Cancer-like Mutations Found in Patients with Sporadic Primary Pulmonary Hypertension

Background: Primary pulmonary hypertension (PPH) is a rare, progressive, and fatal lung disease that is characterized by increased pressure in the pulmonary artery and the formation of lesions in blood vessels of the lungs. The process eventually leads to an enlarged, overworked heart that is unable to pump enough blood to the lungs and can cause heart failure. The etiology of the disease is unknown, and there is no cure other than a heart-lung transplant.

Advance: Investigators recently discovered that the two types of PPH – sporadic and familial – are molecularly distinct conditions. Results from a genomic screening technique called micro array technology revealed that over 300 genes were expressed at different levels (and presumably make different amounts of their respective proteins) in lungs from PPH patients compared to normal lungs. However, the patterns of gene expression differed depending on whether the patient had sporadic or familial PPH. Moreover, genetic alterations in the lesions of sporadic PPH patients were similar to the changes seen in tumors of cancer patients. This is the first report of such mutations in cells that could not be classified as precancerous or cancerous.

Implications: These results demonstrate the power and utility of micro array technology for understanding complex diseases that previously could not be analyzed. The revelation that the symptoms characteristic of PPH can be caused by two distinct sets of molecular changes has profound implications for developing treatments specific for familial or sporadic PPH. In the case of sporadic PPH, researchers may be able to apply what is known about diagnosing, treating, and managing cancer patients to develop appropriate interventions.

Yeager ME, Halley GR, Golpon HA, Voelkel NF, and Tuder RM: Microsatellite instability of endothelial cell growth and apoptosis genes within plexiform lesions in primary pulmonary hypertension. Circulation Research 88: e2-e11, 2001.

Geraci MW, Moore M, Gesell T, Yeager ME, Alger L, Golpon H, Gao B, Loyd JE, Tuder RM, and Voelkel NF: Gene expression patterns in the lungs of patients with primary pulmonary hypertension: a gene microarray analysis. Circulation Research 88: 555-562, 2001.

New Technique for Characterizing Carbohydrates May Lead to New Pharmaceuticals

Background: The complex carbohydrates surrounding our cells interact with proteins and play a critical role in many biological functions. For example, members of the heparin/heparan sulfate-like glycosaminoglycan (HLGAG) family of carbohydrates are thought to have important roles in regulating diverse processes such as development, morphogenesis, cancer progression, blood vessel formation, and anticoagulation. The best characterized member of this family is heparin, commercially available as an anticoagulant. However, other HLGAGs have been less fully explored because the molecules are only available in very small amounts and no practical methods exist for studying them in detail.

Advance: Researchers have developed an accurate, highly sensitive method to isolate, enrich, and sequence tissue-derived complex carbohydrates that bind to specific proteins with clinical significance. The method, which is based on a technique called mass spectroscopy, combines several established, rigorous analytical methodologies into a single procedure.

Implications: This technology will permit direct isolation and characterization of biologically relevant carbohydrates and should aid in the discovery of new drug targets. Many of the proteins that bind to and are regulated by HLGAGs are clinically relevant. By establishing not only which regions of HLGAGs are important for regulating protein functions, but also which portions of the proteins respond to HLGAGs, it should be possible to design pharmaceuticals to mimic HLGAG-mediated processes. Taken with advances in proteomics (the identification of a cell's proteins, their post-translational modifications, and their interactions with one another), it also will be useful for elucidating many extracellular interactions between proteins and carbohydrates that are essential for many biological processes.

Keiser N, Venkataraman G, Shriver Z, and SasisekharanR: Direct isolation and sequencing of specific protein-binding glycosaminoglycans. Nature Medicine 7: 123-128, 2001.

Researchers Establish Requirements of Successful Gene Therapy for Beta-Thalassemia

Background: Beta-thalassemia is a genetic disease characterized by defective hemoglobin production, with morbidity and death due to severe anemia. Therapy for most patients with severe cases includes regular blood transfusions, which place them at risk for complications including infections from blood-borne pathogens and organ damage from iron overload. Bone marrow transplantation is also an option, albeit potentially lethal, if a patient has a suitable donor.
The defective gene that causes beta-thalassemia is known, and researchers are pursuing gene therapy as a potential cure. For any gene therapy to be successful, researchers have to determine the proportion of defective cells that need to be modified and the amount of the corrected protein that the genetically corrected cells must produce. In the case of beta-thalassemia, patients would have to receive genetically modified hematopoietic stem cells that are capable of producing red blood cells containing functional hemoglobin.

Advance: Researchers studying a mouse model of beta-thalassemia projected that patients might benefit even if only 10 percent of their stem cells were corrected. However, they would benefit much more if 20 percent of the stem cells were corrected, assuming that the resulting red blood cells could express the gene to make the correct form of hemoglobin protein at 15 percent of the level "normal" cells do.

Implications: Now that researchers know what needs to be done in order for gene therapy to be a viable option for people with beta-thalassemia, they can begin to focus their efforts on how to best accomplish this in patients without causing toxic side-effects.

Persons DA, Allay ER, Sabatino DE, Kelly P, Bodine DM, and Nienhuis AW: Functional requirements for phenotypic correction of murine β-thalassemia: implications for human gene therapy. Blood 97: 3275-3282, 2001.

Umbilical-Cord Blood Can Be Used for Stem Cell Transplantation in Adults

Background: Graft-versus-host disease (GVHD) is a painful and potentially fatal complication for recipients of bone marrow transplants. It can develop even when doctors are able to find donor cells that appear to be compatible with those of the recipient. There is increasing evidence that donors of stem cells from umbilical-cord blood (UCB) do not need to match recipients as closely as bone marrow donors do, suggesting that transplantation of unrelated UCB may provide a source of stem cells for children with hard-to-match tissue types. However, UCB stem cell transplantation has not been extended to adults because of concerns that UCB units may not contain enough stem cells to reconstitute their immune defenses.

Advance: Researchers, evaluating the safety and efficacy of UCB transplantation in adults with life-threatening hematologic disorders who did not have a matched, related or unrelated, bone marrow donor, demonstrated that UCB from unrelated donors is a feasible source of stem cells for adult patients needing bone marrow transplants. Although infections, bleeding, and other complications killed many of the patients within months, nearly one third survived long term, about the same as among those who receive bone marrow transplants from suitably matched unrelated donors. Moreover, incidence of GVHD was considerably lower in the UCB recipients than would have been predicted if they had received bone marrow transplants.

Implications: Currently, thousands of Americans die each year while awaiting a bone marrow transplant. Stem cell transplantations of cord blood, which is routinely discarded, will expand the options of desperately ill adult patients for whom a suitable bone-marrow donor is not readily available.

Laughlin MJ, Barker J, Bambach B, Koc ON, Rizzieri DA, Wagner JE, Gerson SL, Lazarus HM, Cairo M, Stevens CE, Rubinstein P, and Kurtzberg J: Hematopoietic engraftment and survival in adult recipients of umbilical-cord blood from unrelated donors. The New England Journal of Medicine 344: 1815-1822, 2001.

Laboratory Method Predicts Success of Umbilical Cord Blood Stem Cell Transplants

Background: Because stem cells from placental/umbilical cord blood (UCB) are readily available and can be used in transplants between unrelated, mismatched patients, UCB stem cell transplantation has become a life-saving option for some patients with leukemia and non-malignant blood disorders. Because UCB units generally contain fewer stem cells than can be obtained from bone marrow, they have been used primarily to treat children. If adults suffering from cancer, bone marrow failure, and metabolic diseases are to benefit from UCB stem cell transplants, they must receive UCB units containing enough cells to provide a sufficient "cell dose." The cell dose is the number of stem cells being transplanted relative to the patient's weight; it is a significant predictor of a successful transplant.

Advance: By comparing methods for counting different types of cells with how patients fared following their transplants, researchers concluded that counting hematopoietic colony-forming cells was better for determining the cell dose and predicting the outcome of transplants than counting all nucleated cells.

Implications: Adopting the superior method for calculating a potential recipient's cell dose will reduce the cost, morbidity, and mortality associated with unsuccessful UCB stem cell transplants and allow more patients to benefit from stem cell resources.

Migliaccio AR, Adamson JW, Stevens CE, Dobrila NL, Carrier CM, and Rubinstein P: Cell dose and speed of engraftment in placental/unbilical cord blood transplantation: graft progenitor cell content is a better predictor than nucleated cell quantity. Blood 96: 2717-2722, 2000.

Leukoreduction Does Not Benefit HIV-Infected Patients Requiring Blood Transfusions

Background: A unit of red blood cells will contain some white blood cells (leukocytes) unless it has been further purified by a filtration procedure called leukoreduction. Several studies have shown that transfusions of leukoreduced cells are less likely to cause immune responses in transfusion recipients; leukoreduction may also offer protection against cell-associated viruses. Although widely used in most industrialized countries, the procedure remains controversial in the U.S. because of its projected cost, estimated to be $25-$35 for each unit of blood.

Blood transfusions frequently are used to treat anemia associated with human immunodeficiency virus (HIV) infection. Small studies and anecdotal data suggested that transfusions adversely affect the clinical course of HIV, possibly because white blood cells in the transfused blood promote transfusion reactions or activate HIV production. Therefore, the hypothesis underlying the Viral Activation Transfusion Study was that transfusing leukoreduced blood would be less risky than transfusing untreated blood for HIV-infected patients.

Advance: Researchers comparing the effects of leukoreduced and unmodified red blood cell transfusions on survival and complications of acquired immunodeficiency syndrome (AIDS) found that leukoreduction does not improve survival, decrease serious HIV-related complications, or reduce transfusion reactions in HIV-infected patients requiring blood transfusions. Moreover, some evidence suggested that those receiving leukoreduced blood may have fared worse than those who received unmodified blood.

Implications: This study suggests that there is no need to offer leukoreduced blood transfusions to persons infected with HIV. In an era of high-level scrutiny of blood safety issues and cost-consciousness, failure to demonstrate an advantage for a procedure with potential adverse effects and additional financial cost is a useful, if unexpected outcome. The results emphasize the importance of conducting rigorously controlled studies of effects of leukoreduction in different patient populations before adopting the universal leukoreduction policy being considered by the U.S. Public Health Service.

Collier A, Kalish LA, Busch MP, Gernsheimer T, Assmann SF, Lane TA, Astmuth DM, Lederman MM, Murphy EL, Kumar P, Kelley M, Flanigan TP, McMahon DK, Sacks HS, Kennedy MS, and Holland PV: Leukocyte-reduced red blood cell transfusions in patients with anemia and human immunodeficiency virus infection: the viral activation transfusion study: a randomized controlled trial. The Journal of the American Medical Association 285: 1592-1601, 2001.

Hearing Aid Clinical Trial

Background: Sensorineural hearing loss is among the most prevalent disabilities reported in the U.S., affecting an estimated 20-26 million people and present in about 40 percent of individuals older than 65 years. It adversely affects cognitive and social function, as well as the general quality of life, and has been linked to depression and dementia. While hearing aids are the most widely used form of assistance, only about 20 percent of those who could benefit from hearing aids wear them. Moreover, surveys have suggested that about 50 percent of users are dissatisfied with their hearing aids, calling into question the benefit of hearing aids.

Advance: The NIH and the Department of Veterans Affairs (VA) recently completed a clinical trial to compare the efficacy of three different hearing aid circuits. Efficacy was measured in a variety of listening situations using tests of speech understanding, sound quality, and patient rank-order ratings. The three hearing aid circuits together account for 70 percent of the U.S. hearing aid market. Each circuit provided significant benefit in quiet and noisy listening situations.

Implications: The NIH/VA Hearing Aid Clinical Trial, the first large-scale double-blind, multicenter clinical trial, showed efficacy of hearing aids in both quiet and in noise for a wide range of individuals with hearing loss. Because concerted efforts were made to recruit patients into the study from both sexes and all racial groups, the study sample was a good representation of U.S. adults who are candidates for hearing aids.

Larson VD, Williams DW, Henderson WG, Luethke LE, Beck LB, Noffsinger D, Wilson RH, Dobie RA, Haskell GB, Bratt GW, Shanks JE, Stelmachowicz P, Studebaker GA, Boysen AE, Donahue A, Canalis R, Fausti SA, Rappaport BZ, and for the Participants of the NIDCD/VA Hearing Aid Clinical Trial Group: Efficacy of 3 commonly used hearing aid circuits: a crossover trial. The Journal of the American Medical Association 284: 1806-1813, 2000.

Abilities in Auditory Pitch Recognition are Largely Inherited

Background: Auditory pitch recognition is a complex process that allows us to determine the pitch or tone of a sound. This process relies on both the ear, which receives the sound signal traveling through the air, and the brain, which performs substantial processing of this signal to produce the pitch we perceive. Individuals with problems in pitch recognition are sometimes referred to as "tone deaf." Severe deficits in pitch recognition can be associated with speech and language disorders.

Advance: It was long known that tone deafness can run in families. However, it was unknown whether this disorder was due to genes inherited in these families, or to a common environment shared by family members. To answer this question, NIH scientists performed a large study of twins. They administered the Distorted Tunes Test, which measures people's ability to recognize incorrect notes in popular melodies, together with the American Academy of Otolaryngology's Five Minute Hearing Test questionnaire. Over 600 twins were tested, and the results show that on the Distorted Tunes Test, identical twins are much more alike in their scores than fraternal twins. Translated into numbers, about 70-80 percent of an individual's score is due to their genes, and 20-30 percent is due to their environment or other factors. In addition, individual's scores on the Distorted Tunes Test are almost completely independent of their scores on the Five Minute Hearing Test. This indicates that an individual's pitch recognition abilities are largely independent of their hearing.

Implications: The discovery that individual differences in pitch recognition are mostly genetic opens up the possibility of using genetic methods and the information from the Human Genome Project to find the genes essential for the pitch recognition apparatus. Identifying such genes and how they function will open a new window into the sound processing apparatus of the brain. It could also help our knowledge of speech and language disorders that are currently poorly understood and not easily treated.

Drayna D, Manichaikul A, de Lange M, Snieder H, and Spector T: Genetic correlates of musical pitch recognition in humans. Science 291: 1969-1971, 2001.

Characterization and Functional Classification of 15,000 Mouse Genes

Background: New technologies are providing answers to questions about how genes control cell and tissue function at an ever accelerating pace. Arrays of DNA for specific genes permit the comparison of tens of thousands of genes at one time to determine which are turned on or off in a particular cell or condition. A collection of 15,000 mouse genes has been developed, with emphasis on inclusion of genes active in placenta and embryo development. To facilitate extensive use of this gene collection in aging and other life science research, the set (currently named the "NIA mouse 15K cloned gene set"), has been made freely available as a resource to the scientific community. This collection has been distributed to more than 100 research institutions world-wide.

Advance: The use of this powerful biological tool can be enhanced by clearly defining the characteristics of individual genes in the collection. Nearly complete sequences of each gene in the 15K gene set are available. By comparing the sequence information with genes that have already been well studied, scientists may be able to determine the function of these genes in mice. So far, 4,027 genes have been assigned to biologically important functions: apoptosis (cell death), cell cycle, matrix/structural proteins, energy/metabolism, transcription/chromatin, protein synthesis/translational control, signal transduction, heat shock stress protein, and DNA replication.

