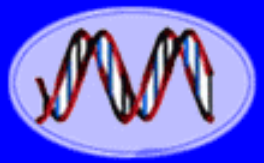
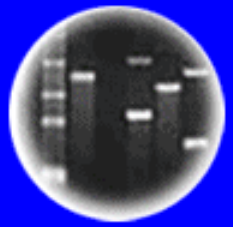


DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria



DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

HC70A & SAS70A Winter 2012
Genetic Engineering in Medicine, Agriculture, and Law

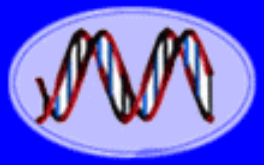
Professors John Harada & Bob Goldberg

Lecture 8

Human Genetic Engineering and Gene Therapy

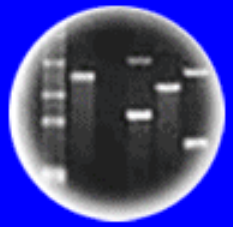
UCLA

UC DAVIS
UNIVERSITY OF CALIFORNIA



DNA
Genetic Code of Life

Entire Genetic Code of a Bacteria



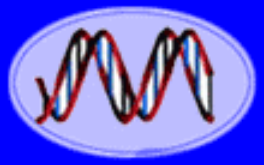
DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

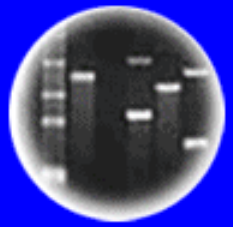
THEMES

1. Review Genetic Engineering Applications (Bacteria to Animals & Plants)
2. Human Gene Therapy-Genetic Engineering Humans
   - a. What is Gene Therapy?
     - i. Germ Line
     - ii. Somatic Cell
   - b. Types of Somatic Cell Gene Therapy
     - i. Ex Vivo Gene Therapy
     - ii. In Vivo Gene Therapy
   - c. Example of Ex Vivo Gene Therapy
     - i. Severe Combined Immunodeficiency (SCID)
     - ii. Using Retroviruses For Gene Therapy
     - iii. Leukemia
     - iv. β-Thalasemmia
   - d. Examples of In Vivo Gene Therapy
     - i. Leber Congenital Amaurosis
     - ii. Hemophilia B
     - iii. Brain Tumors
     - iv. Cystic Fibrosis
   - e. Gene Therapy Trials & Recent Advances
   - f. Problems and Issues With Human Gene Therapy



DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria



DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

THEMES (continued)

3. Using Gene Therapy to Deliver "Molecular Drugs"
   a. Anti-Sense and RNAi
   b. Ribozymes

Some Uses of Genetic Engineering
Review Of Genetic Engineering Applications
Parts One & Two

1. Bacteria
2. Fungi
    a. Drugs
    b. Fermentation
3. Animals
    a. Mouse Model-Knock-Outs-Human Gene Functons
    b. Farm Animals-Drugs
4. Plants
    a. Spectrum of Genes Engineered
    b. Specific Examples of Genetically Engineered Crops
    c. The GMO Crop Landscape
    d. Reasons For Opposition to GMO Crops
5. GMO "Logic" Based on Science & What We Know About Genes & Gene Function

