left to discover about now the various snake neurotoxins alter normal synaptic activity and phenotype, Dr. Rosetto said. For example, there is ongoing debate about the possible role of Phospholipase A2 enzymes in NMJ blockade, although these enzymes are known to cleave phospholipids in cell membranes and to be players in membrane trafficking. There is only a partial correlation between PLA2 activity and the neurotoxicity of various snake toxins, and no overlap of surface residues required for neurotoxicity with those essential for PLA2 activity.</p>

<p align=""left"">In the mouse hemidiaphragm preparation and in cultured neurons, Dr. Rossetto's group tackled this problem by comparing the effects of SPANs and their hydrolysis products, lysophospholipids and fatty acids, on neuromuscular junctions. They observed that an equimolar mixture of these two breakdown products has essentially the same biological effects as the snake toxins. These results draw attention to the possible role of local lipid changes in synaptic vesicle release, Dr. Rossetto said, and provide new tools for future studies of exocytosis.</p>

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<p align=""left"">Structure of the aquaporin-0 mediated membrane junction</p>

<p align=""left"">Thomas Walz</p>

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<p align=""left"">During development, the lens of the eye takes shape and gains function as an elaborate network of junctions is built. Fiber connexins are as basic as two-by-fours in lens construction, but it now turns out that another protein, aquaporin-0 (AQP0), is needed to connect them to one another. Because aquaporins are mainly thought of as proteins that admit water to cells, AQP0's role in lens development would have been easy to overlook.</p>

<p align=""left"">The lens continuously synthesizes the classic, pore-forming version of AQP0.Early in lens development, aquaporin channels allow circulation of nutrients and waste. As the lens matures, assuming the more rigid geometry needed for vision, aquaporin assumes new duties as an architectural element in junction formation.</p>

<p align=""left"">In the May 13, 2006, issue of Nature, Dr. Walz and coworkers published a 1.9 Å resolution structure of junctional AQP0, determined by electron crystallography of double-layered two-dimensional crystals. When the researchers compared junctional and water-pore forms of the protein, they observed that junction formation required a conformational switch in an extracellular loop. This shape change apparently resulted from proteolytic cleavage of the cytoplasmic N- and C-termini.</p>

<p align=""left"">In junctional AQP0, what had been the water pathway narrows so dramatically that water molecules can no longer squeeze through. In the more mature lens, AQP0 forms

tetramers and interacts with lipid molecules to form junctional crystals. Four molecules of the protein in one layer match up with four in the other, locked into a rosette-like shape by proline residues not found in other aquaporins. Dr. Walz's team is now looking more closely at the switch that transforms AQP0 from a water pore to a junction protein, and at the forces that hold the junction crystals together.</p>

<p align=""left"">Tipping the Iron Balance</p>

<p align=""left"">Nancy C. Andrews</p>

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<p align=""left"">The more closely Dr. Andrews scrutinizes the genetic underpinnings of hereditary hemochromatosis, the more ornate it appears to be. She used her Symposium lecture as an opportunity to step back, survey the big picture, and explain how mutations in five different genes contribute to iron-overload disease.</p>

<p align=""left"">In a healthy person, iron is absorbed from food by cells lining the gut. It is released at a tightly controlled rate and stockpiled by red blood cells and macrophages; when tissues and organs need iron, they can take it up from the bloodstream. In hemochromatosis, toxic amounts of iron accumulate in the liver, pancreas and heart – where levels are normally low – and macrophages are mysteriously empty of their usual mineral cargo.</p>

<p align=""left"">Mutations in HFE, the "classic" hemochromatosis gene, or in TFR2 (which encodes the transferrin receptor), are held accountable for most cases of

adult-onset disease. Yet only a fraction of patients homozygous for mutations in these genes develop clinical disease, so obviously these two genes don't tell the whole story. Environmental influences also come into play: physicians know that disease severity varies with factors such as alcohol intake, dietary iron consumption, and menstruation.</p>

<p align=""left"">In addition, Dr. Andrews and other investigators have identified three more genes whose products are also involved in iron homeostasis: ferroportin, hepcidin, and hemojuvelin. "Mutations in two genes –hepcidin and ferroportin – have major effects in hemochromatosis," she said. "The other three fine-tune the connections."</p>

<p align=""left"">There is a clear, inverse correlation between hepcidin level and how sick a patient becomes. Hepcidin normally binds ferroportin, regulating how much iron it releases from stockpiles in intestinal cells and macrophages. Without this restraining factor, ferroportin keeps pouring iron into the bloodstream. Dr. Andrews said these are the two most important contributors to hemochromatosis disease, with the other three genes playing secondary roles. Mutations in the classic HFE gene interfere with transferrin receptors, causing gut cells to store too much dietary iron in the first place. TRF2 abnormalities inhibit release of soluble hemojuvelin, which in turn depresses hepcidin and contributes to disease.</p>

<p align=""left"">Dr. Andrews and her coworkers are refining their model for hemochromatosis and using animals to search for therapeutic alternatives to bloodletting, which is all doctors have to recommend at present. Ideally, researchers will identify a small molecule that can mimic hepcidin's effects</p>

<p align=""left"">Cell-cell signaling through regulated proteolysis</p>

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<p align=""left"">Cholesterol metabolism, Alzheimer's disease, and cellular responses to stress are only a few examples of complex phenomena that are difficult to study in higher animals. Yet all three involve a signaling mechanism that Dr. Rudner is elucidating with help from a far simpler creature. The mechanism is called regulated intramembrane proteolysis, or RIP, and the organism is Bacillus subtilis.</p>

<p align=""left"">Cells respond to their environment and to each other by transferring information across their membranes, which allows the cell to respond appropriately to what's happening on the outside. RIP is a type of messaging that relies on special, hydrophobic proteases that can operate in the lipid-filled interior of the cell membrane itself. These proteases sequentially cleave membrane-bound substrates, releasing transcription factors.</p>

<p align=""left"">Take away B. subtilis's food supply, and the organism differentiates into a spore, a dormant cell type that can endure for hundreds – perhaps thousands of years – until conditions improve, said Dr. Rudner, who received an Armenise junior faculty grant in 2003. The beauty of using a simple animal model is that this remarkable transformation – in which a moving and eating organism turns into a seemingly inanimate pellet – requires a scant handful of genes. And one of these, SpolVFB, is "a founding member of a family of membrane-bound metalloproteases involved in RIP," Dr. Rudner said.</p>

<p align=""left"">Sporulation begins with an asymmetric cell division that generates a large mother cell and a small forespore. Initially the forespore is engulfed by the mother cell, and although the two will follow completely different programs of gene expression, their developmental programs are temporarily coordinated by signal transduction pathways involving the membranes of both. In one pathway, a precursor called pro-sigK is synthesized by the mother cell, then cleaved by the membrane-embedded enzyme SpolVFB into sigmaK, a transcription factor. Until its activation, SpolVFB is locked in a complex with two other integral membrane proteins, SpoIVFA and BofA. The release of SpolVFB from this inactive state is triggered by a signal protein, SpoIVB, which is produced in the forespore and secreted into the space between the mother-cell and forespore membranes. This triggering protein, also called IVB, is known to be a serine protease but its mechanism for activating sigmaK has been poorly understood.</p>

<p align=""left"">Dr. Rudner's team discovered that IVB sits at the apex of a branched pathway that governs RIP, and they have evidence that it activates pro-sigmaK directly by cleaving SpoIVFA at multiple sites and indirectly by cleaving and activating a second serine

protease known as CtpB. CtpB, in turn, activates processing by also cleaving SpoIVFA. This is a one-two punch typical of RIP signaling, and it bears a striking resemblance to serine protease cascades important for blood clotting, dorsal-ventral patterning in Drosophila, and other physiologic processes, he said.</p>

<p align=""left"">Formation and maturation of glutamatergic synapses</p>

<p align=""left"">Carlo Sala</p>

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<p align=""left"">Baseball promoters and post-synaptic neurons in search of excitement may have the same motto: If we build it, they will come. Neuroscientists have long debated whether synapses form at sites bombarded with signals by pre-synaptic neurons, or whether the post-synaptic side initiates the process by building a receiving station that somehow draws incoming signals. Based on extensive studies of excitatory neurotransmission in mammalian hippocampal neurons, Dr. Sala – who received the Foundation's first Career Development Award in 2001 – believes the second is more likely.</p>

<p align=""left"">No thought or movement would be possible without activity at millions of excitatory and inhibitory synapses, each with characteristic components that determine their specificity. In the mammalian brain, glutamate is the primary neurotransmitter that nerve cells use to send excitatory messages. On the receiving side of the glutamatergic synapse is a dense, fibrous mass made of hundreds of molecules, including neurotransmitter receptors, scaffolding proteins, adhesion molecules, and signal transduction enzymes; collectively this is called "the postsynaptic density," or PSD.</p>

<p align=""left"">There are many scaffolding proteins in the PSD, and three of them – PSD-95, GKAP and members of the Shank family – form a complex. Dr. Sala's team has demonstrated that PSD-95 and GKAP are needed for correct folding and synaptic localization of Shank. In the February 2006

issue of Neuron, Dr. Sala and collaborators at the University of British Columbia reported that when this complex is joined by neuroligin1 (NLG1), a synaptic adhesion molecule, they attract a pre-synaptic terminal which matures into an excitatory synapse. The magnetic activity of this complex may be clinically important: a shortage of scaffold proteins is associated with some forms of mental retardation, and flaws in the stabilization of post-synaptic structures may relate to autism.</p>

<p align=""left"">Glutamateric synapses are typically located at the tips of dendritic spines, and Dr. Sala's lab is now using a proteomic approach to screen some 2,000 proteins that may be involved in dendritic spine morphogenesis and protein synthesis.</p>

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<p align=""center"">Session 2: Cell Cycle and Gene Regulation</p>

<p align=""center"">This session focused on two chapters in the life story of cells: the first two speakers provided intimate looks at what happens as mitosis begins; the remaining three explored various mechanisms that determine when and where genes are expressed. But all the talks illustrate the value of the Foundation's support for basic research, according to Symposium co-organizer Steve Harrison, because these researchers are making discoveries that shed light on classic genetic observations, such as X-chromosome inactivation, and because they could pave the way to new treatments for several common cancers.</p>

<p align=""left"">The spindle assembly checkpoint: reality and fiction</p>

Andrea Musacchio

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<p align=""left"">Cells double their DNA during the synthesis, or S phase of their life cycle, and then must exercise great care as they split this genetic material into equal parts before dividing. Lax quality assurance allows chromosomal instability and aneuploidy, problems characteristic of cancer cells. Dr. Musacchio's laboratory studies formation of stable kinetochore-microtubule attachments during mitosis, a process monitored by the spindle assembly checkpoint (SAC). If kinetochores are attached improperly, or left unattached, the SAC arrests cells in a prometaphase-like state. If all sister chromatid pairs are properly attached and oriented, metaphase will proceed and the SAC will be switched off – allowing chromosome separation and anaphase, Dr. Musacchio explained.</p>

<p align=""left"">Over the years, he has made a strong case for the importance of the SAC protein called Mad2 in this inspection process. Here, Dr. Musacchio reported that his lab reconstituted the putative Mad2 kinetochore receptor and developed a kinetochore recruitment assay with purified components. They employed a technique called FRAP, an acronym for "fluorescence recovery after photobleaching," to observe dynamic interactions between cytosolic Mad2 and kinetochores. They saw catalytic activation of bound Mad2, followed by its release in a complex with Cdc20.</p>

<p align=""left"">Dr. Musacchio suspects that a stable Mad1-Mad2 complex actually functions as an enzyme, and that a positive feedback loop controls a crucial conformational change in the Mad2 protein. He is using in vitro FRAP to test this hypothesis, and sees this technology as a boon to understanding how Mad2, a protein, interacts with kinetochores, a physical cellular compartment. "In the future, we plan to use the numbers retrieved using in vitro FRAP to gain a quantitative understanding of the checkpoint network," Dr. Musacchio said.</p>

<p align=""left"">Aurora A and cell cycle progression: a centrosome-independent role in regulating mitotic entry</p>

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<p align=""left"">The gene encoding the small serine/threonine kinase Aurora A (Aur-A) is a proto-oncogene associated with cell proliferation in organisms ranging from flies to humans. In experiments with mice, forced overexpression drives aggressive tumor growth; in humans, abnormally high levels of Aur-A can be measured in 65% of breast and 50% of prostate cancers, Dr. Ruderman said. Not surprisingly, several drug companies are working on Aur-A as a possible target for anti-cancer drugs.</p>

<p align=""left"">These efforts are not far along, however, mainly because Aur-A's regulatory activities are just beginning to be understood. Discovered more than 20 years ago as a Drosophila mutation responsible for defects in the mitotic spindle, Aur-A was soon found to be highly enriched in centrosomes, Dr. Ruderman said. Almost all of Aur-A's known functions reflect in some way its presence at centrosomes, which form the poles of the mitotic spindle.</p>

<p align=""left"">Aur-A is required for the recruitment and/or activity of several key centrosomal proteins, for the correct placement of centrosomes at the spindle poles, and for accurate chromosome segregation during the cell cycle. Experiments have shown that the initial activation of cdc2 during the transition from G2 to M occurs at centrosomes, and that Aur-A participates in the centrosomal activation of cdc25B, the phosphatase responsible for this early activation of cdc2.</p>

<p align=""left"">Dr. Ruderman's laboratory seeks to understand, in molecular terms, exactly how Aur-A regulates to the timing of mitotic entry. In order to tease out the roles of various players in this drama, they make cell-free extracts from Xenopus eggs – some with centromeres and some without. Frog sperm is used to initiate normal cell cycling.</p>

