5th Annual Symposium
Giovanni Armenise-Harvard Foundation
For Advanced Scientific Research

Cancer Biology, Genomics, and Post-Genomics

June 13-14, 2001
Lago Maggiore, Stresa, Italy

About the Symposium

For two days in June, 100 scientists who traveled from Harvard Medical School and five leading Italian research institutions convened at the Grand Hotel des Iles Borromees This historic hotel overlooking Lago Maggiore, one of Italy's most famous and beautiful lakes, was the site of the 5th Annual Symposium of the Giovanni Armenise-Harvard Foundation.

President and CEO Dr. Daniel C. Tosteson welcomed participants with his reflections on how the Foundation has evolved and what its future holds.

Dr. Daniel Tosteson

Like the magnificent lake itself, which crosses imperceptibly from Italy to Switzerland, the Foundation is also making a transition. For the first five years of its operation, Armenise research centers at Harvard were paired with corresponding centers in Italy. Within that framework, scientists undertook collaborative projects that were underwritten by the Foundation. "Now, however, the focus of the Foundation's philanthropy in Italy has changed," Dr. Tosteson said at the symposium. There will be more emphasis on rewarding individual talent and somewhat less on institutional ties. To that end, the Foundation has established a new Career Development Program that will support newly independent Italian scientists as they return to a host institute in Italy to establish themselves and their areas of research. Also in keeping with the Foundation's new focus on the individual, the Armenise-Harvard Foundation has begun a Ph.D. Program at Harvard Medical School for talented Italian science graduates. It is the hope of the Foundation that both programs will enable researchers to expand their potential to make significant contributions to their field of research as well as to further collaborative relationships between Italian scientists and those at Harvard Medical School.

The types of research projects that the Foundation plans to emphasize in the coming years "are captured admirably in the program for this symposium," Dr. Tosteson said by way of introducing Dr. Peter Howley, who chaired the program committee for this annual meeting. Previous symposia have been organized by topic area. In contrast, all the 2001 presentations were chosen to illustrate one major theme: Cancer biology, genomics, and post-genomics. There were 20 invited lectures, organized into five sessions that filled two days, and 30 posters of "extraordinary quality." All were chosen to represent the new wave of scientific inquiry in the post-genome world, Dr. Howley said.

Dr. Peter Howley

About one-third of participants were affiliated with the six basic science departments located on the Quadrangle at Harvard Medical School. Italian participants represented the European Institute of Oncology in Milano, the University of Padova, the Institute for Cancer Research and Treatment at the University of Torino School of Medicine, the Dipartmento di Ricerca Biologica e Tecnologica (DIBIT) at Scientific Institute San Raffaele in Milano, and Universita' Di Roma "La Sapienza".

Presentations – Day One

Cancer: An Unfortunate Genetic Collaboration
Philip Leder, Professor and Chairman
Department of Genetics
Harvard Medical School and Howard Hughes Medical Institute

Dr. Leder's opening presentation introduced the first major theme of the symposium; namely, that many cancers result from a series of individual genetic mutations that conspire to eventually produce disease. The path to malignancy often begins with a single genetic change, which does not cause runaway cell proliferation until other mutations disrupt or activate specific pathways. Over the years, Dr. Leder's laboratory has created mouse models that demonstrate how a series of mutations can lead to malignant transformation, and which enable researchers to study individual steps along the way. Because some of these steps may represent opportunities for treatment, these models are being put to a new use: helping scientists screen large numbers of drugs with cancer fighting potential.

Dr. Leder described a series of experiments using a mouse model for breast cancer. These involved using retroviral vectors to insert selected mutations into transgenic mice that already carried an activated, initiating oncogene. Dr. Leder and his colleagues predicted that some of these insertions would alter additional pathways in ways that would – in concert with the transgene carried by the mice – accelerate the development of breast malignancies. Using this approach, the researchers identified specific collaborating partnerships between the fibroblast growth factor (FGF) and the Wnt pathways in murine breast cancer, and have shown that Wnt acts on β-catenin and its targets.

Building on these findings, Dr. Leder's lab has created a series of transgenic mice that develop malignancy following the introduction of such well-known cancer genes as mutated p53, BRCA-1, or Neu (HER-2). The researchers can now create malignant cells with specific combinations of mutations, which are incorporated into microarrays that can be used to screen literally thousands of small molecules. These microarrays, which are an increasingly important tool for drug development, can be used to identify chemicals that most effectively block proliferation. The hope is that some of these will prove safe and effective enough to treat cancer patients in the future.