Implications: The "NIA mouse 15K cloned gene set" contains many genes that are crucial for understanding biological processes in development and aging. All the information about individual genes has been provided to the community through the web site of the National Institute on Aging's Laboratory of Genetics (http://lgsun.grc.nia.nih.gov/). The site has received nearly 2 million hits in its first year, an indicator of the usefulness of the resources provided and the information generated through this study.

Kargul GJ, Dudekula DB, Qian Y, Lim MK, Jaradat SA, Tanaka TS, Carter MG, and Ko MSH: Verification and initial annotation of the NIA mouse 15K cDNA clone set. Nature Genetics 28: 17-18, 2001.

The TAPP Mouse: The First Link between Plaque and Tangle Formation

Background: A key feature of the pathology seen in Alzheimer's disease (AD) is the neurofibrillary tangle (NFT), which is made up of insoluble aggregates of the *tau* protein in brain neurons. Another key feature is the deposition of beta-amyloid (Aβ) in insoluble amyloid plaques outside brain cells. The Aβ fragment is formed by clipping it out of the much larger amyloid precursor protein (APP). Although many scientists believe that excess production of Aβ is the root cause of AD, it is still unclear how this causes other pathologies. In particular, scientists do not understand how excess production of Aβ leads to development of NFTs. To date, a number of transgenic mouse models of AD have been developed by inserting human mutated APP genes into mice. Amyloid plaques accumulate in the same brain regions of these mice that are affected in AD, but NFTs do not develop and there is no brain cell death. A model for tau pathology has recently been developed by inserting into mice the mutant gene for tau, recently associated with another form of dementia called familial frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17). This dementia attacks different regions of the brain from AD. The resulting tau transgenic mice developed NFTs with neuronal loss, in the regions affected in FTDP-17 but not in the regions affected in AD.

Advance: This study reports on the production of double transgenic mice (TAPP mice) resulting from the crossbreeding of the tau mutant mice with the APP mutant mice. These TAPP mice provide an opportunity to examine whether the distribution and/or timing of NFT development is influenced by mutations in APP. The TAPP mice were similar to the previously described mutant APP mice in the number and distribution of Aβ plaques in brain. NFTs were similar, chemically and structurally, to those described previously in the tau mutant mice. However, the distribution and number of NFTs was increased in female TAPP mice compared to their tau mutant parents; there were a higher number of NFTs in regions of brain vulnerable in AD in the TAPP mice. The results suggest that APP or A_ peptide can influence the regional formation of NFTs.

Implications: The neuropathology observed in female TAPP mice indicates that either APP or A_ induces the development of NFTs in regions of brain vulnerable in AD. This is the first animal model in which the elusive connection between amyloid pathology and tangle formation can be investigated. This improved animal model for AD may also be critical for success in developing therapies against NFT formation and the death of neurons in AD brain.

Lewis J, Dickson DW, Lin WL, Chisholm L, Corral A, Jones G, Yen SH, Sahara N, Skipper L, Yager D, Eckman C, Hardy J, Hutton M, and McGowan E: Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP. Science 293: 1487-1491 2001.

Detecting Lead Using DNA

Background: Lead poisoning is the number one environmental hazard to American children(1). Nearly 1 million children in the U.S. have enough lead in their blood to cause irreversible damage to their brains, nervous systems, kidneys, or reproductive organs (2). At higher levels, lead poisoning can cause coma, convulsions, and death. Most of these children absorb lead into their blood from sources in and around their own homes, such as old paint and contaminated dust and soil. Current methods for detecting lead require sophisticated scientific equipment or complicated sample preparation.

Advance: Last year, two chemists developed a simple, inexpensive method to detect and measure lead in the environment. The technique harnesses DNA, the stuff of genes, as a biosensor that literally glows in the dark when it contacts the metal. The scientists designed a specific strand of DNA that twists into a pocket tailor-made to capture lead. The method is extremely sensitive and can measure quantities of the metal over a wide range of concentrations.

Implications: Because DNA is stable and can easily be used in optical fibers and microchips, the technique might be used not only to detect household lead, but also on a larger scale to monitor lead in wastewater and industrial processes. The researchers also state that by varying the DNA and other chemicals, they can modify the technique to detect other metals, including those that are toxic, such as mercury and cadmium, and those that are beneficial to humans, such as calcium and potassium. Finally, the work will teach scientists more about the sequences and shapes of DNA that normally bind metals in the body. The researchers have applied for a patent on the work.

Li J, and Lu Y: A highly sensitive and selective catalytic DNA biosensor for lead ions. Journal of the American Chemical Society 122: 10466-10467, 2000.

Model for Benzene Induction of Leukemia and/or Lymphoma

Background: Benzene is an important chemical compound with many uses in chemistry and production of goods as well as fuels for power. However, due to such large-scale manufacture and use of petroleum-based products containing benzene, this compound has become ubiquitous in our environment. Long-term exposure to benzene is associated in humans with the development of leukemia and lymphoma. Understanding benzene toxicity has been hampered by the lack of rodent models to investigate its toxicity, complex metabolism, and identification of the by-products that are toxic and may cause leukemia or lymphoma (blood diseases that are continuing to increase in our population).

Advance: Investigators used deficiencies or overexpression of genes (oncogenes and tumor suppressor genes) known to be involved in human cancers, to show that low levels of exposure to benzene may cause leukemias and/or lymphomas in rodents. This research, then, provides a viable model for studying the cancer-causing abilities of benzene and developing mechanisms for circumventing its effects.

Implications: Having a suitable model to act as a surrogate for human exposure will enhance our ability to determine both the mechanism of the cause of the cancer and what levels of exposure must be avoided in order to reduce the risk to humans.

French JE and Saulnier M: Benzene Leukemogenesis: an environmental carcinogen induced tissue specific model of neoplasia using genetically altered mouse models. Journal of Toxicology and Environmental Health 61: 377-379, 2000.

Healy LN, Pluta LJ, James RA, Janszen DB, Torous D, French JE, and Recio L: Induction and time-dependent accumulation of micronuclei in peripheral blood of transgenic p53 +/- mice, Tg.AC (v-Ha-ras) and parental wild-type (C57BL/6 and FVB/N) mice exposed to benzene by inhalation. Mutagenesis 16: 163-168, 2001.

Functional Analysis of Mutations in the p53 Tumor Suppressor Gene

Background: The p53 gene is required for regulating many cellular responses to environmental stress, particularly DNA damage. While p53 mutations are commonly associated with tumors, there has been limited opportunity to characterize them and to define the functional consequences of the mutations.

Cancer development is a multistage process involving the inappropriate activation of normal cellular genes to become oncogenes (i.e., genes that normally play a role in the growth of cells but, when over expressed or mutated, can foster the growth of cancer) and the inactivation of other cellular genes called tumor suppressor genes. p53 is the prototypic tumor suppressor gene that is well suited as a molecular link between the causes of cancer, i.e., carcinogenic chemical and physical agents and certain viruses, and the development of clinical cancer. The p53 tumor suppressor gene is mutated in the majority of human cancers. Genetic analysis of human cancer is providing clues to the etiology of these diverse tumors and to the functions of the p53 gene.

Advance: Using yeast as an in vivo test tube, an exquisitely sensitive system has been developed that provides for rapid analysis of subtle changes in p53 function.

Implications: These approaches will be useful in developing therapy strategies as well as evaluating the p53 gene as a target of environmental damage.

Inga A, Monti P, Fronza G, Darden T, and Resnick MA: p53 mutants exhibiting enhanced transcriptional activation and altered promoter selectivity are revealed using a yeast-based functional assay. Oncogene 20: 501-513, 2001.

Inga A, and Resnick MA: Novel human p53 mutations that are toxic to yeast can enhance transactivation, of specific promoters and reactivate tumor p53 mutants. Oncogene (in press 2001).

New Technique Could Enhance Practical Applications of Genetics

Background: The information being generated from the Human Genome Project has tremendous applications in identifying defective environmental response genes that confer disease risks, in screening environmental agents for their ability to alter genetic functions, and for identifying critical molecular components that ensure genetic stability in humans. Unfortunately, current assay techniques are cumbersome and time consuming, delaying our ability to reap the full benefits from the Human Genome Project.

Advance: A new technique has been developed in yeast which has great promise in providing a rapid assessment of large volumes of genes. Rather than traditional, site-directed methods that involve considerable cloning and DNA sequencing, the *delitto perfetto* system provides opportunities to conveniently modify natural chromosomes, cloned human genes, or even yeast artificial chromosomes containing large segments of human DNA. Changes are accomplished by simply adding easily designed genetic material, oligonucleotides, to cells that contain an appropriate DNA cassette in the region to be mutated and counterselecting for loss of the cassette.

Implications: Rapid screening of large quantities of genetic materials are possible with this system. It would allow high throughput analysis of gene function, identification of consequences of disease gene defects, and gene modification to address mechanisms of genome stability.

Storici F, Lewis LK, and Resnick MA: *In vivo* site-directed mutagenesis using oligonucleotides. Nature Biotechnology 19: 773-776, 2001.

Database Developed for Handling and Providing Initial Analyses of Microarray Data

Background: A major challenge facing scientists in today's environmental health, chemical, and pharmaceutic research communities is the development of new approaches for the identification of hazards found in the environment or associated with chemicals and drug exposures. Traditionally, toxicologists have used rodent bioassays to identify potentially hazardous substances including carcinogens, reproductive toxins, immunotoxins, and neurotoxins. These assays often require high doses, take years to complete, and are expensive. Consequently, only a limited number of compounds have been tested. DNA microarray technology is an innovative tool used to monitor the expression levels of thousands of different genes at a time, thereby condensing months of painstaking laboratory tasks into a day's work. This tool is an important development because it is a more effective way to identify environmental hazards and their effects on DNA. Sorting and analyzing vast quantities of data generated using microarray methods is difficult. In the coming age of high-throughput genomics analysis using microarray technology, scientists will rely heavily on robust data systems and statistical procedures to assist in the organization and interpretation of results.

Advance: MAPS (MicroArray Project System), developed for the management and interpretation of microarray gene expression experiment information and data, is a functional database used for storing, compiling, and analyzing microarray results. This relational database features a web interface to access DNA microarray project information, validate replicate gene expression experimental results, and query gene expression data based on gene classifications of interest. The development of this database has been key in a project where the goal is to validate the "toxicogenomic strategy" by demonstrating that compound classification and correct prediction of unknowns can be accomplished using gene expression arrays.

Implications: MAPS is a platform for scientists to not only manage information and analyze microarray gene expression data but also proceed toward discovering heuristic knowledge from biological information and data.

Bushel PR, Hamadeh H, Bennett L, Sieber S, Martin K, Nuwaysir EF, Johnson K, Reynolds K, Paules RS, and Afshari CA: MAPS: a microarray project system for gene expression experiment information and data validation. Bioinformatics 17: 564-565, 2001.

Developing Tools for Toxicogenomics: a DNA database for *Xenopus laevis*

Background: *Xenopus laevis*, the South African clawed frog, has been an important experimental animal for decades that is used for the study of early development. Its developing embryos are also frequently used in toxicology research to study the effects of chemical and other exposures on the developing organism. *Xenopus* was chosen by NIH as one of five important non-mammalian models of human development and disease; one of the highest priority recommendations from a recent workshop on *Xenopus* research needs was the development of databases with information on the organism's genetic sequences, especially those genes important in development.

Advance: To facilitate analysis of gene expression patterns in normal development and development perturbed by toxins, these investigators created and sequenced a large collection of expressed sequence tags, or ESTs, from unfertilized *Xenopus* eggs. ESTs are short pieces of DNA that are used as landmarks in genome mapping. The collection of ESTs generated and characterized in these studies represents much of the mother frog's genetic contribution to the first several hours of development. This collection has formed the basis for ongoing efforts to use "gene-chip" analysis in the *Xenopus* model to evaluate subtle effects of environmental toxins on development.

Implications: Collections of *Xenopus* ESTs, reflecting information on gene expression in this important model system, will serve as a vitally important resource for future efforts in gene discovery, development of transgenic models, gene-chip analyses of normal and perturbed development, and other critical efforts in developmental biology and developmental toxicology.

Blackshear PJ, Lai WS, Thorn JM, Kennington EA, Staffa NG Jr, Moore DT, Bouffard GG, Beckstrom-Sternberg SM, Touchman JW, Bonaldo MF, and Soares MB: The NIEHS *Xenopus* maternal EST project: interim analysis of the first 13,879 ESTs from unfertilized eggs. Gene 267: 71-87, 2001.

New Insight into Diseases of Aging Possible with Fluorescent Nanosensors

Background: Scientists are now thinking that many of the diseases of aging are caused by damage resulting from reactive oxygen species (ROS) derived from a process known as oxidative stress. Just as iron rusts in the presence of oxygen, so can oxygen cause formation of charged particles, called free radicals, that interact with and damage important cellular components like the energy-producing mitochondria. Environmental toxicants are also suspected of setting up conditions for oxidative stress and, in fact, understanding oxidative stress is a fast growing field in environmental health sciences.

It is generally accepted that the cellular supply of oxygen is largely controlled by the flow of oxygenated blood. Due to the lack of sensitive and accurate techniques for the measurement of intracellular oxygen in mammalian cells, however, little is known about the distribution of oxygen inside the actively respiring ("breathing")cell. Active mitochondria consume large amounts of oxygen and compete with enzymes such as monooxygenases for the limited supply in the body. The likelihood that there may be "hypoxic (low oxygen) regions" in the cell may, therefore, be quite reasonable.

Advance: Scientists recently developed sensitive oxygen measuring devices using nanotechnology. Nanotechnology refers to a new field that creates instruments so small that they exist on the size level of a nano, or one billionth, unit. The recent development of oxygen sensing fluorescent nanoprobes may hold the key to mapping alterations in the intracellular distribution of oxygen in cells challenged by toxic chemicals. The utility of these oxygen nanosensors has been demonstrated in cultures of cancer cell lines and will shortly be extended to studies of toxicant-induced oxidative stress in other types of cells – primary cultures of astrocytes and neurons from rodent brains.

Implications: The new generation of oxygen sensors augment the existing array of nanoprobes developed by these researchers. These probes are actively used in understanding mechanisms of mitochondrial dysfunction following exposure to clinically and environmentally relevant chemicals that produce central nervous system damage. Real-time measurement of oxidative stress is a major breakthrough which could lead to a better understanding of the development of cardiovascular diseases, aging, and neurodegenerative diseases.

Brasuel M, Kopelman R, Miller TJ, Tjalkens R, and Philbert MA: Fluorescent nanosensors for intracellular chemical analysis: decyl methacrylate liquid polymer matrix and ion-exchange-based potassium PEBBLE sensors with real-time application to viable rat C6 glioma cells. Analytical Chemistry 73: 2221-2228, 2001.

Tissue Engineered Repairs of Articular Cartilage

Background: Articular (joint) cartilage injuries are frequent, surgically challenging, and despite the best treatment, sometimes progress to end-stage osteoarthritis. The latter relates to the inability of articular cartilage to repair itself. Although many treatments have been proposed and are currently in use, tissue engineering, the use of living cells with or without a biodegradable scaffold (lattice-work to support the growing tissue), shows great promise in the treatment of this injury. Although much of the tissue-engineered cartilage in existence has been successful in mimicking the morphological and biochemical appearance of articular cartilage, it is generally mechanically inferior to the natural tissue.