<p align=""left"">In this system, neither the DNA nor spindle integrity checkpoint pathways are active, making it possible to investigate the basic cell cycle roles of Aur-A free from complications encountered in somatic cells with active checkpoint pathways. When the researchers added</p>

<p align=""left"">active Aur-A to the cycling egg extracts, cdc2 activation and mitotic entry were accelerated. When they depleted endogenous Aur-A or added inactive dominant negative

Aur-A to the system, mitotic entry was delayed but not blocked.</p>

<p align=""left"">But the most striking result, Dr. Ruderman said, was the finding that Aur-A's effects on the timing of the G2/M transition occur even when centrosomes are absent. In the March 31, 2006, issue of Proceedings of the National Academy of Science, her team published evidence that Aur-A not only contributes to centrosome maturation and function, but also plays a centrosome-independent role in the timing of mitotic entry. They are currently pursuing this observation.</p>

<p align=""left"">Reversal of histone lysine tri-methylation by the JMJD2 family of histone demethylases</p>

<p align=""left"">Yang Shi</p>

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<p align=""left"">Without histone, DNA would not be packaged in the "beads on a string" arrangement so familiar to PBS viewers and high school biology students. This scaffolding for DNA was initially spotted in the late 19th Century, but it wasn't until the 1960s that researchers realized this supposedly inert framework was dynamic, and that its function changed when methyl groups latched onto specific sites on its tail.</p>

<p align=""left"">"Histone's complexity was underestimated: methylation regulates chromatin structure, transcription and the epigenetic state of the cell," Dr. Shi said. Today, chemical modification of histone and DNA is a hot topic in biology, because epigenetic factors are now recognized as on-and-off switches for genes in both health and disease. It took half a

century,however, to identify enzymes that can attach methyl groups to the histone tail. The first histone methylase was cloned in 2000, and biologists thought it was nature's equivalent of Super Glue.</p>

<p align=""left"">In 2004, Dr. Shi's lab overturned the idea of permanent, irreversible methylation by cloning an enzyme that could remove one or two methyl groups from a lysine residue. Addition of methyl groups by histone methylases, and removal of single and double methyl groups by demethylating enzymes such as LSD1 and JHDM1, is now seen as business as usual on the histone tail.</p>

<p align=""left"">Less clear was whether any enzyme could pry loose three methyl attachments. Dr. Shi decided to pursue this question because it relates to real human disease: tri-methylated histones are associated with cancers of prostate and breast and with X chromosome inactivation. An all-out effort in the Shi laboratory succeeded in identifying a family of enzymes that can knock methyl groups off trimethylated sites on histones. A report in the May 5, 2006, issue of Cell describes how one such enzyme, JMJD2A, exercises a range of demythylating effects in cultured human cells and in live C. elegans nematodes. Other members of the same enzyme family operate slightly differently, but all are involved in removing methyl groups from specific sites.</p>

<p align=""left"">Dr. Shi and his coworkers were astonished to find that demythylation is involved in the cell's response to DNA damage, and that manipulating methylation can influence life or death decisions in nematode cells. This raises the possibility of devising medical treatments – possibly for cancer – by using small molecules to interfere with demethylase activity. "Understanding biological importance is the next step, and clearly there is more work to be done," Dr. Shi concluded.</p>

<p align=""left"">Regulation of microRNA expression during myeloid differentiation</p>

<p align=""left"">Irene Bozzoni</p>

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<p align=""left"">One of the most amazing revelations from the human genome project is that the enormous human proteome is encoded not by 300,000 genes – as scientists estimated a generation ago – but perhaps by only 25,000 human genes. How so few genes make so many products is a mystery indeed.</p>

<p align=""left"">It now appears that the solution rests not only with DNA, the most celebrated molecule of the 20th century, but with small, non-coding bits of RNA, a molecule traditionally seen as DNA's modest handmaiden. Dr. Bozzoni has helped gild RNA's image by demonstrating the power of micro-RNAs to curb post-transcriptional gene expression. These 20 to 22 nucleotide snippets of RNA were under the radar for a long time. Not only because they're tiny, but because miRNAs were first seen in plants – causing skepticism among mammalian biologists that they could be important in animals. The discovery of a C.elegans miRNA in 1993 was the first ripple in what became a sea change, and hundreds of highly conserved miRNAs are now known to help regulate development, differentiation, and cell proliferation in animals and plants.</p>

<p align=""left"">Hemopoesis is perhaps the best-studied illustration of a multipotent stem cell giving rise to nearly a dozen constantly renewed, terminally differentiated cell types. Blood cancers make this a clinically important process as well, and Dr. Bozzoni studies how lineages are specified in a cell line derived from patients affected by acute promyelocitic leukemia. When these cells are stimulated with retinoic acid (RA) they differentiate into granulocytes; if exposed to tissue plasminogen activator (TPA) they become monocytes that mature into macrophages.</p>

<p align=""left"">Dr. Bozzoni's lab has identified two micro-RNAs, miRNA-223 and miRNA-424, that act differently on the same target depending on what chemical has been added to the APL cells. When RA is added to trigger differentiation into granulocytes, miRNA223 shuts down NFI-A and granulocytes take shape. When TPA is given, in contrast, miRNA424 represses NFI-A translation and the precursor cells turn into monocyte/macrophages, Dr. Bozzoni reported. Her team has identified specific promoters and other factors that control lineage-specific expression of these miRNAs, and has also found targets useful for studying the balance between proliferation and differentiation during myelopoiesis.</p>

<p align=""left"">Now that this methodology has demonstrated its worth, Dr. Bozzoni believes it will shed light on big questions about the evolutionary advantages of using a modest number of genes to generate a vast array of proteins.</p>

<p align=""left"">Caspase-11 regulates cell migration by promoting Aip1/Cofilin mediated actin depolymerization</p>

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<p align=""left"">Even old friends can surprise us. In the early 1990s, Junying Yuan pioneered the study of cell death genes and made important discoveries about the importance of caspase proteases in apoptosis. Her laboratory's intense scrutiny of caspases gradually revealed that members of this family participate not only in cell death, but also in normal development, inflammatory response, and adaptive immunity. Although Dr. Yuan could see that caspases are more versatile than she initially thought, it was still a revelation to find that caspase-11 helps mobilize lymphocytes to rush into battle against an infection. The mild-mannered hospice worker, it turns out, is also on the first-responder team.</p>

<p align=""left"">Caspase-11 belongs to the caspase-1 subfamily of pro-inflammatory caspases, and experiments in mice had established that it a crucial regulator of cytokine maturation and apoptosis. Caspase-11 is a critical activator of caspase-1, which then acts directly to turn pro-interleukin-1b (pro-IL-1b) into mature IL-1b. In caspase-11 knockout mice, IL-1b maturation and secretion is blocked, and the animals do not develop septic shock that would ordinarily result from lipopolysaccharide (LPS) stimulation.</p>

<p align=""left"">Caspase-11 is barely detectable in healthy wild type mice but is strongly induced by LPS stimulation. Caspase-11 is also involved in directly activating caspase-3 and apoptosis under

certain pathological conditions, such as brain ischemia and septic shock. Dr. Yuan knew all this about Caspase-11.</p>

<p align=""left"">The story took an unexpected turn, however, when the Yuan lab screened large numbers of proteins to see which ones caspase-11 interacted with. Much to their surprise, they got a positive hit for actin. Additional experiments using a mouse peritonitis model revealed that caspase-11 isn't the Lone Ranger here; it affects actin only if two other players are on hand: cofilin, a member of a major actin depolymerization factor family, and Aip1, which helps cofilin depolymerize actin and prevents the ends of severed actin fibers from rejoining.</p>

<p align=""left"">Experiments indicated that caspase-11 interacts physically and functionally with Aip1 to promote cofilin-mediated actin depolymerization, Dr. Yuan said. Knocking out either Aip1 function or caspase-11 expression reduced the mobility of T-cells that should have been rushing toward the front lines of infection. These results reveal a novel function of caspase-11 in regulating actin dynamics and cell migration, and future experiments will search for additional levels on which caspase-11 might be able to regulate inflammatory response</p>

<p align=""center"">Session 3: Genes to Disease</p>

<p align=""left"">The Armenise-Harvard Foundation has been backing neurobiology research since its earliest days. As Count Armenise and his late wife confronted the brain tumor that ultimately claimed her life, they realized that treatments delivered at the bedside begin with basic science questions asked – and answered, when experiments go well– at the laboratory bench. The road from laboratory to clinic is long and difficult, and this session featured reports from various milestones along the way. It began with a provocative new idea about nervous system development, went on to consider how cells invade and form networks during normal development and in disease, and concluded with a report on how prions that enter the body in a bite of hamburger burrow into and destroy the brain.</p>

<p align=""left"">Molecular mechanisms of axon and vascular guidance</p>

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<p align=""left"">With 500-year-old drawings by Vesalius and Da Vinci projected on the screen, Dr. Gu asked participants to consider how remarkably well these images have held up. Among the many things these early students of the human body got right was their portrayal of the nervous and vascular systems. They showed nerves and blood vessels traveling together, bundled between ribs in the trunk, reaching upward to the brain and out to the very tips of fingers and toes. Modern anatomists have a name for this – neurovascular congruency – yet surprisingly little is known about the molecular ties that bind these functionally distinct systems.</p>

<p align=""left"">In addition to traveling essentially the same routes, the nervous and vascular systems share other features as well, Dr. Gu said. Both are formed around the same during development and both remodel dynamically. In anatomical terms they are highly-branched and complicated networks, yet both vary in remarkably stereotyped patterns. Functionally, neural activity and vascular dynamics are interdependent in the periphery and tightly coupled in the brain. Intrigued by shared patterns during development and by neurovascular interactions in adults, Dr. Gu wondered whether they were controlled by common growth signals and whether a “molecular glue” holds them together in mature creatures.</p>

<p align=""left"">From the beginning, she had two well-characterized growth cues to work with: class 3 secreted semaphorins, which control axon growth by repelling the growing tip, and a family of potent angiogenesis regulators known as VEGF, or vascular endothelial growth factors. The first hint of a strong molecular relationship between nerves and vessels came from the discovery that they have a common receptor called neuropilin (Npn-1), Dr. Gu said.</p>

<p align=""left"">To understand how Npn-1 functions in vivo as a receptor for structurally distinct semaphorins and VEGF during development, she and her coworkers generated

a neuropilin-1 knock-in mouse (npn-1^{Sema-}) carrying specific mutations that abolished semaphorin, but not VEGF signaling. By combining this mouse with neuropilin-1 conditional knock-outs, Dr. Gu discovered that Npn-1 functions in an unprecedented way: it integrates signals from two structurally distinct ligands and coordinates the development of the heart, vasculature and nervous system. Cross-talk between circulation and nervous systems was also described in a 2003 article in Science, when Dr. Gu reported that a traditional axon guidance cue, Sema 3E, also can guide blood vessel patterning by binding with the Plexin-D1receptor.</p>

<p align=""left"">Finally, the researchers used genetic mouse models to show that the neurovascular congruency of the developing spinal nerves and intersomitic vasculature is due to a co-patterning mechanism. Sema3A/F expressed in somites controls the guidance of the spinal axons via Sema3A/F-Neuropilin signaling; in contrast, somatic-expressing Sema3E controls intersomitic vessel patterning via Sema3E-plexin-D1 signaling. During development, congruency is established by coordinated activity of these ligand-receptor interactions on nerves and vessels. Taken together, these findings demonstrate a firm molecular basis for the intriguing anatomical relationships documented by Vesalius and Da Vinci so long ago.</p>

<p align=""left"">Signaling mechanisms regulating neuronal connectivity</p>

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<p align=""left"">Do brain cells mature and form the connections they do because external siren songs call to them, or does an inner voice guide their paths? Both, as it turns out, appear to be true. Still needed is an explanation for how experience influences formation of dendritic processes and synapses in the developing brain, a question that Dr. Bonni and his colleagues have been grappling with for several years.</p>

<p align=""left"">As granule neurons mature they form axons that become dendrites, and after reaching their destination in the cerebellum these dendrites develop distinctive claw-like structures at their tips. This is where mossy fiber terminals and Golgi neuron axons form synapses. Dr. Bonni's team and other investigators discovered that the transcription factor myocyte enhancer factor 2A (MEF2A) orchestrates differentiation of these dendritic claws. Using confocal microscopy, the researchers saw that using RNAi to knock down MEF2A prevented claw formation in slices of rat cerebellum. Experiments with rat pups confirmed that interfering with MEF2A keeps claws from forming.</p>

<p align=""left"">Next they asked what role activity might play in regulating synapse formation, and used calcium influx to simulate this. The researchers saw that calcineurin removed a phosphate group from MEF2A at Ser408, which is adjacent to a sumoylation site at Lys403, so that Lys 403 became acetylated instead of sumoylated.</p>

<p align=""left"">The researchers exposed claw-deficient rat neurons to both forms of MEF2A and were surprised to find that the sumoylated form – which they would expect to be a repressor, caused the claws to grow, Dr. Bonni reported. The acetylated form, which they expected to promote transcription, actually inhibited claw differentiation.</p>

<p align=""left"">"Our findings define a mechanism underlying postsynaptic differentiation that may modulate activity-dependent synapse development and plasticity in the brain," Dr. Bonni concluded. He believes that such changes in synapse morphology could shed light on the pathology of certain neurodegenerative diseases and psychiatric disorders.</p>

<p align=""left"">Network mechanisms of light adaptation</p>

<p align=""left"">Elio Raviola</p>

<p align=""left"">Department of Neurobiology, Harvard Medical School, Boston, MA</p>

<p align=""left"">It is miraculous that the human eye can perceive light intensities that vary by a factor of one billion: from a single photon in a darkroom to blinding brilliance of the sun. The retina's famous photoreceptors, the rods and cones, do not accomplish this magic on their own. Instead, the raw signals they generate are sorted, processed and integrated by a complex ecosystem of other retinal cell types that form synapses, propagate chemical signals, and form networks.</p>