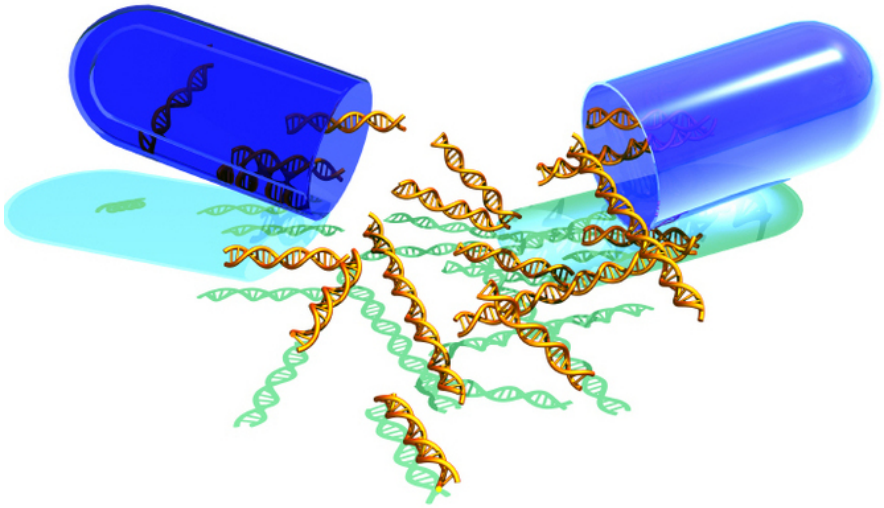
Human Genetic Engineering and Gene Therapy

Gene Therapy

- Germline gene therapy

- Somatic gene therapy
  - Gene supplementation
  - Gene replacement
  - Targeted killing of specific cell-types
  - Targeted inhibition of gene expression

- Issues
  - Regulation
  - NIH Guidelines
  - Human Experimentation
  - Ethics
  - Eugenics



21.4 Principles of gene therapy

Gene therapy involves the direct genetic modification of cells of the patient in order to achieve a therapeutic goal. There are basic distinctions in the types of cells modified, and the type of modification effected.

① ▸ **Germ-line gene therapy** produces a permanent transmissible modification. This might be achieved by modification of a gamete, a zygote or an early embryo. Germ-line therapy is banned in many countries for ethical reasons (see *Ethics Box 2*).

② ▸ **Somatic cell gene therapy** aims to modify specific cells or tissues of the patient in a way that is confined to that patient. All current gene therapy trials and protocols are for somatic cell therapy.

Somatic cells might be modified in a number of different ways (*Figure 21.4*).

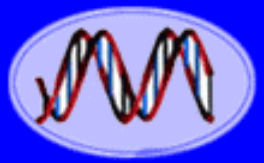
a. ▸ **Gene supplementation** (also called gene augmentation) aims to supply a functioning copy of a defective gene. This would be used to treat loss-of-function conditions (Section 16.4) where the disease process is the result of a gene not functioning here and now. Cystic fibrosis would be a typical candidate. It would not be suitable for loss-of-function conditions where irreversible damage has already been done, for example through some failure in embryonic development. Cancer therapy could involve gene supplementation to increase the immune response against a tumor or to replace a defective tumor suppressor gene.

b. ▸ **Gene replacement** is more ambitious: the aim is to replace a mutant gene by a correctly functioning copy, or to correct a mutation *in situ*. Gene replacement would be required for gain-of-function diseases where the resident mutant gene is doing something positively bad.

c. ▸ **Targeted inhibition of gene expression** is especially relevant in infectious disease, where essential functions of the pathogen are targeted. It could also be used to silence activated oncogenes in cancer, to damp down unwanted responses in autoimmune disease and maybe to silence a gain-of-function mutant allele in inherited disease.

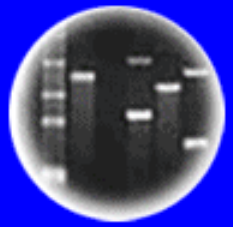
d. ▸ **Targeted killing of specific cells** is particularly applicable to cancer treatment.

Issues
Regulation?
NIH Guidelines?
Human Experimentation?
Ethics?
Eugenics?



DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria



DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

Which type(s) of gene therapy should be allowed?

a. Germline cell gene therapy
b. Somatic cell gene therapy
c. Both
d. Neither

Questions to Consider Before Initiating Gene Therapy

1. Does the condition result from a mutation of one or more genes?
2. What is known about the biology of the disorder?
3. Has the gene been cloned?
4. Will adding a normal copy of the gene fix the problem in the affected tissue?
5. Can you deliver the gene to cells of the affected tissue?