Advance: Given that articular cartilage is a load-bearing tissue, the purpose of this study was to validate the hypothesis that a physiological environment is paramount to producing a tissue that is able to perform the load-bearing and lubrication function of normal articular cartilage. To do this, the investigators compared the material and biochemical characteristics of tissue-engineered articular cartilage grown with or without exposure to deformational loads at normal physiological levels. A custom-designed bioreactor (analogous to an incubator) was used to grow the cartilage constructs in both groups for 4 weeks. The cartilage constructs exposed to mechanical loading had better material properties (stiffness, etc.) than those grown without loading. In addition, those exposed to loading had greater quantities of normal constituents (such as substances called glycosaminioglycans) of articular cartilage.

Implications: Tissue-engineered repairs of articular cartilage injuries hold potential promise. These findings highlight the potential importance of mechanical loading in the successful repair of these injuries. Further research is needed to elucidate the specific loading-induced stimuli to which chondrocytes respond and to optimize bioreactor conditions and loading parameters to achieve a functional articular cartilage substitute.

Mauck RL, Soltz MA, Wang CCB, Wang DD, Chao PHG, Valhmu WB, Hung CT, and Ateshian GA: Functional tissue engineering of articular cartilage through dynamic loading of chondrocyte-seeded agarose gels. Journal of Biomechanical Engineering 122: 252-260, 2000.

Chronic Wounds: Epidemiology and Treatment

Background: Chronic wounds are a major public health problem. They particularly affect the elderly and people with certain predisposing diseases, such as diabetes mellitus. Studies of drugs meant to influence and improve wound healing are difficult to do because the FDA requires complete wound closure as the endpoint. This is not only often difficult to achieve but also can take many months. Validation of markers that appear earlier in the course of wound treatment and correlate with an eventual successful result would improve our ability to conduct such studies. Similarly, epidemiologic studies of chronic wounds that identify predisposing factors would allow identification of higher risk individuals. Changes in routine care for these individuals could prevent the development of some of these chronic wounds and, therefore, prevent this major health impact.

Advance: A meta-analysis (an analysis of a number of already published articles in which the data are combined to produce more statistically significant results) of diabetic foot ulcers and a multicenter study of venous leg ulcers demonstrated that the standard care would result in wound healing if the wounds were small or of brief duration. Large or long standing wounds were not likely to heal within the twenty week time period. In another study of diabetic foot ulcers, individuals treated with a living skin substitute, Graftskin, showed a statistically significant increase in wound healing and decrease in time to complete closure. Adverse reactions were similar in both groups except that osteomyelitis (infection of the underlying bone) and lower-limb amputation, major adverse results of chronic ulcers, were less frequent in the Graftskin group. In a preclinical study, the use of gene transfer of growth factors incorporated into viral vectors (modified viruses used to carry the gene into the skin) increased granulation tissue (the first tissue response in the wound healing process), blood vessel formation and skin covering of the wound, all indications of enhanced wound healing, were achieved.

Implications: These large scale clinical trials and meta-analyses of prior trials indicate that it should be possible to predict the outcome of wound healing studies without waiting for complete closure of the wounds. This should shorten the trials and save money. In addition, they demonstrate the importance of selection of patients when trying to distinguish between those individuals who can be put on standard conservative therapy and those for whom newer or more aggressive treatments should be considered. Newer technologies such as artificial skin equivalent systems can improve the success rate and the rate of healing of existing wounds and minimize or reduce the incidence of severe complications.

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Phillips TJ, Machado F, Trout R, Porter J, Olin J, Falanga V, and The Venous Ulcer Study Group: Prognostic indicators in venous ulcers. Journal of the American Academy of Dermatology 43: 627-630, 2000.

Veves A, Falanga V, Armstrong DG, and Sabolinski ML: Graftskin, a human skin equivalent, is effective in the management of noninfected neuropathic diabetic foot ulcers: a prospective randomized multicenter clinical trial. Diabetes Care 24: 290-295, 2001.

Progress Toward Gene Therapy of Genetic Diseases of Bone

Background: Genetic diseases are caused by chemical alteration, or mutation, of genes. Because most genes specify the structures of proteins, a mutated gene usually either fails to produce any protein at all, or produces an abnormal protein. Osteogenesis imperfecta, one of the most common genetic diseases of bone, can arise from mutations of either type, in the gene for a protein called type I collagen. Often, an abnormal collagen protein causes more severe disease than the simple absence of the protein. In order to correct mutations that result in a deficiency of collagen, it is necessary to develop methods of introducing normal collagen genes into cells that can be permanently established in bone by transplantation. Correction of mutations that produce abnormal proteins requires the development of molecular agents that can inactivate the mutant gene. In either case, it is important that production of the introduced molecule is efficient, but restricted to bone cells.

Advance: Investigators have recently reported progress in both the controlled introduction of genes into bone cells and the design of agents for inactivating disease-causing mutant genes. First, they have shown that a genetically modified virus can carry a special version of the type I collagen gene into bone cells of mice. Importantly, the virus-borne gene was incorporated into the genetic material of bone cells such that it was passed on when the cells divided. Also, the protein specified by the introduced gene was produced in bone cells but not other cells, just as a normal collagen is. In a second line of work, investigators have shown that it is possible to block the production of a protein specified by a particular gene, by introducing a modified version of a naturally occurring molecule called U1 snRNA. Normally, U1 snRNA participates in the processing of messenger RNAs, which are necessary intermediates in the production of a protein. But with specific modifications of its structure, U1 snRNA instead interferes with messenger RNA processing, reducing the amount of protein produced. Most importantly, this effect can be targeted to a particular gene. In the reported work, using genes chosen for easy measurement of their protein products, investigators showed better than 90 percent reduction in protein production by the target genes.

Implications: These results show that it is possible to introduce a gene such that its specified protein is produced only in bone cells, and that it is possible to block the production of proteins specified by genes already present in the cells. Some genetic diseases may be treated simply by introducing a gene for a missing protein. Further, by combining these techniques, it may be possible eventually to block the production of the abnormal proteins specified by some mutant genes. These approaches could apply to many genetic diseases of bone, including osteogenesis imperfecta.

Stover ML, Wang CKL, McKinstry MB, Kalajzic I, Gronowicz G, Clark SH, Rowe DW, and Lichter AC: Bone-directed expression of col1a1 promoter-driven self-inactivating retroviral vector in bone marrow cells and transgenic mice. Molecular Therapy 3: 543-550, 2001.

Beckley SA, Liu P, Stover ML, Gunderson SI, Lichtler AC, and Rowe DW: Reduction of target gene expression by a modified U1 snRNA. Molecular Cell Biology 21: 2815-2825, 2001.

A Natural Resistance to Memory

Background: Certain proteins and processes in brain cells are critical to the formation of memories. Reciprocally, there also are proteins that actually put a brake on learning and memory. One purpose of having a brake on memories could be to keep the background levels low so that only important memories are stored. One candidate protein acting as a brake on memory formation is calcineurin. It is thought that calcineurin antagonizes protein kinase A (PKA), a protein shown to be critical in proper memory formation and in processes, such as long term potentiation, or LTP, involved in learning and memory formation. Advances in technology that allow researchers to not only insert genes prenatally, but to reversibly turn the genes on or off at will, recently permitted novel studies of calcineurin in the mouse.

Advance: If calcineurin is a brake on memory, inhibiting its actions should improve memory. Researchers engineered a calcineurin inhibitor that would be turned on when the animal was given an antibiotic and turned off by removing the same antibiotic. First, they clearly showed that the calcineurin inhibitor was appropriately located in areas of the brain important in learning and memory – the hippocampus and cortex. Next, they showed that stimulation of brain cells, in slices of hippocampus as well as in the intact brain, led to increases in LTP. They further showed that LTP was greatest in animals with the calcineurin inhibitor turned on (indicating the calcineurin is acting as a brake). Calcineurin seemed to directly oppose, or block, a particular learning pathway involving PKA. In behavioral studies of short-term memory, the calcineurin-inhibited mice were better able to recognize when a familiar object had been relocated to a new position, and they were better at recognizing when a familiar object had been replaced with a novel object. In tests of intermediate and long-term memory, the calcineurin-inhibited mice indicated a robust memory for familiar objects; memories lasted more than a week longer than those of "normal" mice that did not have the active inhibitor, or than of mice without the antibiotic. Finally, when tested on their spatial learning and memory ability in a task that required learning and relearning the location of a submerged platform, the calcineurin-inhibited mice were superior performers compared with the control mice and with mice "off" the antibiotic.

Implications: Although pharmaceutical development targeting calcineurin remains a distant goal, the study has therapeutic implications for enhancing memory in individuals who suffer from age-related memory loss or memory loss due to diseases such as Alzheimer's. Cumulative knowledge gained from gene-manipulation studies ultimately may elucidate all critical memory-related proteins and their functions; at which point researchers can develop and target drugs to specific sites in order to enhance memory-related functions.

Malleret G, Haditsch U Genoux D, Jones MW, Bliss TVP, Vanhoose AM. Weitlauf C, Kandel ER, Winder DG, and Mansuy IM: Inducible and reversible enhancement of learning, memory, and long-term potentiation by genetic inhibition of calcineurin. Cell 104: 675-686, 2001.

New Technique Identifies Gene Abnormalities in Schizophrenia

Background: Schizophrenia is a serious disorder of thought and cognition, affecting approximately 1 percent of the population. The age of illness onset is typically in the late teens or early 20's. Clinical symptoms include delusions, hallucinations, and disorganized thought, as well as impaired motivation and decreased emotional expression, leading to employment difficulties and problems in maintaining relationships with friends and family. Most clinicians and scientists agree that a combination of genetic and environmental factors contributes to the disorder. Twin and family studies of schizophrenia have provided strong support for the genetic basis of the disorder by showing that the risk of acquiring the disease increases greatly with increasing familial relatedness to an affected individual. Abnormalities in certain brain regions, particularly the prefrontal cortex (PFC), have been implicated in schizophrenia. But, it has been difficult and time consuming to identify specific genes (or gene products, i.e., proteins) in discrete brain regions that are involved in the disorder.

Advance: A relatively new technique called *microarray analysis* allows for hundreds to thousands of genes in a given brain area to be studied at one time. The technique discerns differences in levels of gene expression between control or "normal" brain samples and, in this case, schizophrenic brain samples (post-mortem). NIH researchers used microarray analysis to assess several thousand genes in order to determine if modest, but consistent changes in gene levels (or expression) occur in the PFC of schizophrenic subjects. Interestingly, they determined that 2.6 percent of all genes examined in schizophrenic subjects were expressed at higher levels and 2.2 percent were expressed at lower levels than in the matched control subjects. Furthermore, they identified a specific group of genes (*PSYN* genes) that were significantly decreased. *PSYN* genes (which lead to the production of proteins involved in the regulation of presynaptic function) are known to be important in communication between brain nerve cells – they are critical to the proper release of chemical messengers from neurons. Three specific *PSYN* genes, called *NSF*, *SYN2*, and *ATPase* were of the most consistently changed in schizophrenic subjects. Three other *PSYN* genes, *STAG5*, *SJAN1*, and *SGYR1* were identified as occasionally changed. Further analysis of these genes by another technique, called *in situ* hybridization (which allows one actually to visualize the expression of a gene in brain tissue) suggests that individual schizophrenic subjects have distinct but consistent patterns of *PSYN* gene decreases. On average, levels of *NSF*, *SYN2* and *STAG5*, *SJAN1* consistently decreased by approximately 40 percent, 37 percent and 16.2 percent and 14.2 percent respectively; but, each schizophrenic subject was distinct with respect to the amount of change in a given gene, showing anywhere from a 4.5 percnet to 54 percent decrease in PSYN levels. Antipsychotic medication (haloperidol) did not, on its own, decrease gene expression in subjects who had received treatment for schizophrenia.

Implications: Because schizophrenia is a complex disease, it is difficult to identify any one gene or gene product involved in its pathology. These data not only support the theory that schizophrenia is a disorder of functionally related groups of genes, but it also specifically identifies genes potentially involved in the disease. This apparent identification of a group of genes (*PSYN*) involved in schizophrenia is a major breakthrough for future research and ultimately for improved therapeutics.

Mirnics K, Middleton FA, Marquez A, Lewis DA, and Levitt P: Molecular characterization of schizophrenia viewed by microarray analysis of gene expression in prefrontal cortex. Neuron 28: 53-67, 2000.

Enhancing Reasoning with Transcranial Magnetic Stimulation (TMS)

Background: Just as brain imaging technology enables physicians and scientists to see the structure of the brain and monitor its activity, transcranial magnetic stimulation (TMS) allows the non-invasive activation or inhibition of brain areas. TMS relies on the principle, from basic physics, that a changing magnetic field induces an electrical field. Thus, a device that produces a precisely controlled magnetic field can painlessly induce an electrical field through the skull in the brain tissue below. By choosing appropriate intensity and repetition rates, the TMS device can either activate or inhibit nerve cells in the brain. TMS was introduced in Great Britain about 15 years ago. Clinicians have used the technology for diagnosing the integrity of conduction along nerve pathways from the brain. In recent years, TMS is increasingly finding application as a tool, complementing brain imaging, for studying how the brain functions and potentially for treatment.

Advance: Brain imaging studies have shown that an area of the cerebral cortex called the left dorsolateral prefrontal cortex (PFC) is active when people perform tasks that require abstract reasoning. However, whether the PFC plays a critical role in the reasoning, or is simply incidentally activated by other brain areas, is not known. To address this issue, researchers asked volunteers to perform puzzles requiring abstract inferences about groups of geometric shapes. Repetitive TMS stimulation using settings that were not quite sufficient to activate the PFC, but enough to enhance its excitability, improved the performance of volunteers on the puzzle tasks. Stimulation of other brain areas did not improve task performance, so these results strongly suggest that the PFC is functionally important for abstract reasoning. This new evidence nicely complements the results from imaging studies and inferences from the behavior of people who have damage to this area of the brain.

Implications: This study is just one example of many that show how activating or inhibiting brain areas with TMS can help understand the human brain. Other TMS studies have examined brain plasticity, that is, how the brain adjusts in health and disease. For example, the area of the cortex that represents the finger shrinks dramatically when a proficient Braille reader takes a 10 day holiday from reading. Some patterns of repetitive TMS stimulation can produce brain effects that far outlast the stimulation itself. For this reason, researchers are exploring the use of TMS as therapy for problems as diverse as depression and improving recovery from brain damage.

Boroojerdi B, Phipps M, Kopylev L, Wharton CM, Cohen LG, and Grafman J: Enhancing analogic reasoning with rTMS over the left prefrontal cortex. Neurology 56: 526-528, 2001.

