

Hereditary Disease Foundation

Pushing for Answers: Strategies for Inactivation of Mutant Genes

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Prepared by Lisa J. Bain

Participants

Robert AbendrothWhyte, Hirschboeck Dudek S.C.111 East Wisconsin AvenueMilwaukee WI 53202(414) 223-5011(414) 223-5000 fax**Lisa Bain**1408 Remington Rd.Wynnewood PA 19096(215) 590-7440lbain@mail.med.upenn.edu**Ronald Breaker**Dept. Molecular, Cellular & Devel. BiologyYale UniversityNew Haven CT 06520(203) 432-9389ronald.breaker@yale.edu**Robert Brown**Day Laboratory for Neuromuscular ResearchMassachusetts General Hospital149 The Navy Yard, 13th StreetCharlestown MA 02129(617) 726-5750(617) 726-8543 faxbrown@helix.mgh.harvard.edu**David Corey**Dept Pharmacol, HHMIU Texas Southwestern Med Ctr5323 Harry Hines BlvdDallas, TX 75235214 648-5096214 648-5095corey@howie.swmed.edu

Maurice FoxMassachusetts Institute of TechnologyBiology Department 68-63077 Massachusetts AvenueCambridge MA 02139(617) 253-4728(617) 253-8699 faxmsfox@mit.edu

William HauswirthDepartment of OphthalmologyUniversity of Florida College of MedicineBox100284Gainesville FL 32610-0284(352) 392-0679(352) 392-3062 faxhauswrth@eye1.eye.ufl.edu

Eric KmiecExperimental Therapeutics & Genetic MedicineJefferson Center for Biomedical Research700 East Butler AvenueDoylestown, PA 18901-2697(215) 489-4903(215) 489-4922 faxEric.Kmiec@mail.tju.edu**Stefan**

KochanekCenter for Molecular MedicineUniversity of CologneKerpener Str. 34D50931 CologneGermany011-49-221-478-3194011-49-221-478-3510 faxstefan.kochanek@medizin.uni-koeln.de

Andrew LiebermanNeurogenetics BranchNINDS10 Center DriveBethesda MD 20892-1250(301) 435-9318(301) 480-3365 faxanlieber@codon.nih.gov**Robert Montgomery**Department of SurgeryJohns Hopkins University School of MedicineBaltimore MD 21205(410) 502-6590(410) 614-

Joachim Engels

Institut fur Organische ChemieJohann Wolfgang Goethe-UniversitatD-60439 Frankfurt am MainGermany011-49-69-7982-9150011-49-69-7982-9148 faxengels@ews1.org.chemie.uni-frankfurt.de

Howard FederoffDepartment of NeurologyUniversity of Rochester601 Elmwood Ave, Box 673Rochester NY 14642(716) 273-2190(716) 756-7665 faxhoward_federoff@urmc.rochester.edu

David FinkDept of Molecular Genetics & BiochemistryUniversity of Pittsburgh School of MedicinePittsburgh PA 15261(412) 624-6506dfink@med.pitt.edu**Paul Fishman**Department of NeurologyUniversity of Maryland School of Medicine22 South Greene StreetBaltimore MD 21201-1595(410) 605-7000, ext 6112(410) 605-7937 faxpfishman@umaryland.edu

2079rmonty@welchlink.welch.jhu.edu**Nicholas**

MuzyczkaDept. of Molecular Genetics & MicrobiologyUniversity of FloridaP.O. Box 100266Gainesville, FL 32610(352) 392-8541(352) 392-3133 faxmuzyczka@college.med.ufl.edu

John RossiBeckman Research InstituteCity of Hope National Medical Center1450 East Duarte RoadDuarte CA 91010(626) 301-8360(626) 301-8271 faxjrossi@smtplink.coh.org

Ethan Signer7th Floor, 230 Park AvenueNew York, New York 10169(917) 368-5498(917) 368-5499 faxsigner@mit.edu

Hereditary Disease Foundation

11400 West Olympic Boulevard, Suite 855
Los Angeles, California 90064
(310) 575-9656

(310) 575-9156**Clifford Steer**Dept Medicine and Cell BiologyUniversity of Minnesota Medical School420 Delaware St SEMinneapolis, MN 55455-0301(612) 624-6648(612) 625-5620 faxsteer001@maroon.tc.umn.edu