<p align=""left"">Among these is a large family of retinal dopaminergic amacrine (DA) cells that have not been extensively studied. These cells are spontaneously active and -- when exocytosis is triggered by voltage changes that cause Ca²⁺ influx through Ca²⁺ channels – able to release both dopamine and GABA, Dr. Raviola said. The release of dopamine, he said, helps prepare all types for retinal cells for bright light.</p>

<p align=""left"">Dr. Raviola's wiring diagram for DA cells was more ornate than the World Cup draw. These cells are equipped with three dendritic arbors: one that receives synaptic input from cone bipolars in the on-sublamina of the inner synaptic layer, one that is postsynaptic to GABAergic amacrines in the off-sublamina of this layer and a third one that spreads throughout the outer synaptic layer. They also exhibit multiple unbranched axons that irradiate in all directions from the cell body and establish GABAergic synapses with A2 amacrine cells, specialized neurons that deliver rod signals to the cone pathway.</p>

<p align=""left"">Recordings of neuron activity in the mouse visual system suggest that DA cells increase their spontaneous discharge of dopamine when exposed to bright light, Dr. Raviola said. This dopamine diffuses throughout the retina and, by sheer volume, causes many of the events involved in neural adaptation to light. At the same time they use GABA to inhibit A2 amacrine cells, which prevents noisy signals originating in saturated rods from gaining access to the cone pathway.</p>

<p align=""left"">Turn off the lights, and GABAergic cells crank up their production, inhibiting DA cells' spontaneous activity and attenuating or suppressing dopamine release. This unblocks the rod pathway so that the rod's specialized ability to pick up tiny amounts of light helps the animal see. In addition to these networks, which respond to changes in ambient light, DA cells are equipped with an autonomous clock that modulates dopamine synthesis and release in anticipation of changes in daily lighting conditions, Dr. Raviola noted.</p>

<p align=""left"">The MET oncogene: control of invasive growth in cancer and stem cells</p>

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<p align=""left"">Like people stuck in a starter home in a ho-hum part of town, many cancer cells aspire to move to a better neighborhood. This is especially true for cells in the suffocating, oxygen-poor center of a tumor. The willing mortgage banker who makes the move possible for many such cells is MET, a proto-oncogene that helps cells cope with undesirable circumstances by providing the mobility required to head for a new and better location. Not only that, but MET also appears to make the new home more hospitable for cells after they metastasize.</p>

<p align=""left"">Under normal, physiologic circumstances, invasive growth is as non-threatening as a lamb, Dr. Trusolino said. Epithelial organ development, angiogenesis, and wound healing, for example, all rely on genetic programs that combine cell proliferation with cell-cell dissociation, migration, and apoptosis protection. MET encodes the receptor for hepatocyte growth factor, HGF, and is one of many players in these necessary and benign patterns of growth.</p>

<p align=""left"">In post-natal life, however, invasive growth is more likely to be a wolf than a sheep. Invasive growth is most likely to be activated in stem and progenitor cells, and in the progression of cancer from malignancy through metastasis. MET expression is upregulated in response to unfavorable microenvironment conditions in solid tumors including those of the cervix and breast. More MET-encoded receptors find more HGF, a protein closely related to blood coagulation factors, and a jolt of HGF “helps cells liberate themselves into high oxygen areas,” Dr. Trusolino said.</p>

<p align=""left"">Interestingly, MET activation turns on hemostasis genes, favoring tumor nesting in the newly colonized territories. This oncogene thus provides a functional mechanistic link between hypoxia, hemostasis and invasive growth. Recent experiments conducted by Dr. Trusolino

and his coworkers focus on disrupting this program. In the May 2005 issue of Molecular and Cellular Biology, they reported that activating the Notch receptor caused transcriptionaldown-regulation of Met, suppressed HGF-dependent Ras signaling,and impaired HGF-dependent cellular responses.</p>

<p align=""left"">The laboratory is currently experimenting with recombinant ""decoy"" proteins, ligand antagonists and antibodies that target the Met kinase receptor, looking for compounds that might have potential as drugs for blocking tumor onset and progression.</p>

<p align=""left"">Recent progress in prion biology</p>

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<p align=""left"">As many as 20 million people have probably eaten meat from cattle infected with Bovine Spongiform Encephalopathy, and yet only about 160 of them have been diagnosed with the human form – popularly known as “Mad Cow Disease” – since the first cases were reported in 1995. “You eat a hamburger, your immune system and the blood brain barrier resists infection, and it takes 160-170 days for prions to penetrate your brain,” Dr. Aguzzi said.</p>

<p align=""left"">A troubling question is whether new variant Creutzfeld-Jakob disease (nvCJD), as this neurological disorder is formally known, will claim more victims as the years pass. He speculated that large numbers of asymptomatic carriers may themselves become sick or may transmit the disease protein, PrP^{Sc}, to others via blood products, organ transplation, or even breast milk.</p>

<p align=""left"">Dr. Aguzzi's laboratory uses transgenic mouse models to investigate how PrP^{Sc}, a structurally abnormal form of a normal cellular protein, travels from the periphery to penetrate the brain. His team showed that the lymphatic system serves as a staging area for the pathologic prion, and that it tags with immune cells that have been summoned to sites of inflammation by cytokines and chemokines. In mice with organ-specific inflammatory diseases, such as hepatitis, the prions gravitated to the damaged organ and accumulated there. In terms of CNS invasion, the key intermediary turned out to be follicular dendritic cells -- abnormal protein accumulates in them before jumping to the nervous system.</p>

<p align=""left"">Because Dr. Aguzzi's institute conducts Switzerland's national CJD surveillance program, he and his coworkers have been able to analyze prion storage in human brain and extraneural tissue. An analysis of samples from patients who died of sporadic CJD between 1996 and 2002 found PrP^{Sc}in the brain tissue of all patients, and in spleen and muscle samples from approximatelyone third.</p>

<p align=""left"">Today, his lab is seeking additional molecular switches responsible for extraneural prion reserves and considering their public health implications. If prions accumulate in breast milk, for example, public health recommendations would be needed to reduce maternal to infant transmission.</p>

<p align=""center"">Session 4: Signaling, Networks, and Cell-cell Communication</p>

<p align=""left"">The life sciences have been transformed by revolutions in thought and technology since the first Armenise-Harvard Symposium was held a decade ago. The concluding session at this 10th anniversary symposium demonstrated how pervasive systems-level approaches have become during the intervening years. Among other things, presentations illustrated the benefits of magical new imaging techniques, high-volume screening and mathematical modeling. In the final analysis, "the idea that there are distinctions between basic science departments is totally passé," HMS Dean Joseph Martin as he brought down the curtain. "It makes no difference what you call yourself: you're going to be talking the same language with your colleagues around the room and around the world."</p>

<p align=""left"">Activation waves and cell motility

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<p align=""left"">All crawling cells use similar mechanisms to move – whether they are neutrophils rushing to kill an infectious microbe or bacteria in search of dinner. Polarity shifts rapidly when the cell moves toward an environmental signal, and actin protrusions extend at the leading edge and retract on the trailing side.</p>

<p align=""left"">Based on experiments with many experimental systems, researchers have proposed that linked positive and negative feedback loops are essential for shifts in cell polarity. Still, it is not obvious how these feedback loops play out in space and time nor do we understand how signaling and cytoskeleton interact to generate polarity and motility, Dr. Kirschner said.</p>

<p align=""left"">For some time, the Kirschner laboratory has been investigating how the Hem-1 complex interacts with Rac and WAVE – two major pathways in neutrophil chemotaxis – when cells change course. In February 2006, Dr. Kirschner's team reported that the Hem-1 complex is needed for Rac activation, actin polymerization and for keeping myosin at bay. Hem-1 is essential for orchestrating polarity – and therefore forward movement – in neutrophils.</p>

<p align=""left"">These findings left Dr. Kirschner wondering about the spacial relationships of Rac, WAVE, and the Hem-1 regulatory complex. When his team used spinning disc confocal microscopy to examine GFP-labeled Hem-1, all they could see was that it was concentrated near the leading edge. "This was not very interesting," he said.</p>

<p align=""left"">Next they turned to total internal reflectance microscopy, or TIRF, a newer technology with a depth of field of one micron and a higher signal-to-noise ratio than confocal microscopy. "We saw propagating wave fronts, all propagating in the direction of the cell's movement," Dr. Kirschner said. This was the first time such a phenomenon had been observed, and subsequent experiments indicated that local positive and negative feedback loops based on actin assembly generate the propagating waves. Other circuits control polarization. This system can turn

on a dime: when the leading edge of a cell encounters a barrier, the waves are extinquished immediately and then start up again in a different direction.</p>

<p align=""left"">Why do cells use waves to move? Dr. Kirschner speculated this particular pattern unifies the leading edge and naturally generates movement in the direction of the waves, propelling the cell forward.</p>

<p align=""left"">Regulation of cell shape: a matter of capping and bundling actin filaments</p>

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<p align=""left"">In contrast to the previous lecture, which focused on actin remodeling in immune cells defending against foreign invaders, Dr. Scita examined cytoskeletal changes found during normal development and in malignant, turncoat cells. What the presentations shared was an interest in changes of cell shape regulated by dynamic, architecturally diverse actin-based scaffolds responding to external cues.</p>

<p align=""left"">Dr. Scita's lab is especially interested in how the barbed ends of actin filaments are capped, and in how filaments are bundled to form protrusions such as microvilli and filapodia. In 2004, his group identified Eps8 as a protein that alters actin dynamics in certain settings by capping the barbed ends of actin filaments. In Nature Cell Biology, they published evidence that Eps8 is essential for normal development of intestinal microvilli in the worm model.</p>

<p align=""left"">Eps8 is so highly conserved that Dr. Scita's team thought it was worth exploring further. As a result, they found that Eps8 also participates in cross-linking and bundling actin filaments. Eps8 binds in a bivalent fashion with IRSp53, a self-inhibited actin bundler that can associate to the Rho-GTPases Rac and Cdc42. Eps8 and IRSp53 mediate actin cross-linking in vitro, and in vivo EPS8 enhances IRSp53-dependent membrane extension to generate neurite-like branched protrusions.</p>

<p align=""left"">Cellular distribution of the IRSp53-Eps8 complex is controlled by binding to Cdc42, Dr. Scita said, supporting the existence of a novel Cdc42-IRSp53-Eps8 signaling pathway. If either IRSp53 or Eps8 was removed, Cdc42-induced filopodia were significantly diminished. He concludes that the synergic bundling activity of the IRSp53-Eps8 complex, regulated by Cdc42, is essential for generating actin bundles and promoting filopodial protrusions. Some of these cytoskeletal changes are associated with fibrosarcoma and other malignant phenotypes.</p>

<p align=""left"">Genetic control of thyroid organogenesis</p>

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<p align=""left"">Congenital hypothyroidism is a common hereditary defect, affecting approximately one of every 4,000 to 6,000 children born. In 85% of cases, this condition is due to thyroid dysgenesis, where some disturbance during development causes the gland to be absent, in the wrong anatomical location, or far smaller than normal. This can have devastating consequences, as adequate levels of thyroid hormone are required for normal development of body and brain. Untreated hypothyroidism leads to mental retardation and growth failure.</p>

<p align=""left"">The morphogenesis begins with a cluster of cells on the floor of the mouth, which form a bud-like structure that migrates to the base of the neck and reaches its final destination in front of the trachea after about one month of embryonic development (5 days in the mouse). Only

at this final stage do thyroid follicular cells express a series of differentiation markers that appear in a fixed temporal pattern in the mouse embryo: Tg TPO and TSHR genes are expressed first, followed by NIS, and by about the 16th day thyroid hormone is detectable. In humans, as in mice, the gland begins producing thyroid hormone only after arriving at its proper location, Dr. Di Lauro said.</p>

<p align=""left"">Some years ago, his lab discovered that three transcription factors, TTF-1, TTF-2 and Pax-8, are expressed not only in mature thyroid cells but also in their precursors. Dr. Di Lauro's team has since identified another gene, Foxe-1, involved in organogenesis. Knowing about these genes allowed them to home in on the genetic basis of normal and pathological thyroid development.</p>

<p align=""left"">The researchers created knockout mice for each of the transcription factors and monitored their development. In TTF-1^{-/-} or Pax-8^{-/-} embryos, the thyroid bud formed and initiated its migration normally. In TTF-2^{-/-} mice, however, the thyroid bud formed but did not migrate from the floor of the pharynx. Additional problems crop up later in development, when Pax-8 null mice fail to develop a working thyroid gland and Foxe-1 null mice have thyroid ectopy or cleft palate.</p>

<p align=""left"">Because migration of the bud to its permanent location at the base of the neck is so important, the Di Lauro team set out to identify target genes of TTF-2. They used laser capture microdissection to isolate thyroid precursor cells and pharynx endodermal cells from tissue sections of wild type and TTF-2 null mice. They have identified some 53 additional genes that could be involved in migration.</p>

<p align=""left"">Although the four transcription factors can contribute independently to defects in organogenesis, Dr. Di Lauro suspects that in nature these defects are probably polygenic. His lab has generated mouse lines that are multiply heterozygous for null alleles of titf1, titf2 and pax8, none of which – when hererozygous – results in dysgenesis. They found that only double heterozygous titf1^{+/-} pax8^{+/-}mice had impaired thyroid function, indicated by high TSH and low T4 hormone levels in blood. Moreover, defects only occurred in the C57/B6 mouse strain. These data are the first evidence of a polygenic origin of congenital hypothyroidism, Dr. Di Lauro said.</p>

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<p align=""left"">Oscillations and variations – p53 dynamics in single living cells</p>

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<p align=""left"">A classic theme in biology is figuring out how a cell takes in signals from the outside world, processes them, and decides what to do next. This struck Dr. Lahav as a perfect application for systems biology, which among other things seeks to understand the control of signaling pathways in complex networks. This approach marries experimentation and modeling and requires precise quantitative information about the dynamics of the proteins involved in the cellular response.</p>

<p align=""left"">When cellular DNA is damaged, the tumor suppressor protein p53 swings into action, either patching up the damage or triggering cell death. Its activity is governed by a feedback loop with its negative regulator, Mdm2: p53 transciptionally activates Mdm2, which in turn degrades p53. Previous studies of p53 and Mdm2 dynamics following DNA damage looked at changes in protein levels over time in whole populations of cells, an approach Dr. Lahav said masked the true behavior of the network.</p>

<p align=""left"">Population studies suggested that the cells' collective response to DNA damage was analog, with p53 and Mdm-2 levels rising gradually to keep pace with worsening DNA damage. Her lab devised a method for tracking what happens in a single cell, using fluorescence microscopy to observe the activity of p53 labeled with cyan fluorescent protein and Mdm-2 labeled with yellow.