Stem Cells Show Promise for Treating Diabetes

Background: Transplantation of pancreatic cells may be feasible for treating diabetes, and clinical trials are underway to test this procedure. However, the difficulty of obtaining cells for transplant would limit the availability of this treatment to relatively few people. Scientists recently noticed that some pancreatic cells make a protein, called nestin, that is a hallmark of developing nerve cells. Researchers trying to make nerve cells from stem cells have developed cell culture methods and chemical signals that lead mouse embryonic stem cells to make nestin, so they reasoned that adapting those procedures might coax stem cells to take on characteristics of pancreatic cells.

Advance: Beginning with what had been learned from experiments to make nerve cells from stem cells, scientists developed procedures that led mouse embryonic stem cells to form cells with characteristics of pancreatic cells. The procedures depend on the cell culture conditions and the supply of certain growth factors at appropriate times. Not only do these cells make insulin and other pancreatic endocrine hormones, they also assemble themselves into specialized structures, with multiple cell types, similar to the islets of Langerhans of the normal pancreas. When injected in to diabetic mice, the cells survived and maintained this organization.

Implications: The pancreas-like cells formed from stem cells survived and maintained their structural organization when transplanted into animals. However, these cells made only about 2 percent of the normal amount of insulin, not yet enough to cure diabetes. Still, this is a promising first step towards developing a practical treatment. Further refinements or drugs are likely to increase insulin output in the future. More generally, these experiments illustrate the versatility of embryonic stem cells and the unanticipated benefits are likely to follow from better understanding of the signals that control the fate of stem cells.

Lumelsky N, Blondel O, Laeng P, Velasco I, Ravin R, and McKay R: Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. Science 292: 1389-1394, 2001.

Pesticides and Parkinson's Disease

Background: Parkinson's disease is a neurodegenerative disorder that progressively affects the control of movement. The symptoms include tremor at rest, bodily rigidity, marked slowness and paucity of movement, postural changes, gait disturbances, and difficulty initiating voluntary movement. These problems reflect the death of nerve cells that help control movement, especially cells that lie in a brain region called the substantia nigra and make the neurotransmitter dopamine. In about 10 percent of cases, this disorder is inherited, but what triggers the dopamine cells to die in most people with this disease is not known. Epidemiological evidence has suggested an association with environmental toxins may be a contributing factor.

Advance: Scientists have demonstrated that chronic administration of the common pesticide rotenone to rats causes a syndrome that is very similar to Parkinson's disease. Behaviorally, the rats exhibited slowness of movement and rigidity quite like the human disorder. Rotenone, like Parkinson's, was highly selective in killing nerve cells in the substantia nigra that make dopamine. The detailed pathological effects on cells and molecules were also very reminiscent of the human disorder.

Implications: These results have important implications on several levels. Most obviously, the findings reinforce the importance of examining the contribution of environmental toxins to triggering Parkinson's disease, and studies are underway. On a practical level, rotenone offers a new animal model to help understand how Parkinson's disease progresses and to test treatment strategies. The model complements those arising from genetic manipulations and may help scientists study the slow progression of the disease and test interventions. Finally, because we know how rotenone affects cells, we can infer clues about the processes that underlie Parkinson's disease. In particular, rotenone apparently acts on cells by interfering with the proper functioning of mitochondria, the energy factories of the cell, with the consequent generation of damaging chemicals called free radicals. Several other lines of research implicate improper functioning of mitochondria and free radicals in Parkinson's and other neurodegenerative disorders. So, further studies of rotenone and similar chemicals may help us understand the mechanisms of how this disease progresses and provide further targets for development of drug therapies to slow the disease.

Betarbet R, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov AV, and Greenamyre JT: Chronic systemic pesticide exposure reproduces features of Parkinson's disease. Nature Neuroscience 3: 1301-1306, 2000.

Gene Microarray Analysis of Brain Tumors

Background: Glia, the supporting cells of the brain, outnumber nerve cells about ten to one. Gliomas are the most common brain tumors. Despite increasing progress against many types of tumors, success in treating gliomas remains elusive, largely because these cells invade surrounding brain tissue and evade surgical attempts to remove them. For cancer biologists, studying which genes are more or less active in tumor cells compared with healthy cells provides important clues about why tumor cells grow and invade tissue in a harmful way. Most previous studies of gene expression in gliomas have focused on only a few genes at a time, but thousands of genes might play critical roles and interactions among genes may be important.

Advance: Scientists have now used gene microarray technology to analyze gene activity in brain tumors. These new methods enabled researchers to simultaneously compare the activity of 11,000 genes in gliomas and normal brain tissue. They examined tissue from very aggressive gliomas (glioblastoma multiforma and anaplastic astrocytes), low grade gliomas (astrocytomas) and a type of benign brain tumor (meningiomas) with normal human brain tissue from trauma patients. Microarray experiments produce a wealth of data so a few highlights will serve to illustrate. All of the glioblastomas overexpressed 14 known genes from two broad functional groups: growth factor related genes and genes related to structure or the extracellular matrix which surrounds brain cells. Two genes were heavily expressed in high and low grade gliomas, as well as in tissue surrounding these tumor cells. One of these codes for a receptor by which cells respond to a growth factor called EGF (epidermal growth factor). The other codes for a component of laminin, a protein that is part of blood vessel membranes and may be important for the formation of new blood vessels that supply a tumor and its progression. The study also followed patients and showed that the clinical course of the tumors were correlated with expression profiles of these and several other critical genes.

Implications: Tumors with the same diagnosis can vary greatly in prognosis and in response to therapy. Developing gene expression profile markers will help improve diagnostic prediction and help physicians find the optimal treatment strategies for each patient. The gene expression profiles also provide important clues for understanding why tumors develop, progress, and invade surrounding tissue, and may yield new targets for therapies to stop them.

Ljubimova JY, Lakhter AJ, Loksh A, Yong WH, Riedinger MS, Miner JH, Sorokin LM, Ljubimov AV, and Black KL: Overexpression of alpha-4 chain containing laminins in human glial tumors identified by microarray analysis. Cancer Research 61: 5601-5610, 2001.

Understanding Functional Magnetic Resonance Imaging (fMRI) of the Brain

Background: Advances in the technology of brain imaging have revolutionized treatment of brain disorders and research to understand how the brain works. Functional magnetic resonance imaging (fMRI) reveals not just the structure of the brain but also which areas of the brain are most metabolically active – that is, using the most energy – when people do different mental or behavioral tasks. However, interpretation of these images has been ambiguous. Some nerve cell connections excite, or electrically activate, other parts of the brain, while other nerve cells inhibit, or reduce the activity of other brain areas; but, both types of connections require energy. So, it has been difficult to know whether metabolic changes evident in fMRI images reflect excitatory or inhibitory brain processes.

Advance: Scientists have now found strong evidence that the activation seen with fMRI is more likely to reflect excitation than inhibition of nerve cells in the cerebral cortex. Volunteers performed a simple "go/no go" task in which they had to quickly extend or not extend their thumb depending on which of two tones sounded. Using a technique called transcranial magnetic stimulation (TMS), researchers determined that the area of the cerebral cortex that controls movement of the thumb was excited during the go and inhibited during the no-go episodes of the task. TMS is a noninvasive, painless method for activating or inactivating parts of the brain from outside the skull based on the physical principle that a magnetic field induces an electric field. The evaluation of fMRI brain images made while the volunteers performed the task allowed researchers to evaluate the contribution of inhibition and excitation to the fMRI activation measurements.

Implications: Physicians and research scientists are using fMRI increasingly to monitor brain activity during health and disease. A better understanding of how brain physiology is reflected in fMRI measurements will help make this technology more useful.

Waldvogel D, van Gelderen P, Muellbacher W, Ziemann U, Immisch I, and Hallett M: The relative metabolic demand of inhibition and excitation. Nature 406: 995-998, 2000.

Brain Images of Children Prenatally Exposed to Methamphetamine Reveals Alteration in Brain Chemicals

Background: Methamphetamine is a highly addictive stimulant whose use has reached epidemic proportions in certain regions of the country. Anecdotal reports suggest that some pregnant women may have the perception that this drug is not dangerous. However, data are limited regarding the effects of prenatal methamphetamine exposure on the developing central nervous system. Thus, the health and developmental consequences of prenatal exposure to methamphetamine and its analogs such as MDMA (ecstasy) needs to be more closely examined and the information shared widely.

Advance: Through the first study to use noninvasive neuroimaging techniques in children exposed to methamphetamine in utero, researchers detected neurochemical alterations in the children's brains. The researchers compared two groups of children who were approximately eight years of age. Twelve of the children were exposed to methamphetamine prior to birth and 14 control subjects had no history of exposure to drugs. Children in both groups received a magnetic resonance imaging (MRI) scan and a more localized non-radioactive test that is typically used to study normal brain development and a variety of brain injuries and diseases in children. The researchers found that the children exposed to drugs in utero had increased creatine levels (10 percent) in the two brain regions examined. Creatine is a brain chemical that provides cells with energy to function properly. Thus, it is a marker that researchers use to determine how well a cell is functioning. The significance of the increased creatine levels is not completely clear, but it may imply that the brain is overcompensating for possible damage that methamphetamine may have caused. There were no reported differences in structural or behavioral abnormalities in the methamphetamine-exposed children.

Implications: Neuroimaging of children exposed to methamphetamine in utero has revealed increases in creatine in areas of the brain associated with executive functioning, the ability to perform complex tasks. Therefore, there is a chance that these children will have learning and memory deficiencies. These data about the possible effects of methamphetamine on the developing brain need to be further researched, but current messages about the dangers of methamphetamine need to include information about the possible effects on the unborn child.

Smith LM, Chang L, Yonekura ML, Grob C, Osborn D, and Ernst T: Brain proton magnetic resonance spectroscopy in children exposed to methamphetamine in utero. Neurology 57: 255-260, 2001.

New Technology May Help Increase the Accuracy of Determining Prenatal Drug Exposure

Background: It is difficult to determine with accuracy the prevalence of drug use by pregnant women. Although each year in the U.S. approximately 5.5 percent of, or 221,000, pregnant women report using an illicit drug at least once during their pregnancy, this number is likely an underestimate. For a variety of reasons, including the fact that pregnant women may fear the consequences to themselves or their child of admitting to the use of an illegal substance, as well as the inaccuracy of recall by the women when reporting the drugs used, determining prevalence of drug use is problematic among this population. Since there are numerous early interventions that can be used to help children prenatally exposed to drugs who may suffer subtle learning deficits among other ailments, it is important to identify these children and provide assistance, without stigmatizing them. To better estimate prenatal drug exposure scientists are using new analysis techniques in addition to self-reporting methods.

Advance: Researchers found that they can increase accuracy in identifying children prenatally exposed to cocaine when they analyze meconium (stool) specimens of newborns with gas chromatography/mass spectrometry and conduct a maternal hospital interview. This conclusion is from the first large-scale report of cocaine and other drug use during pregnancy that used both the interview and the meconium analysis approach. This study was part of the four-site Maternal Lifestyle Study (Detroit, Miami, Memphis, Providence) that NIH sponsors with several other agencies. Over a two-year recruitment period, 11,811 mothers agreed to participate. First, a maternal interview was conducted in the hospital to determine past and current drug use and collect sociodemographic information. Then, a physical exam of the infant was conducted and the infant's meconium collected from diapers. Over 8,500 meconium specimens were collected and analyzed for metabolites of illicit drugs such as cocaine, opiates, cannabinoids, PCP, and amphetamines. Prevalence and observed metabolites showed considerable variation across the four sites, and exposure status was higher in low birth weight infants. The prevalence of cocaine/opiate exposure was 10.7 percent with the majority (9.5 percent) exposed to cocaine based on the combination of meconium analysis and maternal self-report. There was 66 percent agreement between positive meconium results and positive maternal report. Only 2 percent of mothers reported that they used only cocaine during pregnancy and mothers who used cocaine were 49 times more likely to use another drug.

Implications: Meconium testing provides an excellent tool for helping determine these numbers. By using meconium testing and self-report data, children prenatally exposed to drugs can be identified more easily and appropriate medical care, early psychosocial interventions, and special education services can be offered. This study also confirms previous studies which suggest that cocaine is not used alone, it is typically used with other drugs, thus prenatal exposure is likely to be a polydrug problem.

Lester BM, El Sohly M, Wright LL, Smeriglio VL, Verter J, Bauer CR, Shankaran S, Bada HS, Walls HC, Huestis MA, Finnegan LP, and Maza PL: The maternal lifestyle study: drug use by meconium toxicology and maternal self-report. Pediatrics 107: 309-317, 2001.

Automated Synthesis of Complex Sugars

Background: The important molecules in any living organism can be broken down into four groups: nucleic acids, proteins, sugars, and others. Major advances in our understanding of the structure and function of both nucleic acids and proteins would have been impossible without automated techniques to synthesize these molecules. Once automated machines were available, it was possible for many researchers to create the molecules they wanted to study in large quantities. A similar automated process for synthesizing complex sugars has been very difficult to develop, but has recently been solved.

Advance: There were two major barriers to creating an automated machine to synthesize sugars. The first barrier was the nature of a sugar. Sugars are much more chemically reactive than the components of a protein or a nucleic acid polymer. Finding ways to block this reactivity has required a great deal of effort. Fortunately, chemists have discovered methods to decrease sugar reactivity by changing the temperature, changing the solvent, and putting unreactive blocking groups on certain parts of the sugar during a reaction. All of these processes have been implemented in a new automated instrument. The second barrier to automated synthesis was to develop a method to define the structure of the growing sugar chain while it was bound to a solid support material. Such analysis is essential to test and verify the automated instrument. Solid-state nuclear magnetic resonance techniques were used to perform these evaluations.

Implications: Changes in the metabolism and structure of sugars have been implicated in many diseases such as infectious disease, disorders of the immune system, and genetic disorders. The automated synthesizer promises to deliver large quantities of normal and abnormal sugars for study. Once these molecules are in the hands of researchers, a much deeper and complete understanding of sugar related-diseases are expected.

Plante OJ, Palmacci ER, and Seeberger PH: Automated Solid-phase synthesis of oligosaccharides. Science 291: 1523-1527, 2001.

Mechanism of Protein Degradation in the Proteasome

Background: While protein synthesis is essential for the life of a cell, so is its degradation. Proteins need to be broken down to supply the building blocks, amino acids, for new protein synthesis or to remove excess or damaged proteins. One way the cell does this is with the help of a large elaborate structure known as the proteasome, a barrel-shaped composite of 28 proteins of two types, called alpha and beta. The breakdown of protein takes place within the central chamber of the barrel formed by the beta proteins. Access to the chamber by proteins destined for degradation is through a small barrel opening, which is restricted by parts of the alpha units (the tails) that form a gate across the opening of the chamber. Regulatory particles called 11S can bind at either end of the barrel and affect the protein cleavage activity of the proteasome.

Advance: The three-dimensional structure of the proteasome with the 11S particles attached has been viewed using electron microscopy, and now can be seen in greater detail as a result of an x-ray crystallography study. X-ray crystallographic data were collected at the Synchrotron Radiation Structural Biology Resource and used to solve the detailed structure of the complex. Scientists were able to compare this structure with the lower resolution electron microscopy model, and with the x-ray structures of the proteasome alone and the 11S particle alone. The structures viewed in the various states and by different methods were very similar, but now can be seen together at a more detailed level. The arrangements of the subunits, and the nature of their interactions, have revealed a likely mechanism for the actions of the complex. For example, a comparison of the structure of the proteasome alone with the structure of the proteasome-11S complex shows that the open-gate form of the proteasome-11S complex is achieved by the alpha subunit tails assuming different shapes.