Semaphorin 4D Receptor Controls Invasive Growth By Coupling With Met Tyrosine Kinase.
Silvia Giordano
Institute for Cancer Research and Treatment
University of Torino Medical School

Dr. Leder set a theme by establishing that oncogenes act in concert to produce malignant transformation. Dr.Giordano discussed a related kind of malevolent teamwork, in which two different ligand-receptor pairs join forces to promote invasive growth by cancer cells. The first pair is semaphorin 4D and plexin B1. Semaphorins are soluble signals that guide axon growth in neurons, and which bind to plexins, a family of receptors found not only in the nervous system, but also in other types of cells. The second pair consists of a scatter factor, hepatocyte growth factor (HGF), and its receptor Met, a tyrosine kinase from the MET/RON/SEA oncogene family. Although HGF and Met are important during normal embryonic development, they have also been associated with malignancy when mutated in certain ways.

Although the biological activities of sema 4D and plexin B1 in nerve cells are well understood, their function in other settings has been unclear. Working with epithelial cells, Dr. Giordano and her colleagues showed that sema 4D could trigger a complex program for invasive growth, including cell-cell dissociation, anchorage-independent growth and branching morphogenesis. The researchers recognized that these same events in epithelial cells can be launched by the binding of scatter factors, such as HGF, to tyrosine kinase receptors such as Met. They also noticed striking structural homology between Met-type receptors and plexins, notably in the extracellular domain.

More surprising was the discovery that plexin B1 and Met form a complex, and that when sema 4D binds to its receptor, plexin B1, this stimulates the tyrosine kinase activity of Met, resulting in tyrosine phosphorylation of both receptors. Taken together, these events triggered branching morphogenesis and invasive growth by epithelial cells. Semaphorin had no effect, Dr. Giordano observed, on cells where Met was not expressed – indicating that this tyrosine kinase receptor is essential for semaphorin-stimulated invasive growth.

Functional Analysis of BRCA1 and 2.
David M. Livingston
Dana-Farber Cancer Institute and Harvard Medical School

BRCA1 and 2 have become famous as “breast cancer genes,” because germ-line mutations in these genes predispose women to development of early onset breast and/or ovarian cancer. BRCA1 and 2 are highly conserved genes found in a variety of animals as well as humans, and Dr. Livingston and his colleagues have analyzed protein function using a frog embryogenesis model. As a result, his lab has uncovered an unusual and important partnership between the BRCA1 protein and the protein encoded by a similar gene called BARD1.

Prior to these experiments, BRCA1 and 2 were known to have tumor suppressing activity and to encode proteins that interact physically with each other and with other proteins as well – some known, some not. The full range of function for these pairings was unclear, although there was ample evidence that cells need BRCA 1 and 2 "on patrol," Dr. Livingston said, to protect the integrity of their genome. His lab has been analyzing mechanisms these proteins use to maintain genome integrity, as this is the likely key to their tumor suppressing activity.

In frog embryos, the researchers discovered collaboration between the BRCA1 protein and the protein encoded by BARD1, a gene also known to play a role in tumor suppression. It turns out that the two associate to form a heterodimer, and this structure maintains the high levels of the two proteins required for normal development. If the proteins were prevented from associating in this way, and the level of either was depleted during embryogenesis, a range of growth defects and deformities occurred in the frogs.

These findings raise provocative questions about what else BRCA1 may be up to. There is evidence that this protein interacts with products of at least seven other genes associated with cancer, some known to cause malignancy and others known to suppress tumor growth. Dr. Livingston speculates that BRCA1 is an "influence peddler" that plays key roles in genome maintenance, proliferation control, and cell differentiation by regulating the activities of other proteins, rather than acting on its own. Future research will explore this idea.

Oligomerization of RAR and AML2 transcription factors as a novel mechanism of oncogenic activation
Saverio Minucci
Department of Experimental Oncology, European Institute of Oncology

Dr. Minucci's research is part of a long-term exploration of specific molecular events in the pathogenesis of acute promyelocytic leukemia (APL), particularly chromosomal rearrangements of the transcription factor RAR that affect patient survival and response to treatment with retinoic acid. His lab studies the biological activities of a fusion protein that is formed by RAR and the product of a nuclear gene called *PML,* which prolongs survival and blocks cell differentiation. Now they have found that events in other acute leukemias mimic this joint activity.

Dr. Minucci and his colleagues had previously shown that oligomerization of RAR, through a self-association domain present in PML, activates transcriptional co-regulators that in turn recruit histone deacetylases (HDACs). HDACs are required for transcriptional repression of PML-RAR target genes, and for the transforming potential of the fusion protein. More recently, they found evidence that nuclear PML recruits p53, which when modified protects against apoptotic cell death. It now appears that the PML-RAR complex has two mechanisms of action – one where p53 activity prolongs survival; the other in which abnormal HDAC recruitment blocks differentiation.