Bruce Sullenger Department of Experimental Surgery Duke University Medical Center Box 2601 Med Ctr Durham, NC 27710 (919) 684-6375 (919) 684-6492

fax sulle001@mc.duke.edu **Allan Tobin** Brain Research Institute University of California, Los Angeles 2506 Gonda Hereditary Disease Foundation 11400 West Olympic Boulevard, Suite 855 Los Angeles, California 90064

(310) 575-9656

(310) 575-9156

allantobin@hdfoundation.org

Douglas Turnbull Dept. of Neurology University of Newcastle-upon-Tyne Newcastle-upon-Tyne NE2 4HH United Kingdom 011-44-191-22285650 11-44-191-2228553 fax D.M.Turnbull@ncl.ac.uk

Alice Wexler Hereditary Disease Foundation 11400 W. Olympic Blvd., Suite 855 Los Angeles, CA 90064 (310) 575-9656 (310) 575-9156 fax

Nancy Wexler Departments of Neurology and Psychiatry College of Physicians and Surgeons Columbia University 1051 Riverside Drive, Unit 6 P.I. Annex 324 New York NY 10032 (212) 543-5667 (212) 543-6002 fax

Hereditary Disease Foundation
11400 West Olympic Boulevard
Suite 855
Los Angeles, California 90064
(310) 575-9656

Center, Box 951761 Los Angeles CA 90095-1761 (310) 825-5061 (310) 206-5855 fax atobin@mednet.ucla.edu

(310) 575-9156

nancywexler@hdfoundation.org

Jan A. Witkowski Ph.D. Banbury Center Cold Spring Harbor Laboratory PO Box 534 Cold Spring Harbor NY 11724-0534 (516) 367-8398 (516) 367-5106 fax <http://www.cshl.org/banbury> Watson School of Biological Sciences <http://www.cshl.org/gradschool/ImageArchive> on the American Eugenics Movement <http://vector.cshl.org/eugenics/>

Lai-Hsing Yen Department of Neurology Yale University School of Medicine 333 Cedar St New Haven CT 06520 (203) 785-5049 (203) 785-5098 fax xyen@biomed.med.yale.edu

Scott Zeitlin College of Physicians & Surgeons Columbia University New York NY 10032 (212) 304-7157 (212) 304-7158 fax soz1@columbia.edu

Abstract:

A diverse group of scientists met for three days in May 1999, at a symposium co-sponsored by the Hereditary Disease Foundation and the Amyotrophic Lateral Sclerosis Association and its Greater New York Chapter. They discussed a wide array of tools and technologies being developed both to understand the basic biological mechanisms of these two diseases and develop strategies for correcting mutant genes. Three major challenges emerged: first, to understand more fully the pathogenic mechanisms underlying these diseases and identify the appropriate targets for gene therapy approaches; second, to develop specific tools that will correct the mutant genes; and third, to deliver these constructs to the relevant targets. Approaches discussed included viral and non-viral vectors engineered to deliver constructs such as ribozymes, chimeric oligonucleotides, and peptide nucleic acids. It will most likely be not one, but a combination of these strategies that will ultimately prove effective.

Opening a meeting of some 24 scientists who had gathered to discuss new strategies for the treatment of genetic diseases, Nancy Wexler told of her recent visit to a community in Venezuela with a high incidence of Huntington's disease. Since 1981, Wexler has been studying a large pedigree of families there to learn more about the genetics and progression of the disease and she has seen the toll it takes. It was only six years ago that these families helped her identify the gene for HD, a discovery that offered renewed hope that a cure could be found. Yet with treatments still not

Although successful therapeutic approaches for the treatment of HD and ALS are undoubtedly several years away, gene therapy techniques for other genetic diseases have moved closer to reality as the result of the development of new tools for targeting genes and repairing defects. It is the promise of these new tools that invigorated the assembled scientists. But for both HD and ALS, the first hurdle will be to more fully understand the

available, people wonder how she is able to sustain hope. "The only thing that makes it tolerable is to keep pushing for answers," she said.

Three days later, answers remained elusive but optimism was high and the search appeared robust. In recent years, an array of new technologies and refinements of existing methods have emerged that are helping scientists better define the molecular complexity of genetic disease and develop new strategies for targeting and correcting genetic defects. Moreover, the very act of convening a group of brilliant minds seemed to spawn new ideas and new collaborations.