Implications: There exists a larger regulatory unit, called 19S, which is also known to bind to the proteasome at one end while the 11S binds to the other. The 19S unit unfolds and delivers proteins into the barrel for degradation. Since the 11S is now known to enhance the release of protein fragments by opening the gate, it may reduce the extent of cleavage of the protein and release larger fragments. This is a likely mechanism by which protein fragments, called antigens, that are recognized by the immune system are generated. Failure to generate antigens will lead to severe immunological diseases.

Whitby FG, Masters EI, Kramer L, Knowlton JR, Yao Y, Wang CC, and Hill CP: Structural basis for the activation of the 20S proteasomes by 11S regulators. Nature 408: 115-119, 2000.

New Approaches to Integrated Protein Separations and Mass Spectrometry

Background: Technical developments in mass spectrometry and new knowledge about complete collections of genes, called genomes, present previously unimaginable opportunities for sophisticated experiments in biochemistry. These experiments are aimed at studying in a systematic way, all of the proteins present in a biological sample, whether tissue, body fluids, or cultured cells. Their goals are to improve our understanding of diseases, and to identify new protein and gene targets for development of therapies. To facilitate these experiments, new techniques for separating, quantifying, and identifying proteins are constantly evolving.

Advance: Separation of the thousands of proteins found in complex biological samples has previously been extremely cumbersome. Researchers have designed a novel approach for separating very complex mixtures of protein fragments using a multi-stage separation system that can be automated and connected directly to a mass spectrometer. Experiments thus can be performed more quickly with lower levels of detection. This new system can detect 1,484 proteins from yeast in a single experiment.

Other investigators have pursued similar sophisticated combinations of protein separation methods and mass spectrometry. Scientists at the Pacific Northwest Laboratory have combined an advanced instrument called a Fourier-transform ion cyclotron resonance mass spectrometer with other devices and succeeded in detecting more than 100,000 peptides, representing over 1000 proteins, in a single experiment. The UCSF Mass Spectrometry Resource has developed a way to improve the effectiveness of interfacing separations with mass spectometry by drying sample fractions into arrays of spots and doing the analysis more slowly. With this new technology, samples can be analyzed more carefully and in significantly greater detail, without missing any protein fragments.

Implications: Significant advances are being made rapidly in the analytical technology and methods for high-throughput protein analysis. As mass spectrometry continues to evolve, and integrated protein analysis technologies are developed, this will make it possible to associate minute protein abnormalities with particular diseases and open the door to significant improvements in diagnosis and therapy.

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Washburn MP, Wolters D, and Yates JR. Large-scale analysis of the yeast proteome by multidimensional protein identification technology. Nature Biotechnology 19: 242-247, 2001.

Electron Microscopy Used to Model the Fine Structure of Insulin-secreting Cells

Background: The beta cells of the pancreas are the sole source of insulin secretion in humans. Death of the beta cells, or their failure to secrete adequate amounts of insulin, result in severe forms of diabetes. Therefore, a more thorough understanding of insulin secretion promises to aid in the design of therapeutics to alleviate the symptoms of some forms of severe diabetes. The Golgi apparatus is a structurally complex membranous cellular component that has a central role in all forms of secretion, including secretion of insulin. Electron microscopy was used to image the Golgi apparatus in beta cells to study its structure and function, and to gain a fundamental understanding of insulin secretion.

Advance: To preserve the native structure of the Golgi apparatus, specimens were prepared for electron microscopy by rapid freezing in a high pressure freezer, followed by dehydration and chemical fixation at low temperature (termed freeze-substitution). In a collaboration between two Biomedical Technology Resource Centers, electron microscopy data were collected at the Resource for Visualization of Biological Complexity in Albany, New York, and processed and visualized at the Boulder Laboratory for Three-Dimensional Fine Structure of Cells and Tissues in Colorado. Each of these two research resources houses one of the few intermediate voltage cryo-electron microscopes for biology in the U.S. Analysis of the 3D model confirmed that the Golgi apparatus in beta cells is a continuous ribbon-like structure that consists of seven flattened compartments called cisternae, each of which contains frequent openings. Surprisingly, three different kinds of cellular structures were observed that traverse the complete Golgi stack.

Implications: The observed structural features have implications for the movement of molecular materials through the Golgi apparatus without passing through each cisterna. The data indicate a possible role for lipid and protein exchange between another membranous cellular constituent, the endoplasmic reticulum, and the Golgi apparatus. Microtubules, components of the cellular support network, form numerous contacts with the Golgi cisternae and the endoplasmic reticulum, possibly playing a direct role in membrane trafficking. These results lay the groundwork for future studies that promise to more precisely define the mechanisms for controlling protein traffic through the Golgi apparatus and thereby for insulin secretion from the beta cells. This work was possible only through access to the high-end cryo-electron microscopes. Understanding the function of the Golgi apparatus is not only important for defining the mechanism of insulin secretion. If the Golgi apparatus is defective, it will affect the proper secretion of many other proteins and may lead to metabolic and immunological disorders.

Marsh, BJ, Mastronarde DN, Buttle KF, Howell KE, and McIntosh JR: Organellar relationships in the Golgi region of the pancreatic beta cell line, HIT-TI5, visualized by high resolution electron tomography. Proceedings of the National Academy of Sciences USA 98: 2399-2406, 2001.

New Imaging Technique Allows Researchers to Follow Stroke in Progress

Background: Diffusion Tensor Magnetic Resonance Imaging, or DT-MRI, is a form of magnetic resonance imaging (MRI) that provides more detailed anatomical information about tissue structure, composition, architecture, and organization in a living human being than conventional MRI. This kind of information was, until now, only available at autopsy.

DT-MRI measures the random motion of water molecules, referred to as diffusion. Although water appears still to the naked eye, individual water molecules are constantly in motion, colliding with each other and with other molecules in tissues at high speeds. These high-speed collisions cause the water molecules to spread out or diffuse; for example, a drop of dye placed at the center of a beaker of water slowly spreads apart in a spherical pattern. Water in tissues that contain a large number of fibers such as skeletal or cardiac muscle and brain white matter, diffuses fastest along the direction of the fibers, and slowest at right angles to them. In contrast, water diffuses in a spherical pattern in tissues that contain few fibers. DT-MRI converts such complex information about how water diffuses in tissues into intricate three-dimensional color representations of the tissues. The ability to measure these different rates of diffusion along different directions is one of the features that distinguish DT-MRI from other imaging methods.

Advance: The NIH scientists who invented DT-MRI have produced detailed maps of the human brain that clearly show the main white matter pathways. The scientists are applying this new technology to assess and diagnose neurological and developmental disorders. The recent innovation allows scientists to examine Wallerian degeneration, the permanent degeneration of nerve tissue, often associated with chronic stroke, by identifying and differentiating white matter pathways that suffer from a lack of blood supply during the event. Moreover, DT-MRI allows scientists to perform detailed studies of the brain's structure, which previously could only be examined using labor-intensive and invasive methods.

Implications: Using DT-MRI to visualize neurological pathways has made this technology the most successful imaging technique yet available to examine a stroke in progress or to successfully identify Wallerian degeneration. Preliminary studies also show DT-MRI to be useful in following early developmental changes in gray and white brain matter, changes that cannot be detected by other means. In addition, researchers testing new medication to prevent or treat brain damage that results from stroke can also use DT-MRI to evaluate the treatment. Thus, this new ability to study the brain's pathways noninvasively facilitates not only the study of neural pathways of the human brain, but is proving to be valuable in diagnosing and possibly treating a range of neurological diseases.

Basser PJ, Pajevic S, Pierpaoli C, Duda J, and Aldroubi A: In vivo fiber tractography using DT-MRI data. Magnetic Resonance in Medicine 44: 625-632, 2000.

Pierpaoli C, Barnett A, Pajevic S, Chen R, Penix L, Virta A, and Basser P: Water diffusion changes in Wallerian degeneration and their dependence on white matter architecture. NeuroImage 13: 1174-1185, 2001.

Computer Technique Helps Researchers Diagnose Cervical Ectopy

Background: Cervical ectopy is a condition where vascular, mucus producing cells normally found on the inner part of the cervix migrate to the outer cervix. This condition is common among adolescent girls, pregnant women, and women who use combined oral contraceptives. Many studies have reported an association between this condition and certain sexually transmitted diseases (STDs). This is because the area of cervical ectopy is enriched with blood vessels, making it fragile and possibly more vulnerable to infectious agents at the surface of the cervix. To date, however, researchers have not established whether increasing levels of cervical ectopy also increases susceptibility to STDs, nor have they identified conclusively the impact of hormonal contraceptives on the extent of cervical ectopy. The absence of conclusive evidence is due, in part, to the difficulty in documenting the extent of cervical ectopy observed by a physician during pelvic examination.

Advance: As part of a study to evaluate the role of hormonal contraception in the development of cervical ectopy and cervical infections, NIH-supported researchers conducted pelvic examinations on over one thousand women, aged 15-44, who sought gynecologic care at two health centers. In the course of the study, the researchers developed a more accurate technique for recording the extent of cervical ectopy by first placing a standard reference dot on the cervix, then photographing the cervix (colpophotography), and finally using a computer software technique (computerized planimetry) to objectively calculate the extent of cervical ectopy on the photographs. Researchers surmised that this new technique was more accurate than previous efforts that only photographed the cervix and then used trigonometric calculations to calculate the extent of the cervical ectopy. In addition, researchers found that the computerized measurements were significantly more reliable than simple visual assessments where clinicians looked at the cervix unaided by a magnifying device.

Implications: The refinement of this technology has important implications for women's reproductive health. While visual assessments of the cervix are still useful for clinical settings, the high reliability of this technology will enable researchers to use it in large-scale, longitudinal studies to more conclusively define the risks posed by contraceptives or other pharmaceuticals that may affect the cervix. In particular, this new technique will allow researchers to determine if different contraceptive methods or other pharmaceuticals used by women may directly influence their risk of STD infection, and ultimately their risk of pelvic inflammatory disease and possibly secondary infertility.

Morrison CS, Bright P, Blumental PD, Yacobson I, Kwok C, Zdenek S, and Pan Z: Computerized planimetry versus clinical assessment for the measurement of cervical ectopia. American Journal of Obstetrics and Gynecology 184: 1170-1176, 2001.

Methods for Unraveling Protein Structure and Function

Background: Proteins are the fundamental components of all living cells. They exhibit an enormous amount of structural diversity, enabling them to carry out a diverse range of biological functions. Proteins help us digest our food, fight infections, control body chemistry, and in general, keep our body functioning smoothly. Scientists know that the critical feature of a protein is its ability to adopt the right shape, or structure, and that identifying a protein's structure is key to understanding its biological function and its role in health and disease. Illuminating a protein's structure also paves the way for the development of new agents and devices to treat a disease. Yet solving the structure of a protein is no easy feat. It often takes scientists working in the laboratory months, sometimes years, to experimentally determine a single structure. Therefore, scientists have turned towards computers to help predict the structure of a protein based on its amino acid sequence. The basis for this concept is that a strong conservation of protein three-dimensional shape across large evolutionary distances – both within single species and between species – has been demonstrated again and again. The challenge now lies in developing computational methods for accurately and reliably understanding this intricate relationship.

Advance: NIH scientists recently introduced a new technique for gaining insight into a protein's three-dimensional structure, called CDD, or the Conserved Domain Database and Search Service. CDD is a collection of sequence alignments and profiles representing protein domains, or distinct functional and/or structural units of a protein, conserved in molecular evolution. Typically, domains are identified as either recurring sequence or structure units. CDD includes domains from *Smart* and *Pfam*, two popular public domain web-based tools for studying sequence domains as well as alignments of domains to experimentally determined three-dimensional protein structures in the NIH's publicly available Molecular Modeling Database. CD Search may be used to identify conserved domains in a protein query sequence. After identifying a query sequence, DART (the Domain Architecture Retrieval Tool) may be used to display the functional domains that make up the protein and to provide a list of other proteins with similar domain architectures. NIH has also developed a helper-application, called Cn3D, that can display a three-dimensional view of the known structure, together with an alignment display which maps conserved residues from the protein onto the known structure.

Implications: Together, CDD, DART and Cn3D allow researchers to statistically correlate structure and sequence information and to visualize and manipulate this information as well. For example, using these tools, a scientist can quickly locate the residues in a protein's structure that correspond to known disease mutations as well as manipulate and color code different alignment settings. This feature helps reveal possible functional sites within the protein and can help determine how a protein binds to a particular ligand – a molecule that is bound or is able to bind selectively to one or more sites on another molecule. If relevant structures are then detected by further structure-structure comparison, a researcher can view three-dimensional superpositions to identify interactions with other ligands. Such data may also reveal information on the evolutionary history of and relationship between macromolecules.

http://www.ncbi.nlm.nih.gov:80/Structure/

New Developments in the Evolutionary Classification of Proteins from Complete Genomes

Background: The recent explosion in genome sequencing has led to a rapid enrichment of the protein databases, both in terms of number and variety of protein sequences. The function of the majority of these proteins remains unknown. By classifying proteins according to their degree of sequence similarity, which generally reflects an evolutionary relationship, computational biologists are able to predict a likely function for many proteins, determine their evolutionary origin, and predict the three-dimensional structure. The database of Clusters of Orthologous Groups of proteins (COGs), developed by NIH investigators, is designed to classify proteins from completely sequenced genomes on the basis of orthologous relationships, or the relationships of evolutionary descent from a common ancestor. Each COG consists of proteins that likely share a common function or domain, which in turn has a role in a given cellular process or processes. The purpose of the COGs database is to serve as a platform for functional annotation of newly sequenced genomes and for studies on genome evolution. The database has a companion program, COGNITOR, developed to fit new proteins into COGs. COGNITOR may also be used to annotate newly sequenced genomes and allows researchers to predict the function(s) of individual proteins or protein sets.

Advance: NIH investigators recently added several new developments to the existing COGs database to increase the utility of the system for annotation of complete genomes. New features added to the system encompass both the addition of a number of new genomes, including proteins from two eukaryotic genomes, as well as new functionalities. For example, the database now links to information pages containing structural and functional details for each COG as well as to literature references. Improvements to the COGNITOR program include the addition of evaluation criterion for assigning new proteins to COGS.

Implications: A complete understanding of the biology of an organism necessarily starts with the knowledge of its genetic makeup. Proteins encoded in a genome must be identified and characterized, and the presence or absence of specific sets of proteins must be noted to determine potential biochemical or functional pathways used by the organism. The recently improved COGs system provides researchers with a unique and specialized set of tools for doing just this. A researcher can use COGs to annotate proteins found in complete genomes, to examine specific phylogenetic patterns between COGs, and to analyze evolutionary relationships between member proteins. The COGs database demonstrates the present utility and future potential of complete genome comparisons in identifying gene products produced by a particular organism and in predicting their structure and function. Using this database researchers can also identify a gene that is common to all organisms within the three domains of life, as well as a gene that is unique to a particular domain, thereby gaining meaningful insights into the organization and evolution of biological systems.