The researchers hypothesized that the same mechanisms might also be at work in non-APL forms of acute myelocytic leukemia. Experiments confirmed that oligomerization and altered recruitment of HDACs are also responsible for transformation by the fusion protein AML1-ETO, found in a type of AML. AML1-ETO expression blocked retinoic acid (RA) signaling, suggesting that HDAC recruitment may be a common theme in AMLs – and thus an opportunity for treatment. Dr. Minucci's team used a mouse model to show that chemical inhibition of HDAC, in addition to standard retinoic acid treatment, prolonged survival. The HDAC-inhibitor they used was valproic acid, a common anti-seizure medication. The combination of retinoic and valproic acids is currently being tested in a Phase II clinical trial.

RecQ helicases provide links between premature aging and cancer.
David Sinclair
Department of Pathology, Harvard Medical School

Defects in RecQ-like DNA helicases are responsible for genetic instability in Bloom syndrome (BS), Rothmund-Thomson syndrome (RTS) and Werner syndrome (WS). Early in life, people with WS develop not only gray hair and wrinkles, but also typical diseases of old age such as cancer and cardiovascular disease. Dr. Sinclair is a yeast biologist who specializes in the genetic underpinnings of aging, and he is using this simpler organism to shed light on the connection between premature aging and cancer in WS patients.

There is evidence that the premature senescence of fibroblasts cultured from WS patients is due to a defect in the telomere, the protective tip that guards chromosomes against erosion. The enzyme telomerase preserves telomere length when cells are in their prime, then declines as they age. In WS fibroblasts, senescence could be averted by introducing telomerase catalytic subunit (hTERT) into the culture. When the telomeres of yeast chromosomes reach a perilously short length, a parallel mechanism saves some of them from premature senescence. Others, however, are rescued by a yeast helicase called Sgs1 that causes the yeast chromosomes to develop abnormally long telomeres.

In about 10% of human cancer cells, a poorly understood sequence of events called the "ALT" pathway, for "alternative lengthening of telomeres," is thought to immortalize cells by adding to their telomeres even when no telomerase is present. The Sgs1 gene in yeast is homologous with the WS gene, which encodes a DNA helicase known as WRN. Dr. Sinclair's findings about Sgs1 raise the possibility that the human WRN helicase is an "ALT" factor, and that it could be the missing link between premature aging and early onset of cancer in WS patients. Future studies in animal models may help elucidate the *in vivo* function of the mammalian RecQ-helicases as well as the mechanisms by which cancer cells proliferate in the absence of telomerase.

Integration of receptor trafficking and signaling through the protein complex RN-tre/Eps8
Letizia Lanzetti
European Institute of Oncology

Like other tyrosine kinase receptors, epidermal growth factor receptor (EGFR) is the entry point into pathways important for signal transduction, endocytosis, and many other key cellular functions. Small GTPases of the Rho family are known to be involved in signal transduction, while members of the Rab family are established players in endocytosis. Dr. Lanzetti and her colleagues set out to investigate how signaling relying on these two classes of GTPases might be integrated. They studied individual intracellular proteins that are substrates for tyrosine kinase activity, and found unexpected collaborations among them.

Her laboratory has a long-standing interest in Eps8, a protein that plays a role in endocytosis. When it acts in this capacity, Eps8 collaborates with Rab5, a gatekeeper located just beneath the cell membrane to mediate trafficking. This is part of the Ras pathway that is normally involved in the organization of actin into ruffles when tyrosine kinase receptors are activated. In the present series of experiments, Dr. Lanzetti found that alternative complexes between Eps8 and E3b1 or RN-tre connect signaling pathways for ruffle formation and endocytosis. E3B1 is an adaptor protein that couples Eps8 with the sos1 protein, which in turn activates the small GTPase called Rac. In this configuration, Eps8 contributes to the formation of ruffles.

In a second configuration, the SH3 domain connects Eps8 with RN-tre, a GTPase activator protein. Dr. Lanzetti's experiments show that when RN-tre is complexed with Eps8, it inhibits Rab5, an important gatekeeper in the early stages of endocytosis. Knockout experiments show that RN-tre alone does not have this activity. At the same time, RN-tre diverts Eps8 from its Rac-activating function, which inhibits actin reorganization into ruffles. Thus it appears that when RN-tre is complexed with Eps8, signaling through Ras and Rac are affected as well as trafficking through Rab5.

Human papillomavirus E6 activation of cellular tyrosine kinases
Peter M. Howley
Department of Pathology, Harvard Medical School

Human papillomavirus is one of the most medically important microbes, due to its causal role in cervical cancer. Of more than 100 strains that have been identified, about two dozen are associated with genital tract lesions and a subset of these put infected women at high risk for cervical cancer. The dangerous strains make oncogenic proteins that transform cells by selectively inactivating tumor suppressor genes, including p53.