The symposium, cosponsored by the Hereditary Disease Foundation and the Amyotrophic Lateral Sclerosis Association and its Greater New York Chapter, brought to the Banbury Center at Cold Spring Harbor Laboratory, a group of neurologists, chemists, biochemists, cell biologists, surgeons, and geneticists from as far away as Frankfurt, Germany. They shared information about successes, challenges, newly developed techniques and dreams. Three major challenges emerged as the symposium progressed: First, it will be necessary to understand more fully the pathogenic mechanisms of these two diseases and the targets of gene therapy approaches. Once this information is available, specific tools that will correct the appropriate genes will need to be developed. Finally, strategies will need to be devised that will deliver these tools to the relevant targets.

Identifying targets

pathogenic mechanisms and identify the appropriate targets for attack.

While the genetic defect in HD is known to be an expansion of polyglutamine repeats in the HD gene, how that leads to pathology is unclear. Andrew Lieberman of NINDS has been studying the polyglutamine repeat disease Spinal and Bulbar Muscular Atrophy (SBMA), a familial motor neuron disease caused by a CAG repeat expansion in the androgen receptor gene on the X chromosome. Since

the gene for SBMA (also known as Kennedy's disease) was discovered, CAG polyglutamine repeat mutations have been identified in otherwise unrelated genes in seven other neurodegenerative diseases. All of these diseases share a common pathological feature: neuronal intranuclear inclusions of ubiquitin positive protein. In the case of SBMA, the inclusions are immunopositive for the androgen receptor.

According to Lieberman, although the inclusions are found in the neurons that become dysfunctional and die, "that is not to say they are causative. Their role in neuronal dysfunction is somewhat controversial and needs to be defined experimentally." In animal models, while loss of function mutations in the androgen gene do not result in neurological features, transgenic mouse and fly models all suggest that polyglutamine expansion results in a toxic gain of function which appears to lead to cell death. Lieberman suggested three possible mechanisms: interference with transcription or RNA processing; sequestration by the aggregates of other proteins with long polyglutamine tracts, such as transcription factors; or interference with nuclear proteolysis.

Based on these possibilities, Lieberman suggested several approaches to treating polyglutamine disease including: blocking expression of the disease gene, blocking proteolytic processing and nuclear reuptake, blocking polyglutamine aggregation or some of the downstream effects of that process, or conducting drug screens using the animal and cell culture models available.

The genetic mechanisms involved in most cases of ALS are even less clear than those in HD. ALS is a neurodegenerative motor neuron disease that affects people during their years of peak productivity. According to Bob Brown of Massachusetts General Hospital, the disease is completely untreatable and leads to death usually within three to five years. Histologically, the death process in motor neurons is evidenced by deposition of aggregated threads of ubiquitin positive material. Only about 10% of ALS cases are inherited. Of these, about one-fourth have mutations in the gene for superoxide dismutase

Scott Zeitlin of Columbia University agreed that polyglutamine diseases all result from a toxic gain of function. He has been studying the normal function of the HD gene to determine whether loss of function of the normal allele would have any detrimental effects. He described a conditional knockout of the HD gene using cre-recombinase transgenes, which create a conditional null mutant.

This HD knockout mouse is smaller, less aggressive, has a reduced life span, and shows various signs of central nervous system malfunction: fore and hindlimb claspings, difficulty grasping an elevated wire rod, and difficulty walking on a narrow plank. Symptoms are progressive. Histologic staining of the cortex striatum at 12 months of age reveals disorganization. Mosaic null mutants exhibit retarded growth and microtia (lack of an external ear) as well as problems with thymus development. Brain development is retarded and there are craniofacial abnormalities and gastroschisis. According to Zeitlin, this phenotype is similar to that seen with amniotic rupture, supporting his hypothesis that huntingtin function is necessary for nutrition of the embryo.

Despite these abnormalities seen with the knockout mice, Zeitlin notes that there is little known about the function of the HD gene in adult neurons. This knowledge will be essential to understand as strategies are developed to treat HD since reducing the expression of the mutant gene may also interfere with normal huntingtin expression.

(SOD), which results in alteration of free radical homeostasis.