Ex Vivo vs In Vivo Somatic Cell Gene Therapy

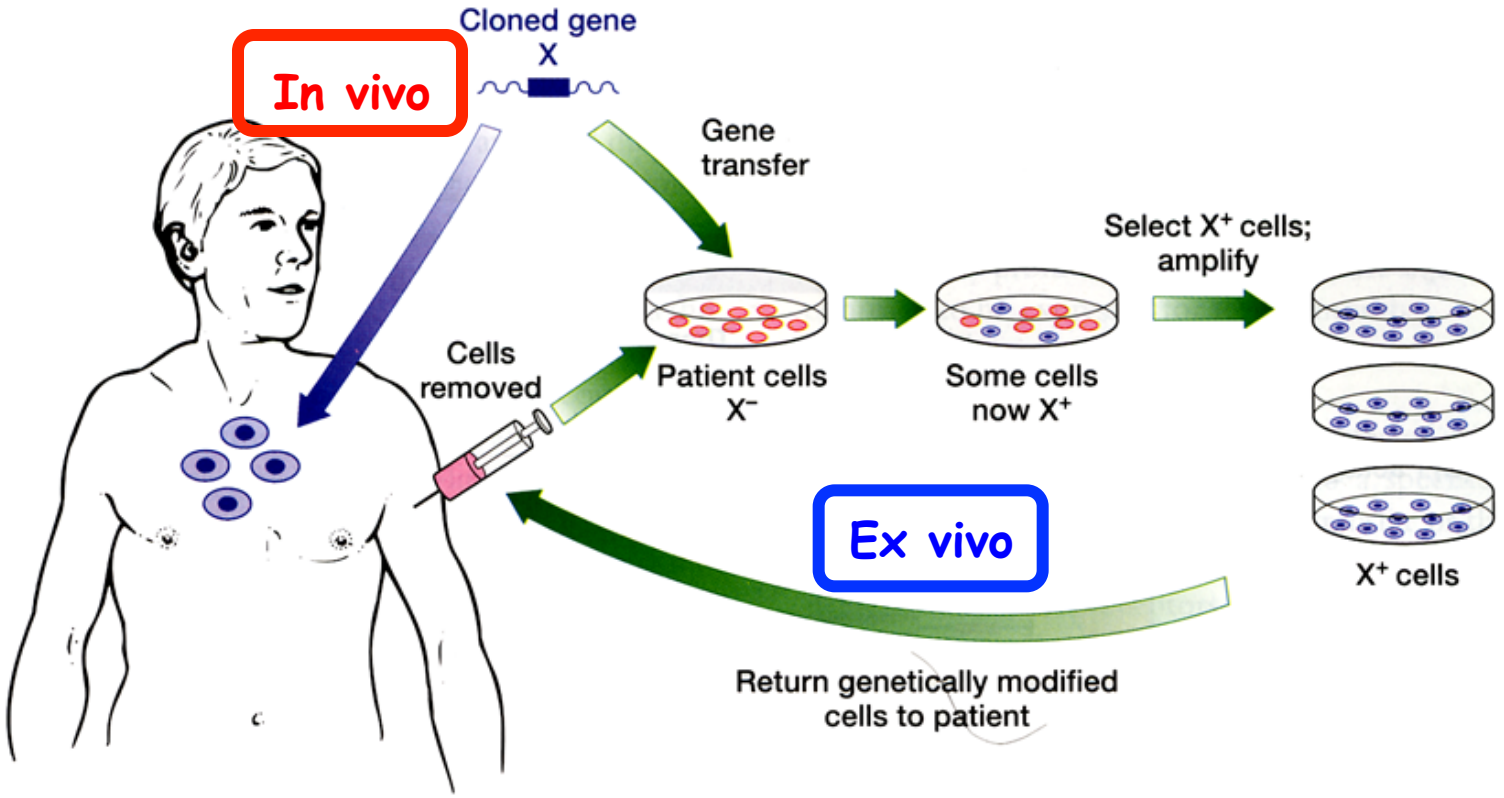