Extensive research on one of these cancer-causing HPV proteins, E6, has been carried out in Dr. Howley's laboratory over the years. He and his colleagues showed that E6 subverts the normal activity of a cellular protein they call E6 associated protein (E6-AP), causing

it to mark p53 protein for proteolytic destruction. Interestingly, this happens only in the presence of the viral protein E6; in uninfected cells, E6-AP is a protein ligase that doesn't interact with p53 at all. This caused the researchers to wonder what else E6-AP might be doing that contributes to oncogenic transformation.

The observation that levels of Fyn, a Src-family tyrosine kinase, are high in cells infected with cancer-causing HPV provided an important clue. A long series of experiments in cells and in mice were needed to show that overexpression of E6-AP in HPV-infected cells caused levels of an enzyme Csk to fall. This enzyme turned out to be a player in the ubiquitin pathway, whose role is to flag the oncogenic Fyn protein for destruction. This is a second major role for E6-AP in malignant transformation: it not only inactivates helpful p53, but also elevates potentially dangerous Fyn.

Inducible transgenic models for the study of spinocerebellar ataxia type 1 (SCA1) and for the study of other neurological disorders
Antonio Servadio
DIBIT – Institute San Raffaele

Spinocerebellar ataxia type 1 (SCA1) is an inherited neurodegenerative disease caused by excess glutamine repeats in the gene that codes for ataxin-1. Healthy genes have less than 41 glutamine repeats; mutations result in as many as 83 and there is a direct correlation between the number of repeats and disease severity. In the brain, the disease destroys Purkinje cells and pontine and olivary nuclei. SCA1 resembles Huntington's disease: onset is late although the genetic defect is present from birth, and once the disease starts it is progressive and devastating. Dr. Servadio set out to find whether it was possible to reverse pathogenic changes associated with abnormal ataxin-1.

His laboratory approached this question by creating a mouse model in which the timing and severity of disease progression could be manipulated. Such a model not only could contribute to the understanding of disease pathogenesis, but also could be useful in evaluating the possible efficacy of novel therapies. Dr. Servadio's team used the "Tet-off" system to create an inducible model of SCA1. They put expression of recombinant ataxin-1 under control of the synthetic tTA transactivator. In this system, giving tetracycline to the mice would inactivate the pathogenic ataxia-1 gene, while withdrawing the antibiotic would allow disease to progress. The researchers also refined their model by breeding transgenic mouse strains that enabled them to regulate expression of mutated ataxin-1 in specific brain regions.

Unfettered ataxin-1 expression resulted in smaller litters, reduced body size, cranio-facial dysmorphology, and performance deficits in newborn mice. None of these defects were observed when ataxin-1 transgene was repressed by continuous tetracycline administration. On rare occasions, some birth abnormalities could be reversed by administering tetracycline and shutting down ataxin-1. In mice that were several months old, SCA1 could also be induced by withdrawing tetracycline and allowing ataxin-1 to flourish. These tools, which enable scientists to manipulate the onset, severity, and

progression of symptoms, are expected to be widely used in the study of this disease. Dr. Servadio's lab created these models with support from Telethon (Italy) and from the National Ataxia Foundation (Minneapolis, MN).

New technologies for measuring and modeling functional genomics
George M. Church
Lipper Center for Computational Genetics, Harvard Medical School

One of Darwin's greatest insights was that each individual cell, and each living organism, constantly strives to leave behind the maximum number of progeny. Cancer cells, unfortunately for patients, proliferate better than most. And as Dr. Leder suggested in the symposium's opening talk, many different genes play a role in runaway cell growth. New technology such as RNA and DNA microarrays now enable scientists to "fingerprint" pathological states such as cancer, and to determine the full spectrum of genes that are involved in cell proliferation and its regulation. Dr. Church and his colleagues at the Lipper Center for Computational Genetics are also developing functional genomics methods that enable them to tell not only which genes are expressed, but also to quantify expression and determine when it occurs in the cell cycle.

When Dr. Church's group used DNA microarrays to study gene expression in cancer cells, they found that the cells have only small growth advantages at first, but over time these are exponentially amplified to optimize their growth. Because DNA must make RNA in order to generate proteins, they used RNA microarrays to determine when specific genes were transcribed and in what quantity. Whether these transcription patterns can be observed *in vivo* is an important question, of course, and the researchers are using animal models to find out. They're using mass spectrometry to track *in vivo* protein expression.

In the post-genome era, Dr. Church believes that genetic fingerprints of different tumors will lead to more precise diagnosis and treatment. Treating all cancers the same, as clinicians know, results in toxicity and failure to respond. The ability to profile individual malignancies could change that.