Their single cell studies showed that p53 does not rise gradually, but instead is expressed in a series of discrete pulses. The mean height and duration of each pulse held steady, and did not vary with the amount of DNA damage, Dr. Lahav reported. They used controlled doses of radiation to stress the cells, and saw the number of pulses in each cell rise as DNA damage worsened.</p>

<p align=""left"">These experimental results suggested a mathematical model for the p53 oscillator, which enabled her to make predictions about patterns of signaling and inhibition. The model indicated that the p53 signaling pathway repeatedly tests for DNA damage, acting as a 'digital clock' that releases well-timed quanta of p53 until the damage is repaired or the cell dies. "Between pulses the cell may be re-evaluating the situation," Dr. Lahav said. Cells may make a finite number of attempts to fix what's wrong, then trigger apoptosis if the damage is irreparable.</p>

<p align=""left"">Additional experimental observations support the idea that p53 pulses are important for regulating the expression of target genes, controlling downstream pathways, and guiding cell fate decisions. The oscillatory nature of this network increases the strength and flexibility of the damage response, illustrating what Dr. Lahav calls "the strength of indecisiveness."</p>

Signaling networks in Drosophila

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<p align=""left"">Dr. Perrimon has long been interested in understanding signaling networks in Drosophila cells, especially networks involved in patterning and morphogenesis. His goal is to identify all the components cells use to receive and integrate signals from their environment, and to organize them into pathways and networks.</p>

<p align=""left"">After the fruit fly genome was sequenced in 2000, he decided that the genetic tools his lab used amounted to shining a flashlight beam on pieces of these networks but did not illuminate the big picture. He set out to change this by creating the Drosophila RNAi Screening Center, which was launched in May 2004. Dr. Perrimon and his colleagues have created a library of 21,000 small, double-stranded RNAs that can systematically turn down or turn off each of the fly's 21,000 genes. RNA interference, or RNAi, makes the process of gene discovery faster, easier, more reliable, and genome-wide.</p>

<p align=""left"">The screening center is available to scientists around the world who want to conduct whole-genome screens in Drosophila. So far, RNAi screening at HMS has identified nearly 300 genes that may provide insights into human development and disease. The yield so far includes genes involved in cancer, obesity, severe combined immunodeficiency disease and others that shape the host responses to bacterial or viral infections.</p>

<p align=""left"">In addition to leading the screening center, Dr. Perrimon continues to investigate how fly embryos develop. His team recently used RNAi to identify a gene defect that interferes with normal muscle growth in fly embryos. They are also using information about cellular architecture to characterize how subcellular localization of components in signal transduction pathways influences network output, and to explore how cell polarity is involved in this process.</p>

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<p align=""left"">Small molecules and biological information transfer</p>

Jon Clardy

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<p align=""left"">“A theme of this meeting – and of life itself – is the flow of information in living organisms, and understanding this information flow at the molecular level energizes a significant fraction of biological research,” Dr. Clardy said, opening the final talk of this year’s Symposium. But when most biologists say “molecular level,” what they really mean is the macromolecular level – how information passes from DNA to RNA to make proteins, and how cascades of phosphorylation and dephosphorylation regulate cellular processes.</p>

<p align=""left"">While this is an important way to understand how life works, Dr. Clardy says, it underestimates the tremendous importance of small molecules. Their ranks include sex hormones, neurotransmitters, and THC, to name only a few, and he sees small molecules as biology’s preeminent messengers. He went on to describe two organisms where understanding the role of small molecules in “lifestyle choices” could open the door to broader biological insights.</p>

<p align=""left"">The fungus Candida albicans is dimorphic, capable of existing in either a yeast-like or filamentous form. In humans, the filamentous form causes everything from diaper rash and thrush to infections of the vagina, intestine, or even heart valves. Genetic studies show that the ability to change forms is central to the development of these maladies, and that a small molecule turns harmless yeast into the pathogenic filamentous form. Initially this molecule

was one of many that Dr. Clardy's team isolated from uncultured bacteria living in the wild, and he was struck by its structural resemblance to other signaling molecules. Now that it is known to turn C. albicans mean, Dr. Clardy hopes to find small molecule therapeutics that will interfere with its biosynthesis or be antagonists for its receptor.</p>

<p align=""left"">The Clardy lab works with another dimorphic organism, Caenorhabditis elegans, which has a normal larval stage when the living is easy but forms a "dauer" larva under adverse conditions. The prevailing wisdom has been that transformation into this skinny, long-lived state is triggered by a single small molecule called the dauer pheromone, Dr. Clardy said. Dropping food into the dish causes nematodes to emerge immediately from the dauer state.</p>

<p align=""left"">When Dr. Clardy's team looked more closely, however, they identified three molecules involved in dauer dynamics. Two of these, combined at physiologic concentrations, caused dauer larva to form; when food was added to the dish, the nematodes could not exit the dauer state unless the third molecule was present. This third molecule resembles the human arachadonic acid receptor, which binds THC as well as a prostaglandin precursor. C. elegans, Dr. Clardy said, turns out to be a wonderful model for other signaling systems besides the dauer phenomenon.</p>

<p align=""left"">The researchers are now conducting systematic searches for all the small molecules that activate all the sensory neurons of C. elegans and for natural ligands for all the nematode's hormone receptors</p>";"Symposium 2006: Catania, Italy";"";publish;closed;open;"";symposium-2006-catania-italy;"";"";2013-02-14 13:45:50;2013-02-14 13:45:50;"";0;http://armeniseharvard.sypdevelopment.com/?p=248;0;post;"";0

249;1;2012-09-14 12:55:34;2012-09-14 12:55:34;"<h4>10th Annual Symposium

Celebrating a Decade of Extraordinary Science

June 12-15, 2006, Grand Hotel Baia Verde, Catania, Italy</h4>

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About the Symposium

the giovanni ARMENISE HARVARD foundation

The 10th Annual Symposium of the Giovanni Armenise-Harvard Foundation Symposium, entitled Celebrating a Decade of Extraordinary Science, demonstrates that grief can give rise to greatness.

Twelve years ago, Count Giovanni Auletta Armenise lost his beloved wife, Dianora Bertacchini, to a brain tumor that not even the best efforts of physicians at Massachusetts General Hospital could defeat. During her time at this Harvard Medical School teaching institution, the couple realized that improved treatments for this and other devastating diseases will be possible only if basic science research flourishes.

Following his wife's death, the Count did more than mourn her loss. He worked closely with Daniel Tosteson, then Dean of Harvard Medical School, to set up a Foundation that would promote investigation of profound questions about the workings of life itself. Not only that, but they devised plans for creating new connections between basic scientists in Italy and at HMS. These two men "set a path that we could follow, evolve and expand upon," current HMS Dean Joseph Martin said in his introductory remarks at this milestone symposium.

In the United States, the Giovanni Armenise-Harvard Foundation supports seven multidisciplinary research centers that involve more than 50 faculty members on the HMS quadrangle. These centers collaborate with Italian institutes and individual scientists, help train Italian postdoctoral fellows and junior scientists, participate in international research seminars and organize public conferences in Boston on a range of topics including neuroscience, systems biology, and aging.

Since 1997, 27 rising young researchers have received HMS Junior Faculty Awards underwritten by the Foundation. These two-year grants help recipients generate scientific publications and attract significant additional funding for their laboratories. Award-winners Azad Bonni, Tom Walz, David Rudner and Gahlit Lahav gave invited podium presentations at this year's Symposium.

At Massachusetts General Hospital, the Foundation has provided support to 13 researchers studying neuro-oncology and related disorders; on hand for the Symposium was two-time recipient Verne Caviness.

In Italy, the Foundation has invested more than $12 million in scientific research since its inception. During the first five years, 56 scientists at five Italian research institutes received support from Collaborative Research Grants. This program made it possible for researchers in Rome, Milan, Turin

and Padua to pursue research questions with new collaborators on the HMS Quad. Armenise support also helped create a new structural biology facility in Milan. Three of the original principal investigators – Jacopo Meldolesi, Giulia De Lorenzo, and Tullio Pozzan – participated in the 10th Annual Symposium.

Today, one of the Foundation's proudest accomplishments is making it possible for young Italian scientists to establish laboratories and launch independent careers in their home country. Eight promising newcomers have received Career Development Program grants since 2001, and seven participated in the Symposium: Alberto Bacci, Stefano Casola, Davide Corona, Stefano Gustincich, Claudia Lodovichi, Carlo Sala and Rosella Visintin. All lead independent laboratories that are magnets for other young researchers, creating new opportunities for work and learning in Milan, Rome, Padua, Palermo and Trieste. Several of the career development awardees received graduate or postdoctoral training at HMS, and the Foundation presently underwrites two PhD candidates on the Quad. In all of Italy, this is one of only two initiatives aimed at helping this nation retain some of its best and brightest minds.

Dr. Martin emphasized that none of these programs would exist without the "incredible partnership" between Count Auletta Armenise and Dan Tosteson. Unfortunately, he told attendees, the former HMS Dean was injured in a fall on the eve of the Symposium and unable to participate. The Count ,however, was in the front row when his contributions to Italian science were formally recognized with the Targa della Presidenza della Repubblica, an award Italy's President bestows only on people or institutions of outstanding merit. This surprise presentation was made on the President's behalf by Tullio Pozzan, one of the original Armenise investigators in Italy. Pozzan is a professor at the University of Padova and the Venetian Institute for Molecular Medicine.

In addition to fueling research, the Foundation has been stimulating the flow of science news to the Italian public since 2000. The Science Writer Fellowship program has enabled 15 Italian journalists to attend annual symposia and travel to HMS, where they learn about science, make invaluable contacts and research articles while being hosted by the Office of Public Affairs. Fellows Daniela Cipolloni and Luca Sciortino participated in the 2006 Symposium; Antonio Carlo Larissa will join them at HMS.

Approximately 95 scientific participants traveled to the historic port city of Catania, on Silicy's northeast coast, to celebrate the Armenise-Harvard Foundation's first decade. Participants represented 15 centers, institutes and universities in Italy, three in the United States, and one in Switzerland. They gathered on the sunny terraces of the Grand Hotel Baia Verde in Catania, overlooking black lava cliffs and the blue Ionian Sea. This is one of three seas whose waves break on

Sicily's coasts, and the island's language and culture are marked by a succession of different rulers – Greeks, Romans, Byzantines, Arabs, Normans and Spaniards.

Like Sicily itself, Celebrating a Decade of Extraordinary Science featured an exceptionally diverse and multicultural scientific program. Over the years, the Foundation has helped scientists explore topics in cancer biology, neuroscience, infectious disease, structural biology, genetics and genomics, systems biology, and integrative biology and physiology. The Symposium's 24 featured speakers and 18 poster presenters who gave participants at least a taste of all those topics and more. The scientific program was organized by Steven Harrison, Peter Howley, Elio Raviola and John Flanagan of HMS. Following the keynote address, lectures were grouped into four sessions:

Membranes

Cell Cycle and Gene Regulation

Genes to Disease

Signaling, Networks, and Cell-cell communication.

This report is structured along the same lines, and provides a brief introduction to each session and summaries of individual presentations.

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

<p align=""left"">Keynote Address</p>

<p align=""left"">Fragile X Syndrome: Molecular mechanisms and Therapeutic Implications</p>

Stephen T. Warren

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The 1991 discovery of the gene responsible for Fragile X syndrome put Stephen Warren and his colleagues on the front page of the New York Times. This deserved headlines because defects in this gene, called FMR1, affect approximately one in 4,000 boys born worldwide – making Fragile X syndrome the most common heritable form of mental retardation in males. At the time, doctors were well aware that these boys' lives were often complicated by autism, hyperactivity and attention problems.