Over 70 dominant mutations in SOD-1 have been identified, most of which are missense mutations, indicating that the mutant gene must be present in the disease state. Mutations appear to contort the molecule and alter the active channel, increasing access of a variety of substrates to Cu⁺⁺, an active ion bound to the protein. The net result appears to be the production of highly toxic hydroxyl radicals. In addition, mutant SOD-1, under certain conditions, induces cleavage of caspase-1 and increased levels of IL-1, beta.

In what way these various reactions contribute to the neuronal damage is not yet known, said Brown. Several conceivable mechanisms were considered by participants at the conference, including direct toxicity of IL-1, which is known to be toxic to other types of neurons. Allan Tobin of UCLA wondered if ALS might be a disease of protein solubility. Paul Fishman of the University of Maryland School of Medicine suggested that since, in the SOD mutants, there appears to be an alteration in enzyme function rather than a loss or gain of function, there may be a change in substrate specificity.

New tools for repairing defects

The search for answers about mechanisms of and treatments for these and other hereditary diseases has spurred the growth of a vital tool-making industry for targeting and modifying genes and their products. What became clear by the end of the conference was that no one single approach will suffice to answer the myriad of questions, but that these techniques used in a complementary fashion may turn out to be extremely powerful.

Federoff and colleagues are using HSV plasmid-based vectors to deliver cre recombinase via stereotactic neurosurgery to specific areas of the CNS. These are simple virus vectors in which one can place virtually any promoter and any gene, or multiple genes downstream of the promoter, and then convert it into a virus that can be used to transduce a variety of cells. Cell type specificity is determined by the promoter used. He prepared six or seven virus vectors that express different forms of cre, including a fusion protein with gfp (green fluorescent protein) at the amino terminus to mark cells that have been transduced, and have shown that in the region of interest, about one quarter of the cells have undergone recombination. The next step will be to use the vector to introduce a gain of NGF function. Federoff believes this will turn out to be a powerful and robust method for producing conditional gain of function as well as loss of function mutants and will be useful in examining the role of huntingtin in adult murine neurons.

Currently perhaps the most well-developed strategy for altering genes in humans involves the

Howard Federoff of the University of Rochester is developing a somatic mosaic approach to study nerve growth factor (NGF) function in adult mice. He described his strategy as a “general approach that could be used to produce both a gain of new function as well as remove a preexisting function within the intact central nervous system.”

Alternative strategies to investigate this problem all had problems, explained Federoff. Somatic viral vector gene transfer often yields variable and non-physiological levels of gene product expression; transgenic mice lacking NGF usually die in the perinatal period; and knockouts result in complex phenotypes because of biological redundancy.

“So we thought that it would be valuable to try to develop a method whereby we could create somatic tissues that would be mosaic for a single mutation, and that we could ultimately use this to look at gene product function in the intact nervous system,” he said.

use of ribozymes, RNA molecules with enzymatic activity that can affect genes in a number of ways by disrupting mutant genes or splicing on corrected genes. Bill Hauswirth from the University of Florida College of Medicine described a gene therapy approach that uses ribozymes packaged in an adeno-associated virus (AAV) vector to treat one form of retinitis pigmentosa (RP), a genetic eye disease that affects approximately 100,000 people in the United States. RP is caused by one of a large collection of single gene mutations. The most common form of RP is caused by an autosomal dominant, single point mutation in the rhodopsin gene, which becomes the targeting site for the ribozyme. “Hairpin and hammerhead ribozymes have short targeting arms so a single mismatch would allow you to distinguish between the mutant allele and the wild type allele,” said Hauswirth.

In both cell culture and transgenic animal models, ribozymes directed to RNA transcribed from a mutant (P23H) allele of the rhodopsin gene resulted in a significant reduction in the expression

of mutant RNA but did not affect wild type RNA. More importantly, in the eyes of the rats treated with one injection of the ribozyme at birth, 80% of photoreceptors remain at day 90, compared with only about 50% in the untreated eyes. By 280 days, untreated eyes have no photoreceptors and are totally blind, while treated eyes retain about 45% of photoreceptors and the animals see well.

Said Hauswirth, "If we could achieve this kind of rescue in humans, we could delay blindness for a decade or two; effectively cure the disease." Giving a second injection might yield even better results. He noted, however, that by the time humans with RP come in for treatment they have already lost many photoreceptors. Experiments indicate that the remaining photoreceptors are rescuable, he said. Interestingly, the control ribozyme, which binds but does not cut the mutant gene, also improves vision in the rats. "We think the ribozyme *in vivo* does two things; it has an antisense effect and a catalytic ability to actually cleave and degrade the RNA."