Analysis of gene expression patterns in acute myeloid leukemia
Myriam Alcalay
European Institute of Oncology

Dr. Alcalay's laboratory is one of several at her institution with a persistent interest in chromosomal translocations in acute myeloid leukemia (AML). Recently, her group has been experimenting with various high-throughput DNA screens as a means for identifying the full spectrum of biological activities associated with such translocations. In particular, the researchers have looked for overlapping gene expression patterns for the AML1/ETO fusion protein of M2-AML and the RARα-fusion proteins associated with acute promyelocytic leukemia (APL). At the outset, several similarities were known:
i) they are responsible for the pathogenesis of the disease,

ii) they act by interfering with the differentiation program of target hematopoietic precursors,
iii) *in vivo*, they form oligomers which are part of high molecular weight complexes including histone deacetylases (HDACs),
iv) they function by deregulating transcription from a set of target genes.

Dr. Alcalay and her colleagues used nylon filter arrays containing 18,376 human cDNAs for a preliminary analysis of gene expression patterns of U937 hematopoietic precursor cells. These cells expressed either the PML/RAR or the AML1/ETO fusion proteins, and the researchers exposed them to substances that stimulate differentiation. This filtering method yielded 1,294 putative target genes. This technique was not very discriminating, however, and additional tests showed that most were not true targets. More exacting Northern blot studies winnowed the candidates down to 12 non-regulated and 64 regulated target genes. Of the 64 regulated genes, half are common targets for PML/RAR and AML1/ETO and half of those are repressed by both fusion proteins. The repressed genes are induced during differentiation. A separate series of experiments with Affymetrix DNA chips identified several hundred genes that are repressed by the products of two or more fusion proteins, pointing to numerous common pathways in acute myeloid leukemogenesis. In AML mice, retinoic acid therapy induced expression of some of these same genes, which Dr. Alcalay said confirms their importance.

Presentations – Day Two

Manipulating the Proteome
Joshua LaBaer
Institute of Proteomics, Harvard Medical School

For a generation of biologists, sequencing the human genome was the most important challenge in their field. Since initial sequencing was completed in June 2000, the focus has shifted to another monumental task: identifying all the proteins encoded by genes and defining their function. As the first step toward this goal, the Institute of Proteomics at Harvard Medical School is building a large scale, state-of-the-art repository of all known genes, and predicted open reading frames (sequences that encode proteins), from humans and from other important organisms. Thousands of human and animal genes will be packaged in a uniform, easy-to-use format called FLEXGene™ (Full-Length Expression-Ready). Researchers can order hundreds or thousands of genes from the FLEXGene™ Repository and transfer them, in a single overnight step, into whatever expression vectors their experiments require.

“What took weeks can now be done overnight,” Dr. LaBaer said. A major roadblock in high-throughput protein analysis has been the labor-intensive task of expressing all the needed proteins in a way appropriate for the experiment. The old approach required having the desired cDNAs available, then sub-cloning them in-frame in appropriate

expression vectors. Using traditional methods, this step is tedious for a single gene, and simply not feasible for most labs if hundreds of genes were needed. For genes in the FLEXGene™ Repository, however, large numbers could be done very quickly. Moreover, the cDNAs are configured to allow the preparation of proteins with or without N- and/or C-terminal fusion peptides as needed.

Stock in the Repository is growing at the rate of about 500 clones per week, Dr. LaBaer said, drawn from the human, drosophila, malaria, and *P. aeruginosa* genomes. The rate-limiting step for preparing FLEXGene™ plasmids turns out to be sequence validation, which is essential for quality control. More than 40 institutions and companies have joined Harvard Medical School in backing this project.

Emx2 **and** ***Pax6*** **control regionalization of the cortical primordium**

Antonello Mallamaci
DIBIT - Institute San Raffaele

There are competing theories about how the mammalian cerebral cortex takes shape during early embryonic development. Proponents of the "protomap" model hold that the primordial cortex holds all the information needed to make a cortex, while those in the "protocortex" camp see the primordium as raw material that is shaped into a cortex by external signals. Reality, Dr. Mallamaci said in prefacing his remarks, will probably turn out to be somewhere in the middle.

His laboratory has analyzed how manipulating the expression of two homeogenes, *Emx2* and *Pax6,* affects primary neurogenesis and shapes the profile of the cortical area. The researchers suspected that these genes might help organize the cerebral cortex because they are expressed in a graded fashion in neocortex. In a knockout mouse with no *Emx2* expression, Dr. Mallamaci and his colleagues saw an obvious enlargement of frontal-motor areas as well as a dramatic reduction of occipital-visual areas at the end of cortical primary neurogenesis. Distortions continued later in development as well. When *Pax6* was disabled, the researchers observed complementary abnormalities; for example, the occipital-visual region was abnormally large, not undersized as it was in mice without *Emx2*.