<p align=""left"">What physicians did not suspect was that abnormalities in FMR1 could cause premature menopause in 20% of women carriers, or that some grandfathers of affected boys would develop a neurological disorder easily mistaken for early-onset Parkinson's disease.</p>

<p align=""left"">Dr. Warren has spent 15 years analyzing the molecular basis for Fragile X syndrome, and he now knows that the dose makes the poison. There is a polymorphic CGG-repeat in the 5' untranslated portion of the X-linked FMR1 gene, and healthy people have alleles containing 7-55 copies of this triplet. Carriers who can pass along Fragile X syndrome have 55-200 copies, which the researchers call "pre-mutation" alleles. Patients with the syndrome have "full mutation alleles," typically containing far more than 200 copies. "It is remarkable how unstable this triplet is when transmitted," Dr. Warren said, "three sons of one mother can have drastically different numbers of CGG repeats." One might have Fragile X syndrome; the others no sign of it.</p>

<p align=""left"">Pre-mutation alleles deliver a moderate dose and are associated with RNA-mediated neurodegenerative disorders, found mostly in men. Women who receive the same 7-55 copies are at risk for passing Fragile X syndrome to their offspring and for premature ovarian failure. When more than 200 copies of the CGG triplet are present, the full mutation allele becomes heavily methylated and the chromatin shifts to a heterochromatic state. As a result FMR1 is silenced, unable to make messenger RNA needed to produce FMRP, its encoded protein.</p>

<p align=""left"">Researchers soon realized that the loss of FMRP is responsible for fragile X syndrome, but they "hammered away" for years before understanding how the protein works and why the number of CGG triplets makes such a difference. DNA microarrays, experiments with transgenic flies and mice, and a serendipitous misadventure with lab chow helped Dr. Warren's team identify FMRP is a selective RNA-binding protein that shuttles between the nucleus and cytoplasm. FMRP binds with about 3% of mRNAs expressed in the brain, and appears to suppress translation of these targets.</p>

<p align=""left"">The study of FMRP gained considerable momentum when researchers found that it occurs mainly in association with ribosomes in dendritic spines. This is a location where local control of protein synthesis is critical in synaptic plasticity. After glutamate stimulation, phosphorylated FMRP appears to locally repress translation of associated mRNAs in a dynamic process that may also involve using microRNAs to put the brakes on protein synthesis. Individuals with Fragile X syndrome have essentially had their brake cables severed.</p>

<p align=""left"">Excessive translation of certain mRNAs, particularly following type I mGluR stimulation, probably accounts for the various cognitive, reproductive and neurological abnormalities associated with pre-mutation and full-mutation alleles. Experiments using mouse and Drosophila models of fragile X syndrome indicate that antagonists of mGluR signaling can reduce over-translation and rescue some affected animals, and a screen of some 2,000 small molecules yielded nine compounds that rescue the dFmr1-deficient phenotype in flies.</p>

<p align=""left"">Dr. Warren's colleagues have devised a highly accurate screening test for pinpointing Fragile X syndrome mutations in newborn boys. The researchers expect to begin early clinical testing of candidate drugs about one year from now, and they are hopeful that early intervention might counteract the loss of FMRP.</p>

<p align=""center"">Session 1: Membranes</p>

<p align=""center"">At first glance the cell membrane appears to be a simple container for liquid cytosol and solid organelles, a slippery mix not unlike bubble tea. Look more closely and the bottle is made of a lipid bilayer that loves water on one surface and hates its on the other; zoom in again and the membrane is a teeming, dynamic marketplace where proteins engage in every imaginable commerce with the extracellular world. The opening session of this 10th anniversary Symposium showcased the work of researchers who examine the inner lives of membranes, exploring how they expand, change shape, form junctions, transfer information and are altered by disease proteins.</p>

<p align=""left"">Regulated exocytosis: what's new about enlargeosomes</p>

<p align=""left"">Jacopo Meldolesi</p>

<p align=""left"">Vita-Salute San Raffaele University, San Raffaele Institute and</p>

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<p align=""left"">At the 2003 Armenise-Harvard Symposium in Trieste, Dr. Meldolesi introduced what he described as "a little, brand-new organelle" that races to the plasma membrane in response to calcium stimulation. Unlike other vesicles controlled by regulated exocytosis, which serve to deliver specific payloads to the cell surface, these newly discovered structures were empty. He

dubbed them “enlargeosomes,” because their sole purpose seemed to be expanding the cell membrane.</p>

<p align=""left"">At the time, Dr. Meldolesi’s presentation was cautiously received: these structures had not been reported in the scientific literature, and the classical view of regulated exocytosis was that it exists to shuttle discrete packets of neurotransmitters, hormones, enzymes and other substances into the extracellular space. Dr. Meldolesi insisted that this was not the whole story. After all, even exocytic organelles fuse with the plasma membrane after releasing their contents, expanding the surface area of the cell.</p>

<p align=""left"">A literature review showed that regulated exocytosis is mentioned in about one-tenth of all biomedical publications, mostly the secretory form. Non-secretory exocytoses that enlarge the plasma membrane have been described in reports concerning cell differentiation in plants and animals, wound-healing, cyto-diuresis, phagocytosis and neurite growth. Dr. Meldolesi concluded that non-secretory exocytosis is just as important as the secretory form (see Chieregatti and Meldolesi, Nature Rev. Mol. Cell Biol., 2005).</p>

<p align=""left"">In his own laboratory, enlargeosomes were first identified in a secretion-defective clone of PC12 cells. The Meldolisi team developed a marker for pinpointing where enlargeosome membranes have fused following calcium stimulation. This marker, a high molecular weight, non-transmembrane protein called Ahnak/Desmoyokin (dA), enabled the researchers to document enlargeosome fusion in approximately 15 cell types following [Ca2+]i increase.</p>

<p align=""left"">Dr. Meldolesi and his colleagues used immunocytochemistry to determine the ultrastructure of these vesicles and track exocytosis in response to stimulation. Enlargeosomes are small, only about 60nm in diameter, and their membrane is coated on both sides. The inside is lined with a large protein, Ahnak; the cytosolic surface of the vesicle is coated with annexin2, a protein that binds to other membranes only in response to an influx of [Ca2+]i. The team’s latest finding is that enlarged cells can shrink by “recycling” fused enlargeosome membranes, which they accomplish via a peculiar, non-clathrin-dependent process, Dr. Meldolesi reported. Enlargeosomes remain the chief focus of his laboratory’s investigations, and he is confident that additional findings will continue to emerge. His listeners, more receptive now than in 2003, appeared to agree.</p>

<p align=""left"">Snake neurotoxins, membrane bending and exo-endocytosis.</p>

<p align=""left"">Ornella Rossetto</p>

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<p align=""left"">Some of the world's most dangerous snakes paralyze and kill their victims by catastrophically disrupting synapse function. Because venoms from kraits, taipans and similar poisonous snakes are so powerful, these natural poisons are invaluable tools for studying how cell membranes behave, Dr. Rossetto said.</p>

<p align=""left"">Her laboratory makes extensive use of SPANs, or snake presynaptic phospholipase A2 neurotoxins, both in cell culture experiments and a more complex system using an ex vivo preparation of mouse hemi-diaphragm. As snake neurotoxins intoxicate and ultimately paralyze the neuromuscular junction (NMJ), abnormal bulges form on neuronal extensions and synaptic vesicles rapidly deplete. Dr. Rossetto hypothesizes that these changes come about because snake toxins accelerate exocytosis and inhibit endocytosis at the same time.</p>

<p align=""left"">Working with cultured primary neurons, her team observed that exposure to nanomolar concentrations of four different SPANs (beta-bungarotoxin, taipoxin, notexin and textilotoxin) induced dose-dependent formation of discrete bulges at various sites on neuronal projections. Using a variety of markers, the researchers could see neurotransmitter-carrying vesicles concentrate at the bulges; exocytosis was confirmed by the presence of the lumenal domain of synaptotagmin I, a protein detectable only when vesicles deliver their cargo to the cell surface. At the same time, the cultured neurons released glutamate and lost signals from FM 1-43 dye – a sure sign that vesicles are being recycled after delivering neurotransmitters. These findings support the idea that SPANs cause bulges by boosting exocytosis and slowing endocytic retrieval of emptied vesicles.</p>

<p align=""left"">There is a great deal left to discover about now the various snake neurotoxins alter normal synaptic activity and phenotype, Dr. Rosetto said. For example, there is ongoing debate about the possible role of Phospholipase A2 enzymes in NMJ blockade, although these enzymes are known to cleave phospholipids in cell membranes and to be players in membrane trafficking. There is only a partial correlation between PLA2 activity and the neurotoxicity of various snake toxins, and no overlap of surface residues required for neurotoxicity with those essential for PLA2 activity.</p>

<p align=""left"">In the mouse hemidiaphragm preparation and in cultured neurons, Dr. Rossetto's group tackled this problem by comparing the effects of SPANs and their hydrolysis products, lysophospholipids and fatty acids, on neuromuscular junctions. They observed that an equimolar mixture of these two breakdown products has essentially the same biological effects as the snake toxins. These results draw attention to the possible role of local lipid changes in synaptic vesicle release, Dr. Rossetto said, and provide new tools for future studies of exocytosis.</p>

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

<p align=""left"">Structure of the aquaporin-0 mediated membrane junction</p>

<p align=""left"">Thomas Walz</p>

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<p align=""left"">During development, the lens of the eye takes shape and gains function as an elaborate network of junctions is built. Fiber connexins are as basic as two-by-fours in lens construction, but it now turns out that another protein, aquaporin-0 (AQP0), is needed to connect them to one another. Because aquaporins are mainly thought of as proteins that admit water to cells, AQP0's role in lens development would have been easy to overlook.</p>

<p align=""left"">The lens continuously synthesizes the classic, pore-forming version of AQP0.Early in lens development, aquaporin channels allow circulation of nutrients and waste. As the lens matures, assuming the more rigid geometry needed for vision, aquaporin assumes new duties as an architectural element in junction formation.</p>

<p align=""left"">In the May 13, 2006, issue of Nature, Dr. Walz and coworkers published a 1.9 Å resolution structure of junctional AQP0, determined by electron crystallography of double-layered two-dimensional crystals. When the researchers compared junctional and water-pore forms of the protein, they observed that junction formation required a conformational switch in an extracellular loop. This shape change apparently resulted from proteolytic cleavage of the cytoplasmic N- and C-termini.</p>

<p align=""left"">In junctional AQP0, what had been the water pathway narrows so dramatically that water molecules can no longer squeeze through. In the more mature lens, AQP0 forms tetramers and interacts with lipid molecules to form junctional crystals. Four molecules of the protein in one layer match up with four in the other, locked into a rosette-like shape by proline residues not found in other aquaporins. Dr. Walz's team is now looking more closely at the switch that transforms AQP0 from a water pore to a junction protein, and at the forces that hold the junction crystals together.</p>

<p align=""left"">Tipping the Iron Balance</p>

<p align=""left"">Nancy C. Andrews</p>

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<p align=""left"">The more closely Dr. Andrews scrutinizes the genetic underpinnings of hereditary hemochromatosis, the more ornate it appears to be. She used her Symposium lecture as an opportunity to step back, survey the big picture, and explain how mutations in five different genes contribute to iron-overload disease.</p>

<p align=""left"">In a healthy person, iron is absorbed from food by cells lining the gut. It is released at a tightly controlled rate and stockpiled by red blood cells and macrophages; when tissues and organs need iron, they can take it up from the bloodstream. In hemochromatosis, toxic amounts of iron accumulate in the liver, pancreas and heart – where levels are normally low – and macrophages are mysteriously empty of their usual mineral cargo.</p>

<p align=""left"">Mutations in HFE, the “classic” hemochromatosis gene, or in TFR2 (which encodes the transferrin receptor), are held accountable for most cases of adult-onset disease. Yet only a fraction of patients homozygous for mutations in these genes develop clinical disease, so obviously these two genes don’t tell the whole story. Environmental influences also come into play: physicians know that disease severity varies with factors such as alcohol intake, dietary iron consumption, and menstruation.</p>

<p align=""left"">In addition, Dr. Andrews and other investigators have identified three more genes whose products are also involved in iron homeostasis: ferroportin, hepcidin, and hemojuvelin. “Mutations in two genes –hepcidin and ferroportin – have major effects in hemochromatosis,” she said. “The other three fine-tune the connections.”</p>

<p align=""left"">There is a clear, inverse correlation between hepcidin level and how sick a patient becomes. Hepcidin normally binds ferroportin, regulating how much iron it releases from stockpiles in intestinal cells and macrophages. Without this restraining factor, ferroportin keeps pouring iron into the bloodstream. Dr. Andrews said these are the two most important contributors to hemochromatosis disease, with the other three genes playing secondary roles. Mutations in the classic HFE gene interfere with transferrin receptors, causing gut cells to store too much dietary iron in the first place. TRF2 abnormalities inhibit release of soluble hemojuvelin, which in turn depresses hepcidin and contributes to disease.</p>

<p align=""left"">Dr. Andrews and her coworkers are refining their model for hemochromatosis and using animals to search for therapeutic alternatives to bloodletting, which is all doctors have to recommend at present. Ideally, researchers will identify a small molecule that can mimic hepcidin’s effects</p>

<p align=""left"">Cell-cell signaling through regulated proteolysis</p>

<p align=""left"">David Z. Rudner</p>

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<p align=""left"">Cholesterol metabolism, Alzheimer’s disease, and cellular responses to stress are only a few examples of complex phenomena that are difficult to study in higher animals. Yet all three involve a signaling mechanism that Dr. Rudner is elucidating with help from a far simpler

creature. The mechanism is called regulated intramembrane proteolysis, or RIP, and the organism is Bacillus subtilis.</p>

<p align=""left"">Cells respond to their environment and to each other by transferring information across their membranes, which allows the cell to respond appropriately to what's happening on the outside. RIP is a type of messaging that relies on special, hydrophobic proteases that can operate in the lipid-filled interior of the cell membrane itself. These proteases sequentially cleave membrane-bound substrates, releasing transcription factors.</p>

<p align=""left"">Take away B. subtilis's food supply, and the organism differentiates into a spore, a dormant cell type that can endure for hundreds – perhaps thousands of years – until conditions improve, said Dr. Rudner, who received an Armenise junior faculty grant in 2003. The beauty of using a simple animal model is that this remarkable transformation – in which a moving and eating organism turns into a seemingly inanimate pellet – requires a scant handful of genes. And one of these, SpolVFB, is "a founding member of a family of membrane-bound metalloproteases involved in RIP," Dr. Rudner said.</p>