Tatusov RL, Natale DA, Garkavtsev IV, Tatusova TA, Shankavaram UT, Rao BS, Kiryutin B, Galperin MY, Fedorova ND,and Koonin EV. The COG database: new developments in phylogenetic classification of proteins from complete genomes. Nucleic Acids Research 29: 22-28, 2001.

A Powerful Tool for Identifying Sequence Similarities

Background: Comparison, whether of morphology or of protein sequences, lies at the heart of biology. Recognizing the potential behind this statement, a team of NIH researchers set out to develop a computer program capable of identifying similarities in strings of data housed in various sequence databases. In 1990, NIH researchers released BLAST – the basic local alignment search tool. The introduction of BLAST made it dramatically easier for scientists to rapidly scan huge sequence databases for overt homologies, or similarities, and statistically evaluate the resulting matches. However, not all important homologies are overt. Some of the most interesting are subtle and do not rise to statistical significance during a standard BLAST search. NIH researchers therefore extended BLAST to address the problem of detecting weak yet significant sequence similarities. Position-specific iterative BLAST, or PSI-BLAST, refers to a feature of BLAST in which a profile, or scoring matrix, is automatically constructed from a multiple alignment of the highest scoring hits in an initial BLAST search. Scores are calculated for aligning the various amino acids to each profile position. Highly conserved positions yield large positive or negative while weakly conserved positions yield scores near zero. This profile is then used to perform subsequent BLAST searches. The results of each "iteration," or search, are used to further refine the profile, increasing the sensitivity of the overall search strategy. A limitation to using PSI-BLAST for large-scale protein analysis was that on a small percentage of queries, false positives – segments having a similar sequence yet no direct relationship – could enter the list of matches at one iteration and corrupt the score matrix for subsequent iterations.

Advance: To overcome this obstacle, NIH scientists recently improved PSI-BLAST accuracy by incorporating the use of composition-based statistics to re-evaluate candidate alignments. Composition-based statistics take into account the frequency with which the various amino acids are used in a database sequence and adjust the scale of the query profile accordingly. This change represents a more careful interpretation of the statistical theory behind BLAST which initially assesses the significance of a local alignment between two sequences and then extends the result to database searching. A number of other refinements were also recently implemented in PSI-BLAST, including the use of more accurately estimated statistical parameters; the filtering of database sequences to prevent segments with highly restricted or biased amino acid composition from participating in the construction of profiles; and an improved treatment of gaps within alignments when estimating position-specific amino acid frequencies.

Implications: Both BLAST and PSI-BLAST are molecular biology tools that have been widely adopted by the international biomedical community for enhancing their research efforts. The original BLAST paper was the most cited paper in 1990 and is now being supplanted only by the 1997 paper describing the original version of PSI-BLAST. The modifications described above improve the accuracy of PSI-BLAST searches, and provide scientists worldwide with a more powerful tool for characterizing novel protein sequences and inferring their function from previously studied gene products.

Schaffer AA, Aravind L, Madden TL, Shavirin S, SpougeJL, Wolf YI, Koonin EV, and Altschul SF: Improving the accuracy of PSI-BLAST protein database searches with composition-based statistics and other refinements. Nucleic Acids Research 29: 2994-3005, 2001.

New Modeling Tools for Analyzing Tumor DNA Data

Background: Cancer is a general term used to define a group of many related diseases in which cells have become unresponsive to the normal processes that control cell growth and differentiation. These cells continue to grow and divide, and can spread to other parts of the body, accumulating and forming tumors that may compress, invade, and destroy normal body tissue. Recent advances in the field of molecular biology have enabled researchers to determine that two classes of genes appear to play a major role in triggering cancer, with a third group, called DNA repair genes, emerging as an important new source. DNA repair genes are thought to be the body's front-line defense against mutations, and, as such, play a major role in cancer. Mutations within these genes often manifest themselves as lost or broken chromosomes. It has been hypothesized that certain chromosomal gains and losses are related to cancer progression and that the patterns of these changes are relevant to clinical prognosis. Using different laboratory methods, researchers can measure gains and losses in the copy number of chromosomal regions in tumor cells. Then, using mathematical models to analyze this data, they can predict which chromosomal regions are most likely to harbor important genes for tumor initiation and disease progression. The results of such an analysis may be depicted as a hierarchical tree-like branching diagram, referred to as a tree model of tumor progression.

Advance: NIH scientists, in collaboration with academia, have developed new software methods to produce tree models of DNA changes in tumors. In 1999, NIH researchers derived a tree model inference algorithm based on the concept of maximum weight-branching. Events found near the root of the tree are predicted to be early events in tumor progression and events clustered together in subtrees are thought to be associated with more genetically similar subclasses of tumors. The main mathematical result of the paper is that, using a set of plausible assumptions, the software quickly chooses from an exponential number of models the best possible fit for depicting events leading to disease progression. Researchers next extended this work to develop distance-based trees, in which the events measured were depicted as leaves of the tree. This model is based on a well-known phylogenetic tree-building method that simply counts the number of differences between two datasets and provides a way to simultaneously visualize all the pairwise correlations between pairs of DNA aberrations. This method also allows researchers to make quantitative predictions about which events actually occur early in tumor progression.

Implications: Although the rates for new cancer cases, as well as deaths for all cancers combined, are on the decline in the U.S., cancer continues to be a real threat to human life and health. Identifying and tracking trends in cancer development and progression are crucial underpinnings of efforts to prevent and control this devastating disease. The computational models described here demonstrate progress towards relating tumor progression to the occurrence of DNA gains and losses and have already proven useful in hunting various cancer genes and in cancer diagnoses. For example, NIH researchers have already published promising results using these models to study forms of ovarian cancer, renal cancer, bladder cancer, and breast cancer. Additional studies are currently underway.

Schaffer AA, Simon R, Desper R, Richter J, and Sauter G: Tree models for dependent copy number changes in bladder cancer. International Journal of Oncology 18: 349-354, 2001.

ClinicalTrials.gov

Background: ClinicalTrials.gov provides patients, families, and members of the public easy access to information about the location of clinical trials, their design and purpose, criteria for participation, and, in many cases, further information about the disease and treatment under study. The system was launched on February 29, 2000 and at that time included information on some 4,400, primarily NIH sponsored, trials. It is freely available over the World Wide Web, and patients and others are able to search and browse the system in a variety of ways. Throughout the system there are just-in-time links to NIH's consumer health site, MEDLINEplus, to PubMED,(NIH's interface to the MEDLINE database of some thirteen million citations), and to other NIH consumer health information.

Advance: *ClinicalTrials.gov* currently contains information on more than 5,700 trials for hundreds of diseases, that are being conducted in almost 60,000 locations. Each month there are more than two million hits on the system and over 5,000 unique users access *ClinicalTrials.gov* daily. In the last year a significant number of trials from a variety of sponsors have been added. The majority of the trials are sponsored by Federal agencies, primarily by the NIH, but also including the CDC and the Department of Veterans Affairs. There are approximately 2,000 trials sponsored by universities or non-profit organizations, and some 800 trials are sponsored by private industry.

ClinicalTrials.gov was established as a consequence of Section 113 of the FDA Modernization Act. In March 2000, the FDA issued the first of two draft guidance documents for industry for interpretation and implementation of Section 113. The second guidance document, which outlined implementation procedures, was issued in July 2001 and was open for comment until September 2001. The document indicates that industry should submit information to *ClinicalTrials.gov* through the NIH developed Protocol Registration System (PRS). The PRS is a robust web-based data entry system that allows users to create, modify, and submit protocol records to *ClinicalTrials.gov.* Sponsors provide and maintain information about their clinical trials ensuring that the records are correct and updated in a timely manner. Once completed by sponsors, the records are automatically sent to the FDA for review and to *ClinicalTrials.gov* for inclusion in the public system. A tutorial to assist new users of the system may be accessed at http://prsinfo.clinicaltrials.gov/. A small number of educational resources for clinical trials are available on *ClinicalTrials.gov.* Recent activities have involved developing additional resources and working with four library-based groups to test and promote these resources through a variety of outreach activities. The groups are supported through NIH's National Network of Libraries of Medicine and serve culturally diverse populations in Chicago, Illinois; Baltimore, Maryland; Johnson City, Tennessee; and Washington, DC.

Implications: Clinical trials are medical research studies that seek to evaluate the safety and effectiveness of new drugs, medical procedures, or other means of treating, diagnosing, or preventing diseases. This type of research helps investigators learn how different people respond to medications or other therapeutic approaches, and such investigations may lead to new or

improved treatments. Trials are conducted when there is no proven treatment for a specific disease, or to test which treatment works best for a particular disease or condition. Patients may want to participate in a clinical trial for a variety of reasons. Most often they are hopeful that the experimental treatment will help them. Many patients who participate in clinical trials believe in the importance of medical research, and, after weighing the benefits and possible risks of participating in a trial, decide to participate because this allows them to contribute personally to a possible cure or better treatment for their disease. *ClinicalTrials.gov* provides a single place for patients and others to come to find information about clinical trials. People now have a central, easily accessible source of information for clinical trials no matter where the trials are being conducted, what the particular drugs or diseases under study are, or who is sponsoring the trials.

McCray AT. Better access to information about clinical trials. Annals of Internal Medicine, 133: 609-614, 2000.

Profiles in Science

Background: The NIH is making the archival collections of pioneering biomedical scientists of the 20th century available on its *Profiles in Science* Web site. The site was launched in September 1998 and promotes the use of the Internet for research and teaching in the history of biomedical science. The collections have been donated to the NIH and contain published and unpublished items, including books, journal volumes, pamphlets, diaries, letters, manuscripts, photographs, audiotapes, video clips, and other materials.

Advance: Profiles in Science currently provides access to the collections of six scientists. In the last year the collections of two NIH Nobel Laureates were made available. In November 2000, the papers of Christian Anfinsen, Jr. (1916-1995) were added to the site. Anfinsen was an American biochemist who shared the 1972 Nobel Prize for work that helped explain the structure and composition of proteins in living cells. In May 2001 the papers of Marshall W. Nirenberg (b. 1927) were added. Nirenberg is an American biochemist who shared the 1968 Nobel Prize for his work on deciphering the genetic code. These papers have been added to the four collections already available through *Profiles in Science.* The site was launched with a selection of papers devoted to the life and work of Oswald T. Avery (1877- 1955). Avery was a founder of modern molecular biology and the first person to establish that genetic information is encoded in DNA. In March 1999, the site was expanded with the release of a portion of the extensive Joshua Lederberg (b. 1925) collection. Lederberg received the Nobel Prize for his discoveries concerning bacterial genetics. In November 1999, a selection from the papers of Nobel Laureate Martin Rodbell (1925-1998) was posted, and in May 2000, the Julius Axelrod (b. 1912) collection was made available. Rodbell shared the Nobel Prize for his discovery of G-proteins and the principles of signal transduction in cellular communication, and Axelrod shared the Nobel Prize for his discovery of the actions of neurotransmitters in regulating the metabolism of the nervous system.

Implications: The *Profiles in Science* site is a growing resource for students, educators, and researchers. The site makes the successes of science readily accessible to anyone who is interested in the scientific process and in the people who have dedicated their lives to scientific discovery. The collections are particularly strong in the areas of cellular biology, genetics, and biochemistry, but also reflect issues in such areas as health and medical research policy, the application of computers in medicine, science education, and the search for extraterrestrial life.

McCray AT and Gallagher ME. Principles for digital library development. Communications of the ACM 44: 49-54, 2001.

http://profiles.nlm.nih.gov/

Clinical Proteomics Initiative

Background: Genomics is the study of DNA and the processes that lead to the creation of proteins. Proteomics is the large-scale study of proteins and is the next phase of molecular medicine after genomics. Proteomics offers a means to discover and define disturbances in the protein communication networks that drive cancer growth and spread. Cancer treatments of the future will be designed to return these disturbed protein pathways to their normal state.

Many human cancers, such as prostate, breast, and colon cancer, emerge from precursor lesions, the microscopic tissue abnormalities that precede cancer and may persist for more than 10 years in the patient's organ. In the past, it has not been possible to conduct proteomic analysis on such microscopic lesions.

Advance: The NIH has teamed up with the U.S. Food and Drug Administration to create the first clinical proteomics initiative. Researchers have invented and perfected a set of proteomic technologies that address critical experimental and clinical needs. Examples of successful research outcomes are as follows:

- Use of laser capture microdissection (a technique to obtain pure cells from specific microscopic regions of tissue sections) combined with proteomic analysis to discover more than 132 proteins that are altered during the transition from benign to malignant disease in breast, ovarian, prostate and/or esophageal cancer. These proteins offer new candidates for imaging targets, early cancer biological markers, and targets for therapy.

- Development of protein microarrays (procedures that help determine the genes that are active in a particular cell type) that profile the state of key proteins in signaling pathways. Application of this technology to microscopic human cancer precursor lesions has revealed that suppression in the pathway that triggers cell death is a very early event in cancer development. Using patient biopsies, these arrays have been used successfully to monitor the effects of experimental therapy on the state of protein pathways that regulate growth and death of tumor cells.

- A microversion of an analytic technique used to identify chemical substances has been adopted for use in small samples of tissue cells and blood. This technology reveals characteristic patterns of changing proteins that may constitute a new approach to disease classification and early diagnosis. We are currently applying new "artificial intelligence" type computer analysis of the protein patterns to discover signatures of early ovarian and prostate cancer.

Implications: Early diagnosis alone can have a profound impact on the success of cancer treatment, even with conventional methods. Proteomic analysis of patient blood and tissue constitutes a direct approach to patient-tailored therapy. In the future, the proteomic signature of an individual patient's tumor could be used to design an optimal combination therapy that maximizes efficacy and minimizes side effects.

LumiCyte escalates its pursuit of early cancer detection; new alliance dedicated to improve the lives of millions. PR Newswire. March 27, 2001.

Bichsel VE, Liotta LA, and Petricoin III E: Cancer proteomics: from biomarker discovery to signal pathway profiling. The Cancer Journal 7: 69-78, 2001.

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Marchant J: Know your enemy. New Scientist November 4, 2000.

Paweletz CP, Charboneau L, Bichsel VE, Simone NL, Chen T, Gillespie JW, Emmert-Buck MR, Roth MJ, Petricoin III EF, and Liotta LA: Reverse phase protein microarrays which capture disease progression show activation of pro-survival pathways at the cancer invasion front. Oncogene 20: 1981-1989, 2001.

Szabo L: Doctors at EVMS and elsewhere are sifting through proteins, looking for "fingerprints," in hopes of finding... the key to early cancer detection. The Virginian-Pilot March 8, 2001.

Controlled Growth Factor Release System

Background: One method of stimulating regeneration of tissue damaged by disease or trauma is to deliver growth factors to the damaged tissue. Currently available drug delivery systems, however, were designed to operate under static conditions. Tissues such as bone, muscle, and blood vessels exist in mechanically dynamic environments. Bone and cartilage compress as we walk, muscles contract and stretch, and blood vessels pulse as blood flows through them. A challenge facing tissue engineers is to develop a drug delivery system that responds to mechanical signaling.