A series of experiments with knockout mice lacking both *Emx2* and *Pax6* led the researchers to several conclusions about the role of their gene products in early neurogenesis. It appears that the two work together in a kind of "balancing act," Dr. Mallamaci said, each shaping part of the neocortex while at the same time down-regulating activity of the other, so that different regions develop in the correct proportions to one another. The absence of functional EMX2 or PAX6 proteins not only reduced size of the caudal-medial and rostral-lateral regions, as predicted, but also impaired the WNT signaling center at the medial-caudal edge of the cortical field. These results suggest that pre-neurogenic cortical regionalization may rely on interactions between these two transcription factors and that later abnormalities in the double

knockout mouse model may arise from mistakes in the molecular protomap and distortion of the cortical growth profile.

Understanding host-pathogen interactions using genome-wide expression profiling
Stephen Lory
Department of Microbiology and Molecular Genetics, Harvard Medical School

Scientists interested in infectious disease have unprecedented tools available in the post-genome era, and these may enable them to determine exactly why a pathogen that causes mild symptoms in one person may cause life-threatening illness in another. Dr. Lory's group focuses on interactions between *Pseudomonas aeruginosa* and cultured cells from the human respiratory system, a clinically important interface because *P. aeruginosa* respiratory infections account for more morbidity and mortality among cystic fibrosis patients than any other cause.

Two resources allow them to scrutinize both sides of the infection story. The genome of *P. aeruginosa* has been completely sequenced, and Dr. Lory's team used this database to create DNA microarrays for analyzing global patterns of gene expression during *P. aeruginosa* infection of susceptible individuals. The researchers exposed these microarrays to environmental conditions that mimic the respiratory mucosa, which influenced the expression of intracellular effector proteins secreted by *P. aeruginosa*. These experiments revealed a complex regulatory network , which activated or repressed several known genes and a large number of unknown ones. Using a series of *P. aeruginosa* mutants with defects in several virulence traits, comparisons of mRNA data indicated that various combinations altered the activity of key organelles, such as secretory machinery that manufacture enzymes for penetrating host cells.

Libraries of expressed human genes enabled the researchers to see what infection looked like from the host's point of view. Dr. Lory and his colleagues used DNA arrays of human genes to determine what signaling mechanisms were mobilized by respiratory epithelial cells following *P. aeruginosa* adhesion. Engineered mutants of *P. aeruginosa* were used to assess the contribution of individual virulence factors to the overall host response. This approach will be also used to define the defects in host response in various disease states, such as chronic respiratory infection in individuals with cystic fibrosis. The long-term goals of this research are to identify innate defense mechanisms that might be boosted to help susceptible people fight off respiratory infections.

***In Vivo* Targeting of Tumor Angiogenesis by Lentiviral Vectors**
Michele De Palma
Institute for Cancer Research and Treatment, University of Torino Medical School

The use of viruses as delivery vehicles for gene therapy and immunization has become increasingly important in recent years. In Dr. De Palma's laboratory, a specially packaged form of HIV is being used to learn more about a specific type of cell that is a key player in cancer. Angiogenesis, the formation of new blood

vessels, is now seen as the rate-limiting step in tumor growth. Unlike tumor cells, the cells in angiogenetic vessels appear to be the same no matter what type of tumor the vessels supply with blood. Because they are so distinctive, these vascular cells appear to be a likely target for gene therapy aimed at fighting cancer.

In order to design effective therapy, however, scientists need to know where the cells lining tumor vessels originate and how they grow within the tumor. In order to explore these questions, Dr. De Palma and his colleagues engineered a vector that will selectively express the genes it carries in the endothelial cells of tumor vessels. The vector incorporates a marker gene as well as structural genes from HIV, which facilitate incorporation into the nucleus, all wrapped in the envelope of VSV (vesticular stomatitis virus).

In mice with tumors, the researchers used this new vector to selectively mark ECs engaged in tumor angiogenesis and to trace their origin and growth pattern *in vivo*. As expected, IV infusion of this vector lit up the tumor vessels themselves; it also expressed in cells in the tumor periphery. More surprisingly, Dr. De Palma reported that it penetrated hematopoetic precursors of these endothelial cells. This is the first indication that bone marrow cells contribute to angiogenesis, a finding that will be pursued in future studies.

Epstein-Barr Virus Nuclear Protein 1
Elliott Kieff
Channing Laboratory, Harvard Medical School

Epstein-Barr Virus Nuclear Protein 1 (EBNA1) enables the EBV genome to persist as a transcriptionally active episome, a packet of bacterial DNA can replicate outside the nucleus, in proliferating lymphoblastoid cell lines (LCLs). Dr. Kieff's laboratory has a long-standing interest in EBV nuclear proteins in a less benign role, as contributors to the transformation of normal lymphocytes into hyper-proliferative cells. When one of his colleagues raised the possibility of using EBNA1 and its cognate cis acting element, oriP, in human gene therapy, this raised an obvious safety issue.