<p align=""left"">Sporulation begins with an asymmetric cell division that generates a large mother cell and a small forespore. Initially the forespore is engulfed by the mother cell, and although the two will follow completely different programs of gene expression, their developmental programs are temporarily coordinated by signal transduction pathways involving the membranes of both. In one pathway, a precursor called pro-sigK is synthesized by the mother cell, then cleaved by the membrane-embedded enzyme SpolVFB into sigmaK, a transcription factor. Until its activation, SpolVFB is locked in a complex with two other integral membrane proteins, SpoIVFA and BofA. The release of SpolVFB from this inactive state is triggered by a signal protein, SpoIVB, which is produced in the forespore and secreted into the space between the mother-cell and forespore membranes. This triggering protein, also called IVB, is known to be a serine protease but its mechanism for activating sigmaK has been poorly understood.</p>

<p align=""left"">Dr. Rudner's team discovered that IVB sits at the apex of a branched pathway that governs RIP, and they have evidence that it activates pro-sigmaK directly by cleaving SpoIVFA at multiple sites and indirectly by cleaving and activating a second serine protease known as CtpB. CtpB, in turn, activates processing by also cleaving SpoIVFA. This is a one-two punch typical of RIP signaling, and it bears a striking resemblance to serine protease cascades important for blood clotting, dorsal-ventral patterning in Drosophila, and other physiologic processes, he said.</p>

<p align=""left"">Formation and maturation of glutamatergic synapses</p>

<p align=""left"">Carlo Sala</p>

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<p align=""left"">Baseball promoters and post-synaptic neurons in search of excitement may have the same motto: If we build it, they will come. Neuroscientists have long debated whether synapses form at sites bombarded with signals by pre-synaptic neurons, or whether the post-synaptic side initiates the process by building a receiving station that somehow draws incoming signals. Based on extensive studies of excitatory neurotransmission in mammalian hippocampal neurons, Dr. Sala – who received the Foundation's first Career Development Award in 2001 – believes the second is more likely.</p>

<p align=""left"">No thought or movement would be possible without activity at millions of excitatory and inhibitory synapses, each with characteristic components that determine their specificity. In the mammalian brain, glutamate is the primary neurotransmitter that nerve cells use to send excitatory messages. On the receiving side of the glutamatergic synapse is a dense, fibrous mass made of hundreds of molecules, including neurotransmitter receptors, scaffolding proteins, adhesion molecules, and signal transduction enzymes; collectively this is called "the postsynaptic density," or PSD.</p>

<p align=""left"">There are many scaffolding proteins in the PSD, and three of them – PSD-95, GKAP and members of the Shank family – form a complex. Dr. Sala's team has demonstrated that PSD-95 and GKAP are needed for correct folding and synaptic localization of Shank. In the February 2006 issue of Neuron, Dr. Sala and collaborators at the University of British Columbia reported that when this complex is joined by neuroligin1 (NLG1), a synaptic adhesion molecule, they attract a pre-synaptic terminal which matures into an excitatory synapse. The magnetic activity of this complex may be clinically important: a shortage of scaffold proteins is associated with some forms of mental retardation, and flaws in the stabilization of post-synaptic structures may relate to autism.</p>

<p align=""left"">Glutamateric synapses are typically located at the tips of dendritic spines, and Dr. Sala's lab is now using a proteomic approach to screen some 2,000 proteins that may be involved in dendritic spine morphogenesis and protein synthesis.</p>

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<p align=""center"">Session 2: Cell Cycle and Gene Regulation</p>

<p align=""center"">This session focused on two chapters in the life story of cells: the first two speakers provided intimate looks at what happens as mitosis begins; the remaining three explored various mechanisms that determine when and where genes are expressed. But all the talks illustrate the value of the Foundation's support for basic research, according to Symposium co-organizer Steve Harrison, because these researchers are making discoveries that shed light on classic genetic observations, such as X-chromosome inactivation, and because they could pave the way to new treatments for several common cancers.</p>

<p align=""left"">The spindle assembly checkpoint: reality and fiction</p>

Andrea Musacchio

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<p align=""left"">Cells double their DNA during the synthesis, or S phase of their life cycle, and then must exercise great care as they split this genetic material into equal parts before dividing. Lax quality assurance allows chromosomal instability and aneuploidy, problems characteristic of cancer cells. Dr. Musacchio's laboratory studies formation of stable kinetochore-microtubule attachments during mitosis, a process monitored by the spindle assembly checkpoint (SAC). If kinetochores are attached improperly, or left unattached, the SAC arrests cells in a prometaphase-like state. If all sister chromatid pairs are properly attached and oriented, metaphase will proceed and the SAC will be switched off – allowing chromosome separation and anaphase, Dr. Musacchio explained.</p>

<p align=""left"">Over the years, he has made a strong case for the importance of the SAC protein called Mad2 in this inspection process. Here, Dr. Musacchio reported that his lab reconstituted the putative Mad2 kinetochore receptor and developed a kinetochore recruitment assay with purified components. They employed a technique called FRAP, an acronym for "fluorescence recovery after photobleaching," to observe dynamic interactions between cytosolic Mad2 and kinetochores. They saw catalytic activation of bound Mad2, followed by its release in a complex with Cdc20.</p>

<p align=""left"">Dr. Musacchio suspects that a stable Mad1-Mad2 complex actually functions as an enzyme, and that a positive feedback loop controls a crucial conformational change in the Mad2 protein. He is using in vitro FRAP to test this hypothesis, and sees this technology as a boon to understanding how Mad2, a protein, interacts with kinetochores, a physical cellular compartment. "In the future, we plan to use the numbers retrieved using in vitro FRAP to gain a quantitative understanding of the checkpoint network," Dr. Musacchio said.</p>

<p align=""left"">Aurora A and cell cycle progression: a centrosome-independent role in regulating mitotic entry</p>

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<p align=""left"">The gene encoding the small serine/threonine kinase Aurora A (Aur-A) is a proto-oncogene associated with cell proliferation in organisms ranging from flies to humans. In experiments with mice, forced overexpression drives aggressive tumor growth; in humans, abnormally high levels of Aur-A can be measured in 65% of breast and 50% of prostate cancers, Dr. Ruderman said. Not surprisingly, several drug companies are working on Aur-A as a possible target for anti-cancer drugs.</p>

<p align=""left"">These efforts are not far along, however, mainly because Aur-A's regulatory activities are just beginning to be understood. Discovered more than 20 years ago as a Drosophila mutation responsible for defects in the mitotic spindle, Aur-A was soon found to be highly enriched in centrosomes, Dr. Ruderman said. Almost all of Aur-A's known functions reflect in some way its presence at centrosomes, which form the poles of the mitotic spindle.</p>

<p align=""left"">Aur-A is required for the recruitment and/or activity of several key centrosomal proteins, for the correct placement of centrosomes at the spindle poles, and for accurate chromosome segregation during the cell cycle. Experiments have shown that the initial activation of cdc2 during the transition from G2 to M occurs at centrosomes, and that Aur-A participates in the centrosomal activation of cdc25B, the phosphatase responsible for this early activation of cdc2.</p>

<p align=""left"">Dr. Ruderman's laboratory seeks to understand, in molecular terms, exactly how Aur-A regulates to the timing of mitotic entry. In order to tease out the roles of various players in this drama, they make cell-free extracts from Xenopus eggs – some with centromeres and some without. Frog sperm is used to initiate normal cell cycling.</p>

<p align=""left"">In this system, neither the DNA nor spindle integrity checkpoint pathways are active, making it possible to investigate the basic cell cycle roles of Aur-A free from complications encountered in somatic cells with active checkpoint pathways. When the researchers added</p>

<p align=""left"">active Aur-A to the cycling egg extracts, cdc2 activation and mitotic entry were accelerated. When they depleted endogenous Aur-A or added inactive dominant negative

Aur-A to the system, mitotic entry was delayed but not blocked.</p>

<p align=""left"">But the most striking result, Dr. Ruderman said, was the finding that Aur-A's effects on the timing of the G2/M transition occur even when centrosomes are absent. In the March 31, 2006, issue of Proceedings of the National Academy of Science, her team published evidence that Aur-A not only contributes to centrosome maturation and function, but also plays a centrosome-independent role in the timing of mitotic entry. They are currently pursuing this observation.</p>

<p align=""left"">Reversal of histone lysine tri-methylation by the JMJD2 family of histone demethylases</p>

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<p align=""left"">Without histone, DNA would not be packaged in the "beads on a string" arrangement so familiar to PBS viewers and high school biology students. This scaffolding for DNA

was initially spotted in the late 19th Century, but it wasn't until the 1960s that researchers realized this supposedly inert framework was dynamic, and that its function changed when methyl groups latched onto specific sites on its tail.</p>

<p align=""left"">"Histone's complexity was underestimated: methylation regulates chromatin structure, transcription and the epigenetic state of the cell," Dr. Shi said. Today, chemical modification of histone and DNA is a hot topic in biology, because epigenetic factors are now recognized as on-and-off switches for genes in both health and disease. It took half a century,however, to identify enzymes that can attach methyl groups to the histone tail. The first histone methylase was cloned in 2000, and biologists thought it was nature's equivalent of Super Glue.</p>

<p align=""left"">In 2004, Dr. Shi's lab overturned the idea of permanent, irreversible methylation by cloning an enzyme that could remove one or two methyl groups from a lysine residue. Addition of methyl groups by histone methylases, and removal of single and double methyl groups by demethylating enzymes such as LSD1 and JHDM1, is now seen as business as usual on the histone tail.</p>

<p align=""left"">Less clear was whether any enzyme could pry loose three methyl attachments. Dr. Shi decided to pursue this question because it relates to real human disease: tri-methylated histones are associated with cancers of prostate and breast and with X chromosome inactivation. An all-out effort in the Shi laboratory succeeded in identifying a family of enzymes that can knock methyl groups off trimethylated sites on histones. A report in the May 5, 2006, issue of Cell describes how one such enzyme, JMJD2A, exercises a range of demythylating effects in cultured human cells and in live C. elegans nematodes. Other members of the same enzyme family operate slightly differently, but all are involved in removing methyl groups from specific sites.</p>

<p align=""left"">Dr. Shi and his coworkers were astonished to find that demythylation is involved in the cell's response to DNA damage, and that manipulating methylation can influence life or death decisions in nematode cells. This raises the possibility of devising medical treatments – possibly for cancer – by using small molecules to interfere with demethylase activity. "Understanding biological importance is the next step, and clearly there is more work to be done," Dr. Shi concluded.</p>

<p align=""left"">Regulation of microRNA expression during myeloid differentiation</p>

<p align=""left"">Irene Bozzoni</p>

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<p align=""left"">One of the most amazing revelations from the human genome project is that the enormous human proteome is encoded not by 300,000 genes – as scientists estimated a generation ago – but perhaps by only 25,000 human genes. How so few genes make so many products is a mystery indeed.</p>

<p align=""left"">It now appears that the solution rests not only with DNA, the most celebrated molecule of the 20th century, but with small, non-coding bits of RNA, a molecule traditionally seen as DNA's modest handmaiden. Dr. Bozzoni has helped gild RNA's image by demonstrating the power of micro-RNAs to curb post-transcriptional gene expression. These 20 to 22 nucleotide snippets of RNA were under the radar for a long time. Not only because they're tiny, but because miRNAs were first seen in plants – causing skepticism among mammalian biologists that they could be important in animals. The discovery of a C.elegans miRNA in 1993 was the first ripple in what became a sea change, and hundreds of highly conserved miRNAs are now known to help regulate development, differentiation, and cell proliferation in animals and plants.</p>

<p align=""left"">Hemopoesis is perhaps the best-studied illustration of a multipotent stem cell giving rise to nearly a dozen constantly renewed, terminally differentiated cell types. Blood cancers make this a clinically important process as well, and Dr. Bozzoni studies how lineages are specified in a cell line derived from patients affected by acute promyelocitic leukemia. When these cells are stimulated with retinoic acid (RA) they differentiate into granulocytes; if exposed to tissue plasminogen activator (TPA) they become monocytes that mature into macrophages.</p>

<p align=""left"">Dr. Bozzoni's lab has identified two micro-RNAs, miRNA-223 and miRNA-424, that act differently on the same target depending on what chemical has been added to the APL cells. When RA is added to trigger differentiation into granulocytes, miRNA223 shuts down NFI-A and granulocytes take shape. When TPA is given, in contrast, miRNA424 represses NFI-A translation and the precursor cells turn into monocyte/macrophages, Dr. Bozzoni reported. Her team has identified specific promoters and other factors that control lineage-specific expression of these miRNAs, and has also found targets useful for studying the balance between proliferation and differentiation during myelopoiesis.</p>

<p align=""left"">Now that this methodology has demonstrated its worth, Dr. Bozzoni believes it will shed light on big questions about the evolutionary advantages of using a modest number of genes to generate a vast array of proteins.</p>

<p align=""left"">Caspase-11 regulates cell migration by promoting Aip1/Cofilin mediated actin depolymerization</p>

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<p align=""left"">Even old friends can surprise us. In the early 1990s, Junying Yuan pioneered the study of cell death genes and made important discoveries about the importance of caspase proteases in apoptosis. Her laboratory's intense scrutiny of caspases gradually revealed that members of this family participate not only in cell death, but also in normal development, inflammatory response, and adaptive immunity. Although Dr. Yuan could see that caspases are more versatile than she initially thought, it was still a revelation to find that caspase-11 helps mobilize lymphocytes to rush into battle against an infection. The mild-mannered hospice worker, it turns out, is also on the first-responder team.</p>

<p align=""left"">Caspase-11 belongs to the caspase-1 subfamily of pro-inflammatory caspases, and experiments in mice had established that it a crucial regulator of cytokine maturation and apoptosis. Caspase-11 is a critical activator of caspase-1, which then acts directly to turn pro-interleukin-1b (pro-IL-1b) into mature IL-1b. In caspase-11 knockout mice, IL-1b maturation and secretion is blocked, and the animals do not develop septic shock that would ordinarily result from lipopolysaccharide (LPS) stimulation.</p>