Joachim Engels of Johann Wolfgang Goethe-Universitat in Frankfurt, Germany, reported on work he has done to disrupt the ras oncogene using hammerhead ribozymes and for HIV using a

Sullenger described *in vitro* studies using this approach. He transfected a library of ribozymes via lipofection into red blood cell precursors and let the ribozymes react inside the cells. "Inside of these patient-derived red blood cells, these ribozymes could actually convert the sickle beta-globin RNAs into ones encoding wild type beta-globin or gamma-globin," said Sullenger. Moreover, they do this with high fidelity and moderate efficiency. For this particular disease, moderate efficiency is enough, since repair of only half of the mutant RNAs should effect a cure. However, for HD or ALS, is it not clear what degree of suppression or correction of mutant RNA would be needed.

Bob Montgomery of Johns Hopkins University used a different strategy to develop a gene therapy treatment for Marfan syndrome, an autosomal dominant disease resulting from mutations in the gene for the extracellular matrix

hairpin ribozyme. He compared the chemically synthesized hammerhead ribozymes to recombinant ribozymes generated by a retroviral construct. Addressing a single point mutation in the ras oncogene, for example, he has achieved very high specificity and about a 60% reduction in ras oncogene activity, in the best case. His work suggests that there may be incomplete transformation of cells when the ribozyme is delivered by lipofection or transfection. In addition, studies using a retrovirally-expressed anti-HIV hairpin ribozyme demonstrated the ability of ribozymes to protect and confer a selective advantage on cells. According to Engels, this indicates rescue from apoptosis.

Bruce Sullenger of Duke University is using ribozymes not just to disrupt a gene, but to correct it as well, by using ribozymes that cut the mRNA and splice on a corrected gene. He is using this approach in the development of a treatment for sickle cell anemia, the most common inheritable hematologic disease, caused by a single base change in the beta-globin gene. This trans-splicing approach was necessary to correct the beta-globin defect, said Sullenger, because globin gene expression is so highly regulated that add-back gene therapy approaches do not work.

protein, fibrillin-1. Montgomery and colleagues looked at mutations in a series of patients and found that those with a high ratio of mutant-to-wild-type transcript usually have missense mutations and a full-blown Marfan phenotype, with cardiac, skeletal, and ocular manifestations. Those with a lower ratio usually have frame-shift and/or missense mutations that result in premature termination codons. These patients exhibit the milder, so-called MASS phenotype. "The threshold for the severe phenotype is about a 12% mutant-to-wild-type mRNA ratio, indicating that adding more wild type protein would not overcome the dominant negative effect," said Montgomery.

"So we came up with this sort of crazy idea," he said, which involved knocking out all the endogenous fibrillin-1 with a chimeric ribozyme, engineered for enhanced stability and expression. This was followed by a rescue step that essentially

reintroduces a modified fibrillin minigene in which the second and/or third nucleotides of the target region have been mutated in such a way that the ribozyme no longer recognizes a target sequence in the minigene mRNA, but it still encodes the wild-type amino acid sequence. "In cell culture, this construct has shown promise in restoring the cells' ability to synthesize fibrillin, said Montgomery. The cells grow normally and have a normal phenotype.

John Rossi of the City of Hope National Medical Center continued the topic of ribozymes by discussing strategies for enhancing their intracellular effectiveness. The rate-limiting feature inside the cell is the process of getting the substrate and the ribozyme together, he said. His lab has developed a series of expression cassettes with different promoters that drive ribozymes to be expressed in different cellular compartments. For example, the human beta-actin 3' untranslated region (UTR) results in trafficking of the mRNA to the tips of fibroblasts. "We're simply taking advantage of the 3' UTR trafficking information," he said. "We hook it on to a target and hook it on to a ribozyme to show that we can co-traffic the ribozyme and the target to the same subcellular

"We should be able to hook aptamers onto a ribozyme and get that ribozyme to trigger when it sees the damage, whatever that might be," he said.