In an effort to determine whether EBNA1 and oriP are safe for therapeutic use, Dr. Kieff's group began by determining which components of EBNA1 mediate episome DNA replication and which bind the viral DNA to human chromosomes. The researchers found that EBNA1 C-terminus, aa 379-641 could not bind to chromosomes, but was needed – along with oriP – for episome copying. The EBNA1 N-terminus, aa 1-386, mediated chromosome association and episome persistence. At least two large domains, aa33-88 and 328-382, are both required for wild type chromosome binding. The EBNA1 distribution on chromosomes and salt elution from chromosomes were similar to HMG-I and Histone H1. Dr. Kieff's team engineered chimeric HMG (aa 1-90)-or Histone H1-EBNA1 C-terminus fusion proteins that could substitute for the EBNA1 N-terminus in diffuse, tight, association with mitotic chromosomes. In short- and long-term assays, these fusion proteins mediated tight association of oriP episomes with chromosomes and

functioned much like EBNA1 in maintaining episome replication. These experiments open the possibility of creating a "humanized" episome maintenance system, which could be useful in gene therapy.

They also evaluated EBNA1's oncogenic potential by transgenic expression of EBNA1 in T and B lymphocytes from three lineages of FVB mice. After 18 months or more, there was no increase in lymphomas or enlarged lymphoid organs compared with age-matched control animals. Finally, they tested the potential impact of EBNA1 on lymphoid cell line growth and survival by conditionally expressing dominant negative EBNA1 (DNE) in an cell line where the EBV genome was integrated into cellular DNA. DNE did not affect EBV gene expression, cell growth and survival, or cell gene expression. Neither DNE nor EBNA1 had any effect on oriP dependent luciferase expression from plasmids that had integrated into cell DNA, whereas Gal4-VP16 activated expression from episomes or from integrated DNA. This is important additional evidence that EBNA1 is unlikely to have any direct effect on cell gene transcription, and that therapeutic use is unlikely to cause malignancy.

Activation of Muscle Protein Breakdown In Cancer Cachexia: Discovery of Atrogin-1, a muscle-specific F-box protein highly induced in disease where muscles atrophy.
Alfred Goldberg
Department of Cell Biology, Harvard Medical School

Muscle wasting is a physiological response to fasting, and a characteristic, debilitating feature of cancer cachexia, diabetes mellitus, sepsis, chronic renal failure, spinal injury, and other physiologically stressful conditions. The common thread that links these disease states is that muscle proteins are rapidly lost due to accelerated degradation by the ubiquitin-proteasome pathway. Exactly how skeletal muscle proteins are marked for proteolysis, and what ubiquitinating enzymes are involved, is poorly understood.

In order to gain a comprehensive understanding of transcriptional adaptations leading to enhanced proteolysis during various types of atrophy, Dr. Goldberg's team used cDNA microarrays to compare normal and atrophying muscles. Fasted mice were initially analyzed because the various changes in energy metabolism and protein breakdown in fasting have been well characterized. Surprisingly, food deprivation had no impact on 95% of gene transcripts in muscle. As expected, the researchers saw expected increases in mRNA levels for familiar genes involved in ubiquitination and proteolysis.

The most exciting new finding from the microarrays was a group of novel genes they call atrogins (for atrophy-specific genes), including one that was expressed at high levels in skeletal muscle during fasting, but which fell within hours when feeding was resumed. Dr. Goldberg's group cloned this gene, which they designated atrogin-1. It encodes a protein that contains an F-box domain, which typically functions as a substrate-binding subunit of SCF-type ubiquitin protein ligases. Experiments confirmed that the F-box of atrogin-1 does bind a central component of SCF complexes. Although atrogin-1 is expressed primarily in skeletal muscle, it is also found in cardiac muscle. In addition to

being triggered by food deprivation, its mRNA also increases 7-10 fold in muscles atrophying due to cancer cachexia, diabetes, or renal failure (despite normal food intake). Glucocorticoids, which are essential for the activation of proteolysis in these states, can also induce its expression. These findings indicate that atrogin-1 plays a central role in muscle wasting in several clinically important disease states, Dr. Goldberg concluded.