<p align=""left"">Caspase-11 is barely detectable in healthy wild type mice but is strongly induced by LPS stimulation. Caspase-11 is also involved in directly activating caspase-3 and apoptosis under certain pathological conditions, such as brain ischemia and septic shock. Dr. Yuan knew all this about Caspase-11.</p>

<p align=""left"">The story took an unexpected turn, however, when the Yuan lab screened large numbers of proteins to see which ones caspase-11 interacted with. Much to their surprise, they got a positive hit for actin. Additional experiments using a mouse peritonitis model revealed that caspase-11 isn't the Lone Ranger here; it affects actin only if two other players are on hand: cofilin, a member of a major actin depolymerization factor family, and Aip1, which helps cofilin depolymerize actin and prevents the ends of severed actin fibers from rejoining.</p>

<p align=""left"">Experiments indicated that caspase-11 interacts physically and functionally with Aip1 to promote cofilin-mediated actin depolymerization, Dr. Yuan said. Knocking out either Aip1 function or caspase-11 expression reduced the mobility of T-cells that should have been rushing toward the front lines of infection. These results reveal a novel function of caspase-11 in regulating actin dynamics and cell migration, and future experiments will search for additional levels on which caspase-11 might be able to regulate inflammatory response</p>

<p align=""center"">Session 3: Genes to Disease</p>

<p align=""left"">The Armenise-Harvard Foundation has been backing neurobiology research since its earliest days. As Count Armenise and his late wife confronted the brain tumor that ultimately claimed her life, they realized that treatments delivered at the bedside begin with basic science questions asked – and answered, when experiments go well– at the laboratory bench. The road from laboratory to clinic is long and difficult, and this session featured reports from various milestones along the way. It began with a provocative new idea about nervous system development, went on to consider how cells invade and form networks during normal development

and in disease, and concluded with a report on how prions that enter the body in a bite of hamburger burrow into and destroy the brain.</p>

<p align=""left"">Molecular mechanisms of axon and vascular guidance</p>

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<p align=""left"">With 500-year-old drawings by Vesalius and Da Vinci projected on the screen, Dr. Gu asked participants to consider how remarkably well these images have held up. Among the many things these early students of the human body got right was their portrayal of the nervous and vascular systems. They showed nerves and blood vessels traveling together, bundled between ribs in the trunk, reaching upward to the brain and out to the very tips of fingers and toes. Modern anatomists have a name for this – neurovascular congruency – yet surprisingly little is known about the molecular ties that bind these functionally distinct systems.</p>

<p align=""left"">In addition to traveling essentially the same routes, the nervous and vascular systems share other features as well, Dr. Gu said. Both are formed around the same during development and both remodel dynamically. In anatomical terms they are highly-branched and complicated networks, yet both vary in remarkably stereotyped patterns. Functionally, neural activity and vascular dynamics are interdependent in the periphery and tightly coupled in the brain. Intrigued by shared patterns during development and by neurovascular interactions in adults, Dr. Gu wondered whether they were controlled by common growth signals and whether a “molecular glue” holds them together in mature creatures.</p>

<p align=""left"">From the beginning, she had two well-characterized growth cues to work with: class 3 secreted semaphorins, which control axon growth by repelling the growing tip, and a family of potent angiogenesis regulators known as VEGF, or vascular endothelial growth factors. The first hint of a strong molecular relationship between nerves and vessels came from the discovery that they have a common receptor called neuropilin (Npn-1), Dr. Gu said.</p>

<p align=""left"">To understand how Npn-1 functions in vivo as a receptor for structurally distinct semaphorins and VEGF during development, she and her coworkers generated a neuropilin-1 knock-in mouse (npn-1^{Sema-}) carrying specific mutations that abolished semaphorin, but not VEGF signaling. By combining this mouse with neuropilin-1 conditional knock-outs, Dr. Gu discovered that Npn-1 functions in an unprecedented way: it integrates signals from two structurally distinct ligands and coordinates the development of the heart, vasculature and nervous system. Cross-talk between circulation and

nervous systems was also described in a 2003 article in Science, when Dr. Gu reported that a traditional axon guidance cue, Sema 3E, also can guide blood vessel patterning by binding with the Plexin-D1receptor.</p>

<p align=""left"">Finally, the researchers used genetic mouse models to show that the neurovascular congruency of the developing spinal nerves and intersomitic vasculature is due to a co-patterning mechanism. Sema3A/F expressed in somites controls the guidance of the spinal axons via Sema3A/F-Neuropilin signaling; in contrast, somatic-expressing Sema3E controls intersomitic vessel patterning via Sema3E-plexin-D1 signaling. During development, congruency is established by coordinated activity of these ligand-receptor interactions on nerves and vessels. Taken together, these findings demonstrate a firm molecular basis for the intriguing anatomical relationships documented by Vesalius and Da Vinci so long ago.</p>

<p align=""left"">Signaling mechanisms regulating neuronal connectivity</p>

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<p align=""left"">Do brain cells mature and form the connections they do because external siren songs call to them, or does an inner voice guide their paths? Both, as it turns out, appear to be true. Still needed is an explanation for how experience influences formation of dendritic processes and synapses in the developing brain, a question that Dr. Bonni and his colleagues have been grappling with for several years.</p>

<p align=""left"">As granule neurons mature they form axons that become dendrites, and after reaching their destination in the cerebellum these dendrites develop distinctive claw-like structures at their tips. This is where mossy fiber terminals and Golgi neuron axons form synapses. Dr. Bonni's team and other investigators discovered that the transcription factor myocyte enhancer factor 2A (MEF2A) orchestrates differentiation of these dendritic claws. Using confocal microscopy, the researchers saw that using RNAi to knock down MEF2A prevented claw formation in slices of rat cerebellum. Experiments with rat pups confirmed that interfering with MEF2A keeps claws from forming.</p>

<p align=""left"">Next they asked what role activity might play in regulating synapse formation, and used calcium influx to simulate this. The researchers saw that calcineurin removed a phosphate group from MEF2A at Ser408, which is adjacent to a sumoylation site at Lys403, so that Lys 403 became acetylated instead of sumoylated.</p>

<p align=""left"">The researchers exposed claw-deficient rat neurons to both forms of MEF2A and were surprised to find that the sumoylated form – which they would expect to be a repressor,

caused the claws to grow, Dr. Bonni reported. The acetylated form, which they expected to promote transcription, actually inhibited claw differentiation.</p>

<p align=""left"">“Our findings define a mechanism underlying postsynaptic differentiation that may modulate activity-dependent synapse development and plasticity in the brain,” Dr. Bonni concluded. He believes that such changes in synapse morphology could shed light on the pathology of certain neurodegenerative diseases and psychiatric disorders.</p>

<p align=""left"">Network mechanisms of light adaptation</p>

<p align=""left"">Elio Raviola</p>

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<p align=""left"">It is miraculous that the human eye can perceive light intensities that vary by a factor of one billion: from a single photon in a darkroom to blinding brilliance of the sun. The retina’s famous photoreceptors, the rods and cones, do not accomplish this magic on their own. Instead, the raw signals they generate are sorted, processed and integrated by a complex ecosystem of other retinal cell types that form synapses, propagate chemical signals, and form networks.</p>

<p align=""left"">Among these is a large family of retinal dopaminergic amacrine (DA) cells that have not been extensively studied. These cells are spontaneously active and -- when exocytosis is triggered by voltage changes that cause Ca²⁺ influx through Ca²⁺ channels – able to release both dopamine and GABA, Dr. Raviola said. The release of dopamine, he said, helps prepare all types for retinal cells for bright light.</p>

<p align=""left"">Dr. Raviola’s wiring diagram for DA cells was more ornate than the World Cup draw. These cells are equipped with three dendritic arbors: one that receives synaptic input from cone bipolars in the on-sublamina of the inner synaptic layer, one that is postsynaptic to GABAergic amacrines in the off-sublamina of this layer and a third one that spreads throughout the outer synaptic layer. They also exhibit multiple unbranched axons that irradiate in all directions from the cell body and establish GABAergic synapses with A2 amacrine cells, specialized neurons that deliver rod signals to the cone pathway.</p>

<p align=""left"">Recordings of neuron activity in the mouse visual system suggest that DA cells increase their spontaneous discharge of dopamine when exposed to bright light, Dr. Raviola said. This dopamine diffuses throughout the retina and, by sheer volume, causes many of the events involved in neural adaptation to light. At the same time they use GABA to inhibit A2 amacrine cells, which prevents noisy signals originating in saturated rods from gaining access to the cone pathway.</p>

<p align=""left"">Turn off the lights, and GABAergic cells crank up their production, inhibiting DA cells’ spontaneous activity and attenuating or suppressing dopamine release. This unblocks the rod pathway so that the rod’s specialized ability to pick up tiny amounts of light helps the animal see. In addition to these networks, which respond to changes in ambient light, DA cells are equipped with

an autonomous clock that modulates dopamine synthesis and release in anticipation of changes in daily lighting conditions, Dr. Raviola noted.</p>

<p align=""left"">The MET oncogene: control of invasive growth in cancer and stem cells</p>

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<p align=""left"">Like people stuck in a starter home in a ho-hum part of town, many cancer cells aspire to move to a better neighborhood. This is especially true for cells in the suffocating, oxygen-poor center of a tumor. The willing mortgage banker who makes the move possible for many such cells is MET, a proto-oncogene that helps cells cope with undesirable circumstances by providing the mobility required to head for a new and better location. Not only that, but MET also appears to make the new home more hospitable for cells after they metastasize.</p>

<p align=""left"">Under normal, physiologic circumstances, invasive growth is as non-threatening as a lamb, Dr. Trusolino said. Epithelial organ development, angiogenesis, and wound healing, for example, all rely on genetic programs that combine cell proliferation with cell-cell dissociation, migration, and apoptosis protection. MET encodes the receptor for hepatocyte growth factor, HGF, and is one of many players in these necessary and benign patterns of growth.</p>

<p align=""left"">In post-natal life, however, invasive growth is more likely to be a wolf than a sheep. Invasive growth is most likely to be activated in stem and progenitor cells, and in the progression of cancer from malignancy through metastasis. MET expression is upregulated in response to unfavorable microenvironment conditions in solid tumors including those of the cervix and breast. More MET-encoded receptors find more HGF, a protein closely related to blood coagulation factors, and a jolt of HGF “helps cells liberate themselves into high oxygen areas,” Dr. Trusolino said.</p>

<p align=""left"">Interestingly, MET activation turns on hemostasis genes, favoring tumor nesting in the newly colonized territories. This oncogene thus provides a functional mechanistic link between hypoxia, hemostasis and invasive growth. Recent experiments conducted by Dr. Trusolino and his coworkers focus on disrupting this program. In the May 2005 issue of Molecular and Cellular Biology, they reported that activating the Notch receptor caused transcriptionaldown-regulation of Met, suppressed HGF-dependent Ras signaling,and impaired HGF-dependent cellular responses.</p>

<p align=""left"">The laboratory is currently experimenting with recombinant ""decoy"" proteins, ligand antagonists and antibodies that target the Met kinase receptor, looking for compounds that might have potential as drugs for blocking tumor onset and progression.</p>

<p align=""left"">Recent progress in prion biology</p>

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<p align=""left"">As many as 20 million people have probably eaten meat from cattle infected with Bovine Spongiform Encephalopathy, and yet only about 160 of them have been diagnosed with the human form – popularly known as “Mad Cow Disease” – since the first cases were reported in 1995. “You eat a hamburger, your immune system and the blood brain barrier resists infection, and it takes 160-170 days for prions to penetrate your brain,” Dr. Aguzzi said.</p>

<p align=""left"">A troubling question is whether new variant Creutzfeld-Jakob disease (nvCJD), as this neurological disorder is formally known, will claim more victims as the years pass. He speculated that large numbers of asymptomatic carriers may themselves become sick or may transmit the disease protein, PrP^{Sc}, to others via blood products, organ transplation, or even breast milk.</p>

<p align=""left"">Dr. Aguzzi’s laboratory uses transgenic mouse models to investigate how PrP^{Sc}, a structurally abnormal form of a normal cellular protein, travels from the periphery to penetrate the brain. His team showed that the lymphatic system serves as a staging area for the pathologic prion, and that it tags with immune cells that have been summoned to sites of inflammation by cytokines and chemokines. In mice with organ-specific inflammatory diseases, such as hepatitis, the prions gravitated to the damaged organ and accumulated there. In terms of CNS invasion, the key intermediary turned out to be follicular dendritic cells -- abnormal protein accumulates in them before jumping to the nervous system.</p>

<p align=""left"">Because Dr. Aguzzi’s institute conducts Switzerland’s national CJD surveillance program, he and his coworkers have been able to analyze prion storage in human brain and extraneural tissue. An analysis of samples from patients who died of sporadic CJD between 1996 and 2002 found PrP^{Sc}in the brain tissue of all patients, and in spleen and muscle samples from approximatelyone third.</p>

<p align=""left"">Today, his lab is seeking additional molecular switches responsible for extraneural prion reserves and considering their public health implications. If prions accumulate in breast milk, for example, public health recommendations would be needed to reduce maternal to infant transmission.</p>

<p align=""center"">Session 4: Signaling, Networks, and Cell-cell Communication</p>

<p align=""left"">The life sciences have been transformed by revolutions in thought and technology since the first Armenise-Harvard Symposium was held a decade ago. The concluding session at this 10th anniversary symposium demonstrated how pervasive systems-level approaches have become during the intervening years. Among other things, presentations illustrated the benefits of magical new imaging techniques, high-volume screening and mathematical modeling. In the final analysis, “the idea that there are distinctions between basic science departments is totally passé,” HMS Dean Joseph Martin as he brought down the curtain. “It makes no difference what you call yourself: you’re going to be talking the same language with your colleagues around the room and around the world.”</p>