This might have application for triplet repeat diseases, he continued. "We certainly can recognize those repeat sequences; that's without question," he said. "The problem is, how can you distinguish between 35 repeats and 40 repeats? I think it's doable because nature does things like this all the time, but it's a challenging engineering project."

Lai-Hsing Yen of Yale University has, in fact, been trying to develop RNA-cleaving DNA enzymes that might attack multiple CAG repeats on single-stranded regions of HD mRNA. His laboratory has designed 15 different DNA enzymes targeted to different sites. Two of these led to significant cleavage of mRNA. After modification of the enzyme to reduce cellular degradation, Yen co-transfected the enzyme with the HD gene into cells and then assayed for expression of the huntingtin protein. The enzyme

compartment." Many RNAs have such trafficking abilities, he said. "I think neuron specific mRNAs also have localization signals which might actually traffic them to the end of an axon."

Moving beyond ribozymes

A novel variation of ribozyme technology was discussed by Ronald Breaker of Yale University. Breaker's approach challenges the notion that proteins are necessary for biocatalysis. He proposes that both RNA and DNA may have enzymatic functions. Most textbooks, he said, say that DNA is used only for genetic information storage. "But we now know that's not true. At least in a test tube we can get DNA to do quite a number of things."

Breaker's laboratory has focused on testing theories about the RNA world, such as the notion that at one time all living systems were based on RNA and the idea that primitive metabolism was done by the ribosome. He has taken this one step further, engineering allosteric ribozymes that use small organic compounds to control complex metabolic reactions. While his focus has been on exploring basic biology, he also sees a role for these ribozymes in treating disease.

significantly reduced expression, he said, indicating that the enzyme is capable of operating within the cellular context. He noted, however, that while the enzyme produced preferential cleavage of mutant vs. wild type, the cleavage is inefficient and probably not specific for the HD gene. Another potential problem is getting the enzyme through the blood-brain barrier and into the CNS, he said.

Eric Kmiec of Thomas Jefferson University presented yet another approach for correcting mutant genes using chimeric oligonucleotides composed of both DNA and RNA. According to Kmiec, DNA-RNA hybrids are more stable than DNA-DNA pairings. This led to the hypothesis that the half life of the conjunction site between vector and gene could be increased using chimeras of the two ribonucleotides; and that this might increase the likelihood of gene repair at the target site. In the construct Kmiec has created, RNA is synthesized as a continuum with DNA so that it forms a double D loop structure, which provides

added stability. The RNA sections hold the structure together once the chimera has paired with the target site. “The longer this thing stays in place, the better chance you have for mismatch repair,” said Kmiec. In addition, each end of the molecule has a loop of T residues which give the molecule flexibility. Kmiec’s research indicates that the chimeric oligonucleotide is able to correct point mutations or deletions and to insert or remove single bases. His group is also pursuing the concept of correcting frame-shift mutations.

Cliff Steer of the University of Minnesota has already applied Kmiec’s approach in an effort to treat genetic diseases of the liver. Steer said the process involves two steps: the pairing event is initiated by the RNA portion of the chimera and is followed by a classic mismatch repair event using the DNA strand. Steer has been delivering the

An animal model, the Gunn rat, exists for this disease. The Gunn rat has a single base deletion and produces no enzyme. Using Kmiec’s strategy, Steer reinserted a guanosine base into the mutated gene. The construct was injected into the rat intravenously through the tail vein. Following injection, approximately 20% reinsertion was achieved, resulting in protein expression and reduction in the levels of bilirubin. A second injection produced further reduction in bilirubin levels. Steer is now preparing to begin a clinical trial of this technology among a population of Amish people in Pennsylvania, among whom Crigler-Najjar occurs at a relatively high frequency.

David Corey of the University of Texas, Southwestern Medical Center, has taken a different approach to correcting genes, using synthetic molecules called peptide nucleic acids (PNAs). PNAs are nucleic acid analogs in which a non-ionic backbone replaces the phosphate-sugar backbone found in DNA or RNA. PNAs hybridize with DNA and RNA quickly and with high affinity using Watson-Crick base pairing.

“I think it’s this exceptionally high rate of binding that is the real advantage of peptide nucleic acids, said Corey. Other advantages include the fact that the molecules are relatively easy to synthesize, have high mismatch discrimination,

constructs as compact complexes of chimera and polyethyleneimine (PEI), which are small enough to get into hepatocytes. He has also worked with liposome formulations with galactocerebroside bonded to the surface, which facilitates very efficient entry of the constructs into the cell via receptor-mediated endocytosis.