Advance: NIH-funded investigators developed a model delivery system that responds to mechanical stress by releasing a growth factor. They loaded hydrogel carriers, which are used frequently in tissue engineering, with a growth factor that promotes blood vessel formation. When the scientists compressed hydrogel disks in a device, the growth factor release rate was up to five times higher than the release rate from non-compressed disks; the increase could be regulated by the amount of compression. The researchers also implanted hydrogels under the skin of laboratory mice. Control animals whose implants did not contain growth factor showed no significant blood vessel formation regardless of whether or not they received mechanical stimulation. Animals whose implants contained growth factor showed enhanced blood vessel formation, and the increase in vascularization was significantly greater in animals that also received mechanical stimulation.

Implications: A growth factor delivery system that responds to mechanical signaling may be useful for a number of applications, including regeneration and engineering of tissues as well as more general drug delivery uses.

Lee KY, Peters MC, Anderson KW and Mooney DJ: Controlled growth factor release from synthetic extracellular matrices. Nature 408: 998-1000, 2000.

Mouse Epidermal Stem Cell Sorting

Background: The epidermis (skin) is an epithelial tissue. Epithelial tissues are continuously renewing – some cells die and slough off and are replaced by division of cells that lie underneath. A population of stem cells, which have the ability to divide for indefinite periods, maintain the epidermis and other epithelial tissues. Scientists believe that targeting the epidermal stem cell population with gene therapy or bioengineering techniques could hold promise for treatment of skin diseases. Unfortunately, isolating a pure population of epidermal stem cells from adult tissues has been a difficult task.

Advance: NIH-funded researchers have developed a novel cell sorting method that separates stem cells from other types of epidermal cells. The sorting method yields three categories of mouse epidermal basal cells: the stem cells, transient amplifying cells, and non-proliferative basal cells. The stem cells proliferate for a lifetime while the transient amplifying cells, already committed to becoming skin cells, will divide a finite number of times before they are sloughed off. The non-proliferating basal cells exhibit characteristics of proliferating cells, but do not divide. The scientists were able to identify the stem cells as such because they exhibited certain properties, namely: high proliferative capability, ability to regenerate tissue, and long-term gene expression, or activity. Gene expression was measured by inserting a special 'test' gene into the cells to see how long it would be expressed.

Implications: This novel sorting method yields pure, viable epidermal stem cells that might be useful for bioengineering skin tissue. Additionally, the evidence of long-term gene expression means that these cells could be used in gene therapy for correcting skin diseases. The technology also may be useful for isolating stem cells from other epithelial tissues, including the oral mucosa.

Dunnwald M, Tomanek-Chalkley A, Alexandrumas D, Fishbaugh J, and Bickenbach JR: Isolating a pure population of epidermal stem cells for use in tissue engineering. Experimental Dermatology 10: 45-54, 2001.

Mussel Byssal Thread: A Self-Healing Biomolecular Material

Background: Studies of the marine mussel are providing valuable information about the secrets of its super-tough tendon, called the byssus – information that may someday have human bioengineering applications. The mussel uses its byssus to anchor itself to solid surfaces under the harshest of marine environments. This composite structure is made up of a series of fibrous protein threads with sticky tips that are secreted from the mussel's foot. Distal sites are especially intriguing because they combine features of spider silk and tendon collagen in the same material that lend both elasticity and toughness to the byssus, as well as the ability to regain its original dimensions after recovery from stress.

Advance: NIH grantees have found that the ability of byssus threads to recover after stress is intimately connected to the presence of protein cross-linkages – or intermolecular bridges – in the threads. There was a dramatic reduction in the threads' ability to recover from stretching after they were placed in distilled water and exposed to various chemical agents that removed metals or disrupted protein cross-linkages. In most cases, however, this loss was reversed when the byssus was re-exposed to natural seawater. The scientists conclude that the molecular architecture of byssal threads appears to be able to accommodate a variety of mechanical and chemical disturbances. The capacity to recover from these disturbances is dependent upon the presence of seawater.

Implications: Knowledge of the byssus threads' design, assembly, and composition may lead to the development of a new generation of synthetic restorative materials for tooth and bone.

Vaccaro E and Waite JH: Yield and post-yield behavior of mussel byssal thread: a self-healing biomolecular material. Biomacromolecules (in press 2001).

New Evidence of the Existence of Circulating Skeletal Stem Cells

Background: The bone marrow of post-natal mammals contains two populations of stem cells: hematopoietic stem cells that form the blood elements and stromal stem cells that give rise to all major skeletal tissues, including bone and cartilage. Hematopoietic cells are circulating and non adherent (not able to stick). Stromal cells, however, are adherent, non-circulating, and fibroblastic, capable of forming collagen, the major protein that makes up skeletal tissues. Scientists have speculated that blood may also contain cells that can become adherent and display fibroblastic properties, but the existing data was not definitive.

Advance: NIH researchers have now established the existence of adherent, fibroblastic-like cells in the blood of four types of mammals. These "circulating skeletal stem cells" resemble, but are not identical to, stromal stem cells. The researchers took blood from adult mice, rabbits, guinea pigs, and humans and placed it in petri dishes. They observed some cells that were adherent and clonogenic, meaning a single cell can give rise to colonies. They then transplanted the cells into mice where the cells formed bone. This is the first evidence that adherent, fibroblast-like cells circulating in blood can form bone.

Implications: Further research may establish the ability of these "circulating skeletal stem cells" to give rise to other types of tissues. Additionally, the evidence of their existence offers researchers an accessible source of adult stem cells for potential therapeutic use.

Keznetsov SA, Mankani MH, Gronthos S, Satomura K, Bianco P, and Robey PG: Circulating skeletal stem cells. Journal of Cell Biology 153: 1133-1139, 2001.

Potential Gene Therapy for Dominantly-Inherited Retinal Diseases

Background: Retinal diseases offer promising targets for gene therapy, since over 100 different genes are known to be involved in causing retinal disorders. The accessibility of the retina lends this tissue to interventions from outside the eye. Direct delivery of therapeutic agents to the space between the retina and the adjacent tissue, the retinal pigmented epithelium, has been shown to be practical. Several types of therapy have been attempted using animal models of retinal degeneration. Such therapy has included replacement of missing enzymes, delivery of protective factors called neurotrophins, and introduction of agents that halt the natural process of cell death, or apoptosis. The problem of treating some inherited mutations has proven particularly difficult to model because production of the inappropriate gene product causes profound metabolic disturbance or causes cell death.

Advance: In some inherited diseases, only one of the pair of corresponding genes normally found on a chromosome (one gene on each strand of the DNA) need be mutated to cause the disease. These are called dominantly-inherited diseases. One approach to curing certain dominantly-inherited disorders is to deactivate the mutated gene, leaving the corresponding normal gene partner intact and functional. Normally during gene expression, a messenger RNA (mRNA) molecule is produced that can be read within the cell and ultimately directs the assembly of a gene product. This is the concept behind ribozyme therapy. Ribozymes are small RNA molecules, which act like scissors to cut the mRNA produced from the mutated gene leaving the mRNA from the normal gene intact. Recent experiments in animals have demonstrated that it is possible to deliver ribozymes to photoreceptors cells and decrease the number of mutant mRNA molecules made from a known dominant mutation in the rhodopsin gene. This treatment effectively cured the retinal degeneration for up to eight months. The ribozyme therapy was also effective when administered at a late stage of the disease.

Implications: Dominantly-inherited, single-gene mutations causing retinal degeneration could be cured if a ribozyme molecule could be targeted to the defective mRNA sequence. For this to happen, the exact specific mutation causing the disease must be known. This is happening at an increasing rate thanks to advances in molecular genetics. The demonstration of a long-lasting therapeutic effect that can be instituted at late stages of the disease is particularly important for human retinal degenerations, many of which have a late onset.

LaVail MM, Yasumura D, Matthes MT, Drenser KA, Flannery JG, Lewin AS, and Hauswirth WW: Ribozyme rescue of photoreceptor cells in P23H transgenic rats: long-term survival and late-stage therapy. Proceedings of the National Academy of Sciences USA 97: 11488-11493, 2000.

Improved Imaging of the Human Lens

Background: Conventional magnetic resonance imaging (MRI) is a sensitive diagnostic imaging tool that provides three dimensional images of the body's interior without the use of radiation. A refinement of MRI, called magnetization transfer contrast (MTC) enhancement, has been shown to be sensitive to the accumulation of fluid in tissues (edema) that changes the concentration, movement, and structure of large molecules within those tissues. These changes often occur in cataract formation, in which the normally transparent lens becomes cloudy. MTC-enhanced MRI has successfully detected lens changes in animal models of cataracts that are seen in diabetics. The changes were documented sooner than when using standard MRI and demonstrated tissue changes before they could be seen clinically.

Advance: Researchers attempted to determine the feasibility of creating high resolution MTC-enhanced images of human eyes. Because of the motion of the eye and eyelids and the bony structures surrounding the eye, it was difficult to obtain high resolution images of the fine structures surrounding and within the eye with minimal distortion. Scientists were able to overcome these difficulties and were able to obtain high resolution images with standard and MTC-enhanced MRI. Interestingly, they found that MTC-enhanced MRI was better able to detect nuclear cataract (opacities in the center of the lens) and standard MRI was better at detecting cortical cataract (opacities in the outer areas of the lens).

Implications: MTC-enhanced MRI provides the means to detect subtle changes in the molecular structures of the eye non-invasively. This has important implications for further study of changes within the lens that occur prior to development of an opacity that interfers with vision, as well as providing a potential tool for monitoring the effectiveness of efforts to halt or reverse cataractous changes.

Lizak MJ, Datiles MB, Aletras AH, Kador PF, and Balaban RS: MRI of the human eye using magnetization transfer contrast enhancement. Investigative Ophthalmology and Visual Science 41: 3878-3881, 2000.

Gene Regulation in the Retina

Background: A growing number of eye diseases are reported to have genetic components as the basis of their pathology. The eye is a particularly unique structure that is amenable to treatment with gene therapy. The eye and its tissues are easily accessible for viral vector or drug delivery, and it is an immunologically-privileged site, i.e., it is not subjected to the full range of the body's immune responses. Current research is directed toward using viral vectors or vehicles to transfer normal genes into cells that have defective or missing genes. However, in order for gene therapy to live up to its full potential, it is necessary to develop the means to regulate gene expression. If not enough of a gene product is produced, it may not have the desired effect, while overproduction may be toxic to the system or tissues. Ideally, there should be a means of turning on and turning off gene expression externally, perhaps with an active compound that can be taken orally.

Advance: Scientists have used a portion of a bacterial gene (called Tet-On) that regulates resistance to the antibiotic tetracycline to control expression of genes transferred to the retina. Because tetracycline and its analogs can pass through the blood-retinal barrier, orally administered tetracycline or an analog can activate Tet-on, initiating the process that leads to the synthesis of gene products. These scientists used viral vectors to carry the Tet-On activator and the DNA encoding a green fluorescent pigment that could be easily detected when produced, linked to the appropriate tetracycline response element. After administration of a tetracycline analog to turn on the gene, the researchers found that the fluorescent pigment was detectable in the target retinal tissues. Using this same system, they were also able to transfer a human growth hormone gene and regulate the production of growth hormone.

Implications: This system offers a promising means of delivering replacement genes for those found to be defective or missing, while regulating the expression of those genes or the product of that expression. It opens the possibility of delivering the genes necessary to ameliorate a variety of eye diseases and being able to control them as needed in a similar way to the therapeutic application of conventional drugs.

Dejneka NS, Auricchio A, Maguire AM, Ye X, Gao GP, Wilson JM, and Bennett J: Pharmacologically regulated gene expression in the retina following transduction with viral vectors. Gene Therapy 8: 442-446, 2001.

Inhibition of Neovascularization in the Eye

Background: The abnormal growth or proliferation of blood vessels is a process known as neovascularization. A number of diseases involve neovascularization, including the growth of some tumors, arthritis, and a variety of eye diseases. In the eye, neovascularization can have a devastating impact on vision. In one form of age-related macular degeneration, for example, neovascularization of the choroid, the middle vascular layer between the retina and the white of the eye, leads to the leakage of blood and accumulation of fluid under the macula – the area of the retina responsible for our sharp central vision. People with this form of the disease suffer a rapid and devastating loss of vision.

Advance: Several proteins or drugs have been found to inhibit or stimulate the nonvascular process. Unfortunately, the effects that these substances have on the neovascular process often depend on the tissue in which they are employed. The uniquely specialized tissues of the eye make it especially difficult to predict the effect that a promising protein or drug might have on the neovascular process. One such promising protein is endostatin, a potent inhibitor of blood vessel growth (angiogenesis) in tumors.

In order to test the effects of endostatin on ocular neovascularization and at the same time minimize the difficulties associated with handling the protein and maintaining high levels of the protein in the serum, researchers developed a viral vector to transfer the gene sequence for endostatin coupled with a sequence that creates a signal that can be detected to ensure the endostatin gene has been successfully transferred. They injected this vector system into the bloodstream of an animal model prior to inducing neovascularization by laser treatment. These scientists found that the endostatin gene transfer successfully sustained high levels of endostatin that prevented choroidal neovascularization in the animal model. This is the first report of endostatin inhibition of neovascularization in the eye.

Implications: Endostatin has been shown to inhibit tumor growth and cause tumor regression possibly due to its inhibition of angiogenesis. This report of endostatin's prevention of intraocular neovascularization also has implications that it may also be useful in causing regression of neovascularization that is caused by age-related macular degeneration or other related eye diseases.

Mori K, Ando A, Gehlbach P, Nesbitt D, Takahashi K, Goldsteen D, Penn M, Chen CT, Mori K, Melia M, Phipps S, Moffat D, Brazzell K, Liau G, Dixon KH, and Campochiaro PA: Inhibition of choroidal neovascularization by intravenous injection of adenoviral vectors expressing secretable endostatin. American Journal of Pathology 159: 313-320, 2001.

Decreased Haze and Apoptosis After Photoablation

Background: The excimer laser has been approved for use in the correction of nearsightedness (myopia), farsightedness (hyperopia), and irregularities in the corneal surface (astigmatism) for several years. One surgical technique is known as photorefractive keratectomy or PRK. In PRK, the light energy of the laser removes precise amounts of the corneal tissue (also known as photoablation) to reshape the surface, thereby improving the cornea's refractive properties in focusing light on the retina. Although PRK is effective in reducing refractive errors, there is a small risk, particularly in patients with higher degrees of refractive error, of the development of corneal haze that reduces the transparency of the cornea. In spite of technological improvements in the excimer laser, the mechanism involved in development of corneal haze is not clear and treatment has had limited success.

Advance: The amniotic membrane is the inner membrane of the placenta that surrounds a fetus during development. After birth this tissue is discarded. Researchers have previously reported the use of amniotic membrane in reconstruction of the corneal surface in patients with a variety of eye diseases. Because of the success of these procedures in promoting healing while reducing inflammation and scarring, scientists wanted to know whether transplantation of amniotic membrane after photoablation would reduce or eliminate corneal haze. Animals received a phototherapeutic keratectomy in both eyes, and the cornea of one eye was subsequently covered with a dressing or bandage of amniotic membrane. The uncovered cornea served as the control. Investigators found that the use of amniotic membrane at an early stage in the corneal healing process markedly reduced corneal haze in animals treated with the excimer laser. Investigators also found fewer apoptotic corneal cells (cells with characteristic features of programmed cell death) and fewer inflammatory infiltrates in the corneas covered with the amniotic membrane, suggesting suppression of apoptosis in those corneas.