<p align=""left"">Activation waves and cell motility

Marc W. Kirschner, PhD</p>

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<p align=""left"">All crawling cells use similar mechanisms to move – whether they are neutrophils rushing to kill an infectious microbe or bacteria in search of dinner. Polarity shifts rapidly when the cell moves toward an environmental signal, and actin protrusions extend at the leading edge and retract on the trailing side.</p>

<p align=""left"">Based on experiments with many experimental systems, researchers have proposed that linked positive and negative feedback loops are essential for shifts in cell polarity. Still, it is not obvious how these feedback loops play out in space and time nor do we understand how signaling and cytoskeleton interact to generate polarity and motility, Dr. Kirschner said.</p>

<p align=""left"">For some time, the Kirschner laboratory has been investigating how the Hem-1 complex interacts with Rac and WAVE – two major pathways in neutrophil chemotaxis – when cells change course. In February 2006, Dr. Kirschner’s team reported that the Hem-1 complex is needed for Rac activation, actin polymerization and for keeping myosin at bay. Hem-1 is essential for orchestrating polarity – and therefore forward movement – in neutrophils.</p>

<p align=""left"">These findings left Dr. Kirschner wondering about the spacial relationships of Rac, WAVE, and the Hem-1 regulatory complex. When his team used spinning disc confocal microscopy to examine GFP-labeled Hem-1, all they could see was that it was concentrated near the leading edge. “This was not very interesting,” he said.</p>

<p align=""left"">Next they turned to total internal reflectance microscopy, or TIRF, a newer technology with a depth of field of one micron and a higher signal-to-noise ratio than confocal microscopy. “We saw propagating wave fronts, all propagating in the direction of the cell’s movement,” Dr. Kirschner said. This was the first time such a phenomenon had been observed, and subsequent experiments indicated that local positive and negative feedback loops based on actin assembly generate the propagating waves. Other circuits control polarization. This system can turn

on a dime: when the leading edge of a cell encounters a barrier, the waves are extinquished immediately and then start up again in a different direction.</p>

<p align=""left"">Why do cells use waves to move? Dr. Kirschner speculated this particular pattern unifies the leading edge and naturally generates movement in the direction of the waves, propelling the cell forward.</p>

<p align=""left"">Regulation of cell shape: a matter of capping and bundling actin filaments</p>

<p align=""left"">Giorgio Scita</p>

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<p align=""left"">In contrast to the previous lecture, which focused on actin remodeling in immune cells defending against foreign invaders, Dr. Scita examined cytoskeletal changes found during normal development and in malignant, turncoat cells. What the presentations shared was an interest in changes of cell shape regulated by dynamic, architecturally diverse actin-based scaffolds responding to external cues.</p>

<p align=""left"">Dr. Scita's lab is especially interested in how the barbed ends of actin filaments are capped, and in how filaments are bundled to form protrusions such as microvilli and filapodia. In 2004, his group identified Eps8 as a protein that alters actin dynamics in certain settings by capping the barbed ends of actin filaments. In Nature Cell Biology, they published evidence that Eps8 is essential for normal development of intestinal microvilli in the worm model.</p>

<p align=""left"">Eps8 is so highly conserved that Dr. Scita's team thought it was worth exploring further. As a result, they found that Eps8 also participates in cross-linking and bundling actin filaments. Eps8 binds in a bivalent fashion with IRSp53, a self-inhibited actin bundler that can associate to the Rho-GTPases Rac and Cdc42. Eps8 and IRSp53 mediate actin cross-linking in vitro, and in vivo EPS8 enhances IRSp53-dependent membrane extension to generate neurite-like branched protrusions.</p>

<p align=""left"">Cellular distribution of the IRSp53-Eps8 complex is controlled by binding to Cdc42, Dr. Scita said, supporting the existence of a novel Cdc42-IRSp53-Eps8 signaling pathway. If either IRSp53 or Eps8 was removed, Cdc42-induced filopodia were significantly diminished. He concludes that the synergic bundling activity of the IRSp53-Eps8 complex, regulated by Cdc42, is essential for generating actin bundles and promoting filopodial protrusions. Some of these cytoskeletal changes are associated with fibrosarcoma and other malignant phenotypes.</p>

<p align=""left"">Genetic control of thyroid organogenesis</p>

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<p align=""left"">Congenital hypothyroidism is a common hereditary defect, affecting approximately one of every 4,000 to 6,000 children born. In 85% of cases, this condition is due to thyroid dysgenesis, where some disturbance during development causes the gland to be absent, in the wrong anatomical location, or far smaller than normal. This can have devastating consequences, as adequate levels of thyroid hormone are required for normal development of body and brain. Untreated hypothyroidism leads to mental retardation and growth failure.</p>

<p align=""left"">The morphogenesis begins with a cluster of cells on the floor of the mouth, which form a bud-like structure that migrates to the base of the neck and reaches its final destination in front of the trachea after about one month of embryonic development (5 days in the mouse). Only at this final stage do thyroid follicular cells express a series of differentiation markers that appear in a fixed temporal pattern in the mouse embryo: Tg TPO and TSHR genes are expressed first, followed by NIS, and by about the 16th day thyroid hormone is detectable. In humans, as in mice, the gland begins producing thyroid hormone only after arriving at its proper location, Dr. Di Lauro said.</p>

<p align=""left"">Some years ago, his lab discovered that three transcription factors, TTF-1, TTF-2 and Pax-8, are expressed not only in mature thyroid cells but also in their precursors. Dr. Di Lauro’s team has since identified another gene, Foxe-1, involved in organogenesis. Knowing about these genes allowed them to home in on the genetic basis of normal and pathological thyroid development.</p>

<p align=""left"">The researchers created knockout mice for each of the transcription factors and monitored their development. In TTF-1^{-/-} or Pax-8^{-/-} embryos, the thyroid bud formed and initiated its migration normally. In TTF-2^{-/-} mice, however, the thyroid bud formed but did not migrate from the floor of the pharynx. Additional problems crop up later in development, when Pax-8 null mice fail to develop a working thyroid gland and Foxe-1 null mice have thyroid ectopy or cleft palate.</p>

<p align=""left"">Because migration of the bud to its permanent location at the base of the neck is so important, the Di Lauro team set out to identify target genes of TTF-2. They used laser capture microdissection to isolate thyroid precursor cells and pharynx endodermal cells from tissue sections of wild type and TTF-2 null mice. They have identified some 53 additional genes that could be involved in migration.</p>

<p align=""left"">Although the four transcription factors can contribute independently to defects in organogenesis, Dr. Di Lauro suspects that in nature these defects are probably polygenic. His lab has generated mouse lines that are multiply heterozygous for null alleles of titf1, titf2 and pax8, none of which – when hererozygous – results in dysgenesis. They found that only double heterozygous titf1^{+/-} pax8^{+/-}mice had impaired thyroid function, indicated by high TSH and low T4 hormone levels in blood. Moreover, defects only occurred in the C57/B6 mouse strain. These data are the first evidence of a polygenic origin of congenital hypothyroidism, Dr. Di Lauro said.</p>

<p align=""left"">Oscillations and variations – p53 dynamics in single living cells</p>

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<p align=""left"">A classic theme in biology is figuring out how a cell takes in signals from the outside world, processes them, and decides what to do next. This struck Dr. Lahav as a perfect application for systems biology, which among other things seeks to understand the control of signaling pathways in complex networks. This approach marries experimentation and modeling and requires precise quantitative information about the dynamics of the proteins involved in the cellular response.</p>

<p align=""left"">When cellular DNA is damaged, the tumor suppressor protein p53 swings into action, either patching up the damage or triggering cell death. Its activity is governed by a feedback loop with its negative regulator, Mdm2: p53 transciptionally activates Mdm2, which in turn degrades p53. Previous studies of p53 and Mdm2 dynamics following DNA damage looked at changes in protein levels over time in whole populations of cells, an approach Dr. Lahav said masked the true behavior of the network.</p>

<p align=""left"">Population studies suggested that the cells' collective response to DNA damage was analog, with p53 and Mdm-2 levels rising gradually to keep pace with worsening DNA damage. Her lab devised a method for tracking what happens in a single cell, using fluorescence microscopy to observe the activity of p53 labeled with cyan fluorescent protein and Mdm-2 labeled with yellow. Their single cell studies showed that p53 does not rise gradually, but instead is expressed in a series of discrete pulses. The mean height and duration of each pulse held steady, and did not vary with the amount of DNA damage, Dr. Lahav reported. They used controlled doses of radiation to stress the cells, and saw the number of pulses in each cell rise as DNA damage worsened.</p>

<p align=""left"">These experimental results suggested a mathematical model for the p53 oscillator, which enabled her to make predictions about patterns of signaling and inhibition. The model indicated that the p53 signaling pathway repeatedly tests for DNA damage, acting as a 'digital clock' that releases well-timed quanta of p53 until the damage is repaired or the cell dies. "Between pulses the cell may be re-evaluating the situation," Dr. Lahav said. Cells may make a finite number of attempts to fix what's wrong, then trigger apoptosis if the damage is irreparable.</p>

<p align=""left"">Additional experimental observations support the idea that p53 pulses are important for regulating the expression of target genes, controlling downstream pathways, and guiding cell fate decisions. The oscillatory nature of this network increases the strength and flexibility of the damage response, illustrating what Dr. Lahav calls "the strength of indecisiveness."</p>

Signaling networks in Drosophila

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<p align=""left"">Dr. Perrimon has long been interested in understanding signaling networks in Drosophila cells, especially networks involved in patterning and morphogenesis. His goal is to identify all the components cells use to receive and integrate signals from their environment, and to organize them into pathways and networks.</p>

<p align=""left"">After the fruit fly genome was sequenced in 2000, he decided that the genetic tools his lab used amounted to shining a flashlight beam on pieces of these networks but did not illuminate the big picture. He set out to change this by creating the Drosophila RNAi Screening Center, which was launched in May 2004. Dr. Perrimon and his colleagues have created a library of 21,000 small, double-stranded RNAs that can systematically turn down or turn off each of the fly's 21,000 genes. RNA interference, or RNAi, makes the process of gene discovery faster, easier, more reliable, and genome-wide.</p>

<p align=""left"">The screening center is available to scientists around the world who want to conduct whole-genome screens in Drosophila. So far, RNAi screening at HMS has identified nearly 300 genes that may provide insights into human development and disease. The yield so far includes genes involved in cancer, obesity, severe combined immunodeficiency disease and others that shape the host responses to bacterial or viral infections.</p>

<p align=""left"">In addition to leading the screening center, Dr. Perrimon continues to investigate how fly embryos develop. His team recently used RNAi to identify a gene defect that interferes with normal muscle growth in fly embryos. They are also using information about cellular architecture to characterize how subcellular localization of components in signal transduction pathways influences network output, and to explore how cell polarity is involved in this process.</p>

<p align=""left"">Small molecules and biological information transfer</p>

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<p align=""left"">“A theme of this meeting – and of life itself – is the flow of information in living organisms, and understanding this information flow at the molecular level energizes a significant fraction of biological research,” Dr. Clardy said, opening the final talk of this year’s Symposium. But when most biologists say “molecular level,” what they really mean is the macromolecular level – how information passes from DNA to RNA to make proteins, and how cascades of phosphorylation and dephosphorylation regulate cellular processes.</p>

<p align=""left"">While this is an important way to understand how life works, Dr. Clardy says, it underestimates the tremendous importance of small molecules. Their ranks include sex hormones, neurotransmitters, and THC, to name only a few, and he sees small molecules as biology’s preeminent messengers. He went on to describe two organisms where understanding the role of small molecules in “lifestyle choices” could open the door to broader biological insights.</p>

<p align=""left"">The fungus Candida albicans is dimorphic, capable of existing in either a yeast-like or filamentous form. In humans, the filamentous form causes everything from diaper rash and thrush to infections of the vagina, intestine, or even heart valves. Genetic studies show that the ability to change forms is central to the development of these maladies, and that a small molecule turns harmless yeast into the pathogenic filamentous form. Initially this molecule was one of many that Dr. Clardy’s team isolated from uncultured bacteria living in the wild, and he was struck by its structural resemblance to other signaling molecules. Now that it is known to turn

C. albicans mean, Dr. Clardy hopes to find small molecule therapeutics that will interfere with its biosynthesis or be antagonists for its receptor.</p>

<p align=""left"">The Clardy lab works with another dimorphic organism, Caenorhabditis elegans, which has a normal larval stage when the living is easy but forms a “dauer” larva under adverse conditions. The prevailing wisdom has been that transformation into this skinny, long-lived state is triggered by a single small molecule called the dauer pheromone, Dr. Clardy said. Dropping food into the dish causes nematodes to emerge immediately from the dauer state.</p>

<p align=""left"">When Dr. Clardy’s team looked more closely, however, they identified three molecules involved in dauer dynamics. Two of these, combined at physiologic concentrations, caused dauer larva to form; when food was added to the dish, the nematodes could not exit the dauer state unless the third molecule was present. This third molecule resembles the human arachadonic acid receptor, which binds THC as well as a prostaglandin precursor. C. elegans, Dr. Clardy said, turns out to be a wonderful model for other signaling systems besides the dauer phenomenon.</p>

<p align=""left"">The researchers are now conducting systematic searches for all the small molecules that activate all the sensory neurons of C. elegans and for natural ligands for all the nematode’s hormone receptors</p>";"Symposium 2006: Catania, Italy";"";inherit;open;open;"";248-revision;"";"";2012-09-14 12:55:34;2012-09-14 12:55:34;"";248;http://armeniseharvard.sypdevelopment.com/senza-categoria/248-revision/;0;revision;"";0