Steer is developing this technology to correct the genetic defect in Crigler-Najjar syndrome, a very rare autosomal recessive disease caused by a single base deletion or conversion in the gene for the enzyme uridine diphosphate glucuronosyltransferase (UDPGT). UDPGT is necessary for conjugation of bilirubin prior to excretion. Individuals with this condition have high levels of unconjugated bilirubin at birth. If they survive, they often require a liver transplant.

and a low propensity to bind other proteins that normally bind to nucleic acids. Corey has been using PNAs to study cellular complexity; specifically he has chosen the ribonuclear protein, telomerase, as a target. He suggested that PNAs may be useful for both antisense and antigene inhibition of gene expression, “and that might lead to a better understanding of protein function and cellular signaling.” Whether PNAs will also be a useful tool for correcting mutations is less clear, he said.

Doug Turnbull, of the University of Newcastle-upon-Tyne, however, has already been working with PNAs in an effort to develop treatments for mitochondrial diseases. Mitochondrial diseases present an entirely new set of challenges for toolmakers, both because mitochondrial DNA is inherited differently than nuclear DNA, and because targeting the mitochondrial DNA will require delivery of gene repair constructs into the mitochondria. Targeting domains for delivery into mitochondria have been identified, noted Paul Fishman. Turnbull thinks mitochondrial mutations may have some impact on neurodegenerative disease such as ALS and motor neuron disease. “We’re all acquiring mitochondrial mutations as we age,” he said.

PNAs get taken up by the nucleus as well as the mitochondria, said Turnbull. “Very

preliminary data” suggest that this might be a feasible strategy to correct deletions and point mutations in mitochondria. “But we’ve got a million miles to go,” he said.

Getting the tools where they’re needed

Many of the vectors developed to deliver gene therapy constructs into cells take advantage of the strategies viruses have evolved to introduce their genomes into host cells. One of the most studied of these viral vectors is the adenovirus, a double-stranded DNA virus of approximately 36

Kochanek and colleagues have modified this approach by producing what he calls “gutless” adenoviral vectors. These vectors contain only the genes of interest plus an appropriate promoter, but lack all the other viral genes including those that would induce an immune reaction. They are produced in cell culture using a helper virus, which allows packaging of the vector into an infectious particle. Using this system, Kochanek produced a vector containing the entire gene for alpha-1-antitrypsin including two natural promoters, one of which is active in hepatocytes and the other in macrophages. The vector was injected into mice that are unable to mount an immune response against the human alpha-1-antitrypsin. After intravenous injection in mice, adenoviral vectors predominantly infect liver cells.

After gene transfer of this gutless vector, there was an increase [of alpha-1-antitrypsin] for 3 to 4 weeks and there was complete stabilization of expression for more than one year,” said Kochanek. He showed that the protein was expressed in hepatocytes and that protein expression was driven by the hepatocyte promoter. In addition, no histopathology or toxic damage to the liver was observed even with very high vector doses.

Kochanek cited two features of the gutless approach that open numerous possibilities for gene therapy development. First, the capsid protein can be modified by inserting different ligands in order to target different cell types. This technology will likely allow efficient and specific gene transfer into the many different cell types of the body in the future. Second, promoters can be used which offer either tissue specificity, as in the alpha-1-

kb that normally causes only a mild illness in humans. According to Stefan Kochanek, of the University of Cologne, adenovirus vectors are good transducers of cells because once the virus makes it through the cytoplasmic membrane, transport to the nucleus is very efficient. Yet the effectiveness of these vectors has been limited by the fact that in some cell types there is 1) low uptake of DNA because the adenovirus receptor is not present on the cell surface, and 2) because expression of adenoviral genes following gene transfer generates an immune response in humans. antitrypsin experiments, or drug inducibility. In one series of experiments, Kochanek’s team engineered a vector that delivered human growth hormone with a promoter inducible by the drug, RU-486. Both *in vitro* and *in vivo*, they were able to turn expression of the gene on and off with RU-486.