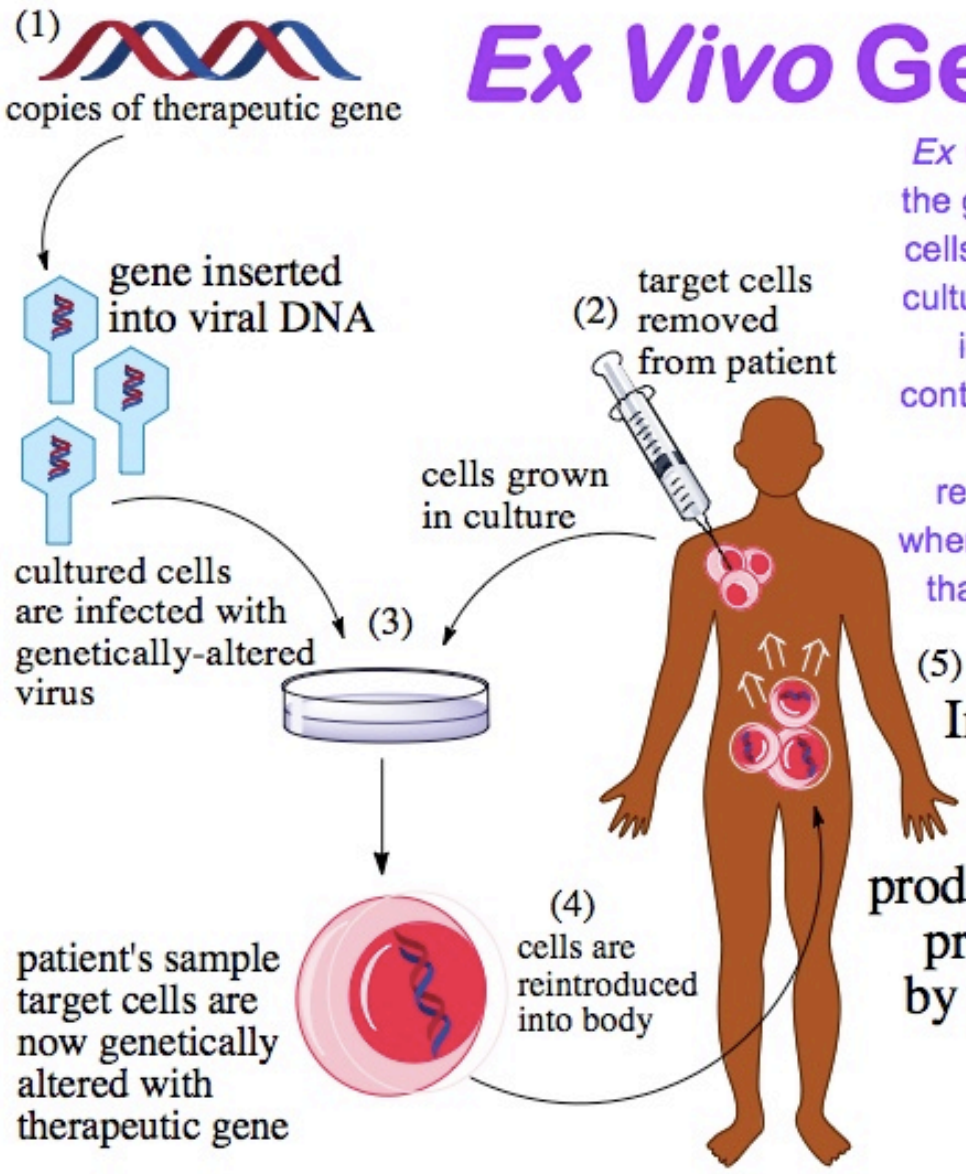


Figure 21.6: *In vivo* and *ex vivo* gene therapy.