Implications: Although amniotic membrane has been used in corneal reconstruction, its use as a bandage or dressing to promote wound healing and reduce inflammation has now been demonstrated. The success of this technique may be useful in reducing corneal haze after PRK, but it also has important implications for use in enhancing corneal healing after damage by injury, disease, or infection.

Wang MX, Gray TB, Park WC, Prabhasawat P, Culbertson W, Forster R, Hanna K, Tseng SCG: Reduction in corneal haze and apoptosis by amniotic membrane matrix in excimer laser photoablation in rabbits. Journal of Cataract Refractive Surgery 27: 310-319, 2001.

Inhibition of CMV Replication

Background: Cytomegalovirus (CMV) is a common herpes virus that usually causes subclinical or mild infection in adults with healthy immune systems. In immunocompromised people, such as AIDS patients or organ transplant recipients, whose immune systems are suppressed by the disease or by medication, the virus can spread throughout the body and cause serious complications. In the eye, the virus causes CMV retinitis, leading to pain and a loss of vision. The inflammatory response itself can cause a decrease in vision. There continues to be a need for innovative therapies and for model systems in which to test them. One such therapy involves antisense oligonucleotides, compounds that block the production of viral proteins within cells by interfering with the messages directing their production.

Advance: Scientists have used two model systems for growing cells in the laboratory for testing two oligonucleotides that have been developed against CMV. One system employs human fibroblasts (skin cells) and the other human retinal pigment epithelial (HRPE) cells, derived from a layer of cells in close contact to the retinal photoreceptors. The researchers found that both oligonucleotides were effective in inhibiting viral damage to the cells. Interestingly, they found the oligonucleotides were effective up to six days after introduction of the virus in the HRPE system but only up to three days in the fibroblast system.

Implications: This study demonstrates not only the antiviral activity of the oligonucleotides being studied, but also their increased potency in the HRPE system. The investigators suggest that this may be due to the slow spread of the virus through the HRPE cells compared to a more rapid spread in fibroblasts. Slow spread of the virus through the HRPE system more closely parallels the slow, progressive spread of the virus seen in patients with CMV retinitis. This may make the HRPE system a more appropriate system for testing other antiviral therapies.

Detrick B, Nagineni CN, Grillone LR, Anderson KP, Henry SP, and Hooks JJ: Inhibition of human cytomegalovirus replication in a human retinal epithelial cell model by antisense oligonucleotides. Investigative Ophthalmology and Visual Science 42: 163-169, 2001.

New Focus on Race Yields Better Data on Cirrhosis

Background: Epidemiology studies tell us, among other things, how big a health problem is and what segments of the population it affects. This ensures that research addresses the groups most affected. One of the ways we glean this kind of information is by examining death certificates and compiling the details they list about age, gender, race, and cause of death.

When the Census Bureau began tabulating these kinds of death-certificate data in 1943, it listed decedents' race simply as "white" or "other." According to these classifications, the death rate of cirrhosis of the liver, whose primary cause is alcohol abuse, was higher for whites than for nonwhites in the early part of the 20th century. By the 1950s, however, more nonwhites were dying of cirrhosis. At the time, the nonwhite population of the U.S. was mostly black.

The high cirrhosis death rate among blacks appeared to be confirmed by data, released in 1968 by the National Center for Health Statistics, which gave more specific death-certificate details about decedents' race. Research based on these new details showed a dramatic increase in cirrhosis deaths among black males, compared with whites and females. The overall death rate from cirrhosis has dropped dramatically, but black males appear to have maintained the lead. Clinicians and researchers thus have focused on black males as being at high risk of death from cirrhosis.

"Hispanic" first appeared as a race option on death certificates in the last half of the 20th century. In 1989, the U.S. Standard Certificate of Death began including "Hispanic." Now that certificates differentiate between white Hispanic, white non-Hispanic, black Hispanic, and black non-Hispanic decedents, a different picture of cirrhosis mortality has emerged. It is reflected in cirrhosis mortality trends tabulated for 1997. If investigators had simply counted cirrhosis deaths among each group, the largest groups would have had the highest rates by virtue of their size. The investigators adjusted the data to represent the true vulnerability of each group, regardless of size.

Advance: Cirrhosis mortality of white Hispanic males exceeded that of black non-Hispanic males during this period (highest and second highest, respectively).

Implications: A study of drinking patterns from 1984 to 1992 revealed that Hispanic males had the highest proportion of new heavy-drinking cases, followed by black males, then white males. The link between alcohol abuse and liver cirrhosis might be one of the factors that accounts for the increase in cirrhosis death rates among Hispanic and black males. Another potential factor is the increasing rate of hepatitis C infection, which is highest among Hispanic males. Patients with both hepatitis C and alcohol abuse tend to have much worse liver damage than would be expected from the combination of the two conditions.

Stinson FS, Grant BF, and Dufour MC: The critical dimension of ethnicity in liver cirrhosis mortality statistics. Alcoholism, Clinical and Experimental Research (in press 2001).

A Computer "Detective" Helps Physicians Locate Colonic Polyps at Virtual Colonoscopy

Background: Colon cancer is the second leading cause of cancer death in Americans. Colonic polyps (small growths in the wall of the colon) are thought to be precursors to colon cancer. The best test currently available for detecting and treating colonic polyps is colonoscopy. However, it is expensive and uncomfortable and only a fraction of Americans have it done despite health guidelines recommending it after age 50. Radiologists have spent the past six years investigating a new way to diagnose colonic polyps, called CT colonography or "virtual colonoscopy". In virtual colonoscopy, the patient undergoes a CT scan of the abdomen and pelvis to look at the colon. A small tube is placed into the patient's rectum and the colon is distended with air. The test takes only a few minutes. Surveys have shown that many patients prefer virtual colonoscopy over conventional colonoscopy.

Advance: Researchers at NIH have taken the first steps towards developing a computer algorithm that analyzes the CT scan and locates possible polyps automatically. The computer algorithm creates a three-dimensional model of the patient's colon. Then the computer inspects each of hundreds of thousands of points on the model of the colon to look for small protrusions that could represent a polyp. Like a detective, the algorithm prunes the list of possible polyps to a manageable few and reports these to the physician. The NIH researchers have proven feasibility of this algorithm on a computer simulation of colonic polyps derived from real human virtual colonoscopy data. They recently validated the algorithm on virtual colonoscopy studies of patients with known polyps, detecting 2/3 of clinically significant polyps 1 cm or larger. Almost all computer-aided diagnosis systems produce false positive diagnoses (i.e., stating disease is present when it really is not). The NIH researchers were able to keep the number of false positive diagnoses under 4 per patient (a physician is likely to be able to eliminate most of these). The computer also found a few polyps missed by the physicians. This is a substantial advance because it shows for the first time in a peer-reviewed publication that computer-aided diagnosis for virtual colonoscopy is feasible for real patients with real polyps. Currently they are assessing the improvement in diagnosis when radiologists incorporate the algorithm into their decision-making process.

Implications: Virtual colonoscopy needs to be cost-effective and accurate if it is to be widely used. One major barrier to cost-effectiveness is that it takes a physician anywhere from 10 to 60 minutes to interpret a virtual colonoscopy study. One major barrier to accuracy is perceptive error (the physician does not locate a polyp that can be seen in retrospect). A computer algorithm such as the one described could address these barriers to cost-effectiveness and accuracy. The algorithm would prescreen the colon for polyps thereby reducing interpretation time and increasing accuracy of polyp detection. The goal: a test that patients prefer and that reduces suffering from colon cancer.

Summers RM, Beaulieu CF, Pusanik LM,Malley JD, Jeffrey RB Jr, Glazer DI, and Napel S: Automated polyp detector for CT colonography: feasibility study. Radiology 216: 284-290, 2000.

Summers RM, Johnson CD, Pusanik LM, Malley JD, Youssef AM, and Reed JE: Automated polyp detection at CT colonography: feasibility assessment in a human population. Radiology 219: 51-59, 2001.

HIV Infection Among Blood Plasma Donors in Rural China

Background: In the early- to mid- 1990s, a common practice among poor rural Chinese farmers was to supplement their salaries by donating plasma, the liquid portion of the blood in which the blood cells are suspended. Repeat plasma donation was allowed every 15 days, while blood donors were required to wait 3 months between donation. As a result, thousands of commercial plasma collection centers were established. For every plasma donation, about 800ml of blood was drawn, and the plasma was obtained by removing the red blood cells. These red blood cells were later re-infused into the donor. Though screening for hepatitis was done among all donors, no HIV screening existed until 1995. Once HIV infection among donors was recognized as a serious problem, the Chinese government temporarily closed the commercial plasma collection centers and issued more stringent regulations. Little is known, however, about the extent of the HIV infections among these plasma donors.

Advance: NIH-supported researchers at the University of California, Los Angeles worked with counterparts at the Chinese Academy of Preventive Medicine to identify and interview 1,517 individuals from 3 counties in rural China who had donated plasma or blood before the introduction of HIV testing in 1995. Donors and their spouses were interviewed and tested for HIV infection. Information collected included demographic characteristics, sexual behavior, recreational drug use, and history of medical and blood/plasma donation. The study found that the HIV incidence among the 1,043 plasma donors was 12.5 percent, while the HIV infection rate among non-donating spouses of plasma donors was 2.1 percent. Among the plasma donors, the risk of HIV infection increased along with the number of donations reported. Only one of the estimated 300 blood donors was HIV infected. While prevalence was inversely related to educational level and was higher in married participants, activities associated with other modes of HIV infection (invasive medical care, high-risk sexual behaviors, and drug use) could not account for increased HIV infection.

Implications: Transmission of HIV through unsafe blood donation has been virtually wiped out in countries with sophisticated medical systems. However, this mode of transmission remains a serious threat for much of the world where procedures to ensure safe donation or transfusion of blood or blood products are inadequate. The commercialization of blood and blood products, as seen in China, is not uncommon in many of these countries and results in an increased risk of HIV infection through donation and unsafe blood supplies. Most importantly, not only are donors and blood product recipients at risk, but so are their sexual partners, spouses, and newborn children. China's experience with blood and plasma donation should underscore the need for other countries to continue efforts towards global safe blood services.

Wu Z, Rou K, and Detels R: Prevalence of HIV infection among former commercial plasma donors in rural eastern China. Health Policy and Planning 16: 41-46, 2001.

The Effects of Chernobyl Radiation on Thyroid Cancer

Background: After the 1985 Chernobyl accident, the numbers of cases of thyroid cancers in children under 18 increased dramatically in areas surrounding the plant. Thus, 59 cases were recorded in the 5-year period prior to the accident compared with 577 in the decade following the accident. As a result of the huge increase, NIH-supported scientists along with those from the Ukrainian Institute of Endocrinology and Metabolism and the U.S. Department of Energy initiated a cohort study of the effects of Chernobyl radiation on thyroid cancer in children, the group most susceptible to radiation-induced cancer. In recent years, the continuation of this study has been jeopardized by the absence of an adequate in-country infrastructure for data management.

Advance: In 2000, NIH scientists requested the assistance of the University of Illinois Data Management Center in Kiev, located at the Institute of Pediatrics, to support the ongoing project. Staff at the Management Center assisted in processing a large backlog of data and established systems to prospectively manage the Chernobyl study data, consistent with expected international standards. Both the U.S. and Ukranian partners have now requested that the University of Illinois establish a satellite data management center on-site at the Institute of Endocrinology to continue to support the binational project as well as to build the capacity of the Ukranian partners. The establishment of this new data center, which will be partially supported through NIH, is in the planning stages.

Implications: The University of Illinois Data Management Center is an example of a scientific "Center of Excellence" that is facilitating high quality research in Ukraine. Other U.S. Institutes that have used the data center include the National Institute for Environmental Health Sciences and the National Center for Health Statistics (European Longitudinal Study of Pregnancy and Childhood in Ukraine - ELSPAC), the U.S. Environmental Protection Agency (Environmental Pollution and Health Status of Children), and the National Institute for Occupational Health and Mine Safety and Health Administration (Morbidity Study of Coal Miners in Donetsk). The accomplishments of the NIH-funded data center show the essential nature of investing in capacity building as a fundamental component of collaborative research between U.S. and developing countries.

Robbins J, Dunn JT, Bouville A, Kravchenko, VI, Lubin J, Petrenko S, Sullivan KM, VanMiddlesworth L, and Wolff J: Iodine nutrition and the risk from radioactive iodine: a workshop report in the chernobyl long-term follow-up study. Thyroid 11: 487-491, 2001.

A Model to Predict Survival in Patients with End-Stage Liver Disease

Background: Many efforts have been undertaken to enhance the efficiency and effectiveness of the nation's organ transplantation programs. In 1998, the Department of Health and Human Services issued the final rule on the Organ Procurement and Transplantation Network defining organ allocation policies and procedures. Principles included allocating organs among transplant candidates ranked according to medical urgency. In order to promote the efficient placement of organs, the role of waiting time was minimized, and emphasis was placed on avoidance of transplants with doubtful outcome. Implementing the new policies requires an index based on a more refined scale to measure disease severity accurately to assess the risk of death in various groups of patients with different etiology and severity of liver disease, as well as geographical diversity. A subsequent congressionally commissioned report from the Institute of Medicine (IOM) and an independent study concluded that waiting time was not an appropriate measure of the fairness of the organ allocation system. The IOM recommended that waiting time as a criterion for organ allocation be discontinued and that a medical triage system be developed to ensure equitable allocation of organs based on disease prognoses rather than waiting times.

Advance: Researchers assessed the validity of the Model for End-Stage Liver Disease (MELD) as a disease severity index for patients with end-stage liver disease awaiting liver transplantation. This model was originally developed to determine the short-term prognosis of patients with cirrhosis undergoing the transjugular intrahepatic portosystemic shunt procedure. The main question that the MELD assessment study addressed is whether the model is able to grade patients according to their risk of death within three months. Calculation of the MELD score is based on clinical criteria such as serum bilirubin, serum creatinine, and other liver function indicators. Results confirmed that the MELD statistical model is a reliable tool to assess short-term mortality risk in patients with end-stage liver disease of diverse etiology and severity.

Implications: The emphasis on severity of liver disease mandated by issuance of the Final Rule requires a disease severity index based on generalizable, verifiable, and easily obtained variables. Using the MELD score, in place of the current classification standard known as the Child-Turcotte-Pugh criteria, eliminates waiting time as a "tie breaker" for candidates awaiting liver transplantation. The population undergoing liver transplantation will most likely shift toward a less favorable prognosis given that patient and graft survival are related to the recipient's degree of illness at the time of transplantation. To its advantage, the MELD score is applicable to pretransplantation prognosis, is easy to use, and is verifiable.

Kamath PS, Wiesner RH, Malinchoc M, Kremers W, Therneau TM, Krosberg CL, D'Amico G, Dickson ER, and Kim WR: A model to predict survival in patients with end-stage liver disease. Hepatology 33: 464-470, 2001.