Another vector that has attracted much interest is the adeno-associated virus (AAV), a single-stranded, transcriptionally-inactive parvovirus that requires help from adenovirus in order to replicate. According to Nick Muzyczka of the University of Florida, help can be provided either by coinfection with adenovirus or by placing adenoviral genes on the AAV plasmid. Muzyczka has been working on developing AAV vectors to treat neurologic diseases by inserting the promoters for neurotrophic factors such as platelet derived growth factor-beta (PDGF-beta). In addition to using these promoters, he said, AAV has a natural tropism for neuronal cells. He delivers the vector by direct injection into various areas of the brain. Injection into the substantia nigra results in non-uniform spread over about 2 mm; whereas injection into the hippocampus results in better spread. With no pathogenicity and good transduction, the approach has the capacity to target specific structures within the brain, said Muzyczka. He added that different types of neural cells exhibit variable tropisms and abilities to be transduced.

Muzyczka has shown that BDNF (brain-derived neurotrophic factor) delivered into the brain with this system results in behavioral effects but no sparing of neurons. “What we’ve got is a technology in which we can inject the

hippocampus and convert 80-90% of the susceptible neurons. Now the question is, what do we want to put in that will modify behavior or susceptibility to disease,” he said.

Another viral vector with natural neural tropism is the herpes virus. David Fink and colleagues at the University of Pittsburgh have demonstrated that their vector can be used to deliver and express genes in both the peripheral and central nervous system. They have produced a non-replicating vector that expresses

One of the advantages of herpesvirus as a gene therapy tool is its large size, which offers the potential to incorporate large genes or multiple genes for deliver to the target. Fink and co-workers have created a vector that expresses five different transgenes from independent loci with independent promoters. “For those of us who are optimistic about the ultimate utility of gene transfer as a therapy, I think there are going to be niches for many different vectors for different applications. One of the things, besides the neurotropism of herpes, that makes it different from some of the others is that if you have to deliver different gene products into the same cell, you can actually do that with this vector,” he said.

One non-viral vector system applicable to neurologic disease exploits the affinity of tetanus toxin for nerve terminals. According to Paul Fishman, of the University of Maryland, tetanus toxin is constructed from three domains. One of these, called fragment-C, is non-toxic and in its recombinant form makes a good delivery vehicle which has high affinity binding, neuron selectivity, a very long intracellular lifetime, and transneuronal, transynaptic transport. One injection into the leg of an animal results in labeling of every motor neuron in the body, said Fishman.

Researchers in Bob Brown’s lab used this technology to deliver SOD to motor neuron cells and showed that the cellular SOD content tripled as a result. Yet while SOD has been shown to be neuroprotective in a stroke model, this approach did not appear to offer protection in an ALS model.

preproenkephalin, which can be used to express the gene in dorsal root ganglion to reduce pain perception in rats. A similar vector expressing nerve growth factor can be used to deliver the peptide into dorsal root ganglion cells, which can prevent nerve cell degeneration in experimental models of peripheral neuropathy. The nuclear localization of the natural latency transcript (LAT) may prove to be useful in the nuclear targeting of transgene products for therapeutic applications.

Fishman said the TetC hybrids localize in endosomal vesicles, on membrane surfaces, and at synapses. Now, he and others are trying to identify proteins, such as thermolysin or glutamate degrading enzymes, that might have a beneficial effect if localized to these compartments. For instance, thermolysin may accelerate A-Beta degradation and thereby reduce amyloid toxicity in a variety of neurodegenerative diseases, including ALS. In collaboration with Bob Brown’s group, he has also made hybrid proteins that use the translocation domain from diphtheria toxin to allow the protein of choice to escape the endosome and have access to cytoplasmic and nuclear targets.

Conclusion

In the closing session of the symposium, Bill Hauswirth reminded the group that research on ALS and HD is where RP research was only a few years ago. An effective therapy, he said, will require an approach that: covers the infected tissue, perhaps with multiple injections; has cell type specificity and lack of toxicity; and provides the appropriate amount of gene expression. He also emphasized the need for good animal models and good non-invasive assays of pathology. Many people commented on the need to combine tools and continue to develop the wide array of technologies.

Despite recognition of the daunting challenges ahead, the symposium generated much excitement. In a post meeting-comment, David Corey wrote “What little is known about the biological mechanisms of these diseases demonstrates that they are exceptionally interesting scientifically.... this scientific fascination probably

won't comfort people suffering with these diseases or their families, but it should attract the attention of a large audience of scientists.”