Structure and regulation of the CDK5-p25^{nck5a} complex
Andrea Musacchio
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During normal brain development, cyclin-dependent kinase 5 (CDK5) teams up with p35, a regulatory protein, to guide newborn neurons into their proper place in the brain. But when CDK5 pairs up with a p25, a fragment of p35, Dr. Musacchio and collaborators at Harvard Medical School have found, the combination is hazardous to the brain. For starters, the two transform a normal brain protein, tau, into a potent toxin that is associated with neuron death in stroke and Alzheimer's. The CDK-p25 duo no doubt alters other proteins as well, and the researchers are working to discover them.

As a structural biologist, Dr. Musacchio's focus is on the crystal structure of the CDK5-p25 complex. It contains a single copy of a helical assembly similar to the Cyclin-box fold, and although this bears a structural resemblance to other cyclins, it displays an unprecedented mechanism for the regulation of a cyclin-dependent kinase. Binding to p25 tethers the unphosphorylated T-loop of CDK5 in a conformation typical of active proline-directed kinases. In the normal pairing of CDK5 and p35 during brain development, residue Ser159 in this loop contributes to the specificity of the CDK5-p35 interaction. When this serine is replaced with threonine, p35 binding is prevented, while the presence of alanine neither affects binding nor kinase activity. His analysis also showed involvement of the activator subunit in substrate recognition, and provided evidence that the CDK5-p25 complex uses a novel mechanism to establish substrate specificity.

The Xenopus Oocyte Progesterone Receptor – One receptor, two different abilities?
Joan Ruderman
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It is well known that steroids work by turning on receptors that are members of a large superfamily of ligand-activated transcription factors. Progesterone is known to cross the plasma membrane, bind and activate the nuclear progesterone receptor, and activate (or repress) the transcription of specific genes important for the growth and functioning of target cells and tissues.

Dr. Ruderman's research focuses on a second, less appreciated scenario for progesterone activity. In addition to regulating gene transcription, steroids such as progesterone can be important players in cytoplasmic signal transduction pathways. The oocytes of the

Xenopus frog sit quietly in G2 arrest for long periods, until they are activated by progesterone. The hormone breaks the G2-arrest of the meiotic oocyte and sets in motion a poorly understood chain of events that involves translational activation of most mRNA, MAP kinase activation, and cell cycle re-entry. These events prepare the egg for a meeting with the sperm, and all of this occurs independently of transcription. Other researchers have shown what when estrogen and progesterone activate signal transduction pathways in somatic cells, they do so via the conventional estrogen or progesterone receptors. But what happens in germ cells?

For years, the identity of the progesterone receptor in Xenopus oocytes has been elusive. But the discovery that other steroid receptors might be dual-function structures led Dr. Ruderman to ask whether the conventional progesterone receptor might activate cytoplasmic signaling in frog oocytes. Her team cloned what appears to be a conventional Xenopus PR (XPR-1) that also has the ability to signal, and their experiments locate it in the cytoplasm, rather than on the surface as expected. Now the researchers are screening for proteins that may function in a signaling pathway downstream of XPR-1, as well as exploring possible cross-talk with membrane receptors.

Transcriptional control of HGF-responsive genes
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Hepatocyte growth factor, more recently known as plasminogen-related growth factor (PRGF), is among the scatter factors that control events involved in normal growth and in malignant transformation and metastasis. In epithelial cells, PRGF activates a genetic program in which cells dissociate from one another ("scattering"), then grow and become invasive. Although other growth factors trigger scattering, Dr. Medico realized that PRGF specifically stimulates invasive growth. He set out to learn why.

To identify genes involved in the onset of invasive growth, Dr. Medico's team compared the transcriptional response of mouse liver cells to PRGF and to epidermal growth factor (EGF). Like PRGF, EGF activates a tyrosine kinase receptor in liver cells; unlike PRGF, it does not trigger invasive growth. Dr. Medico's team used two different commercial microarray platforms to sort through some 20,000 genes that might be involved in PRGF-triggered invasive growth. One technology used high-density spotted cDNAs and the other relied on in-situ synthesized oligonucleotides. Comparing their results winnowed the 20,000 candidate genes down to about 1,000 transcriptionally regulated sequences, with a surprisingly high overlap between the PRGF and EGF responses.

If PRGF and EGF act on so many of the same genes, why does one cause invasive growth and the other not? One clue came from Dr. Medico's functional studies of a major transcriptional target, the extracellular matrix protein osteopontin (OPN). While both PRGF and EGF induce OPN, experiments in cultured cells showed that only PRGF promoted adhesion of the cells to newly synthesized OPN through the CD44 receptor. In these experiments, the researchers could see PRGF stimulate the growth of tendrils that

extended across the surface and scattered. EGF-stimulated cells put out small fingers, but their growth soon stopped. Dr. Medico concluded that PRGF's ability to stimulate invasive growth does not rely directly on transcriptional regulation, but rather on functional interplay among the products of the regulated genes and the activated PRGF receptor.