Where possible, cells are removed from the patient, modified in the laboratory and returned to the patient (*ex vivo* gene therapy; green arrows). This allows just the appropriate cells to be treated, and the cells can be checked before they are replaced to make sure that the desired change has been achieved. For many tissues this is not possible and the cells must be modified within the patient's body (*in vivo* gene therapy; blue arrow).

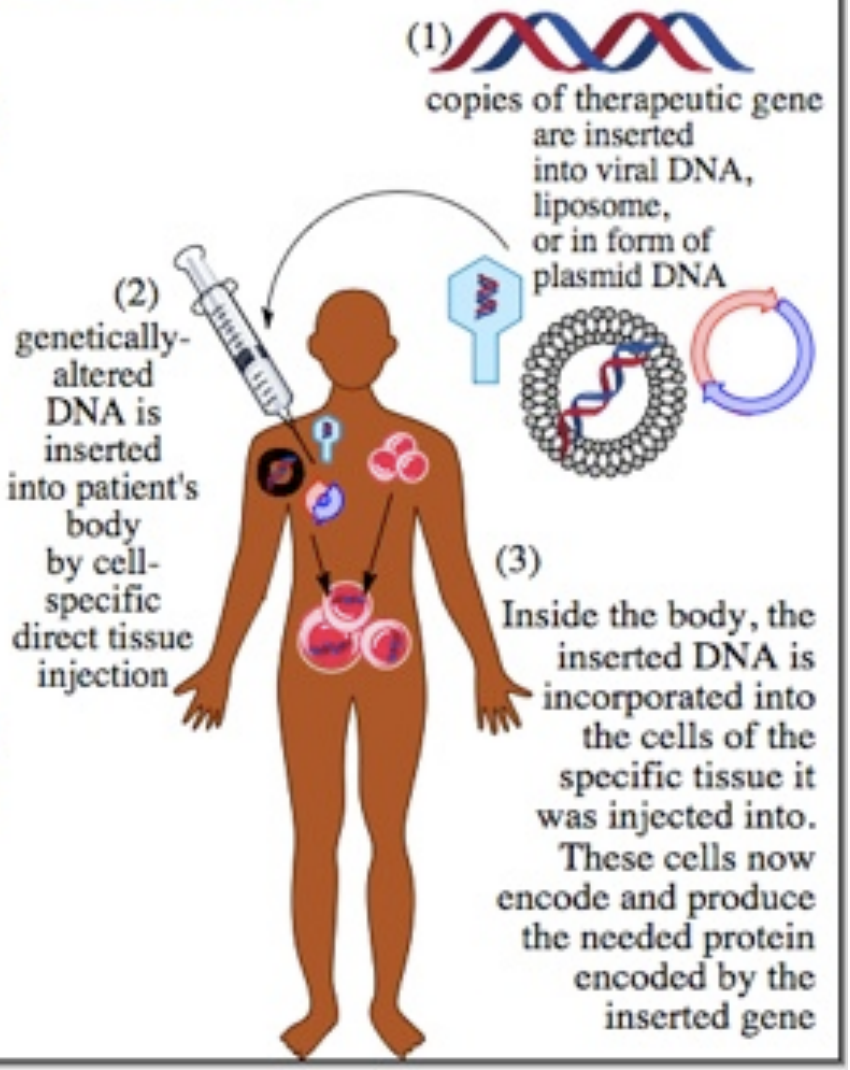
Ex Vivo Gene Therapy

Ex vivo gene therapy is performed with the genetic alterations of patient's target cells happening outside of the body in a culture. Target cells from the patient are infected with a recombinant virus containing the desired therapeutic gene. These modified cells are then reintroduced into the patient's body, where they produce the needed proteins that correspond to the inserted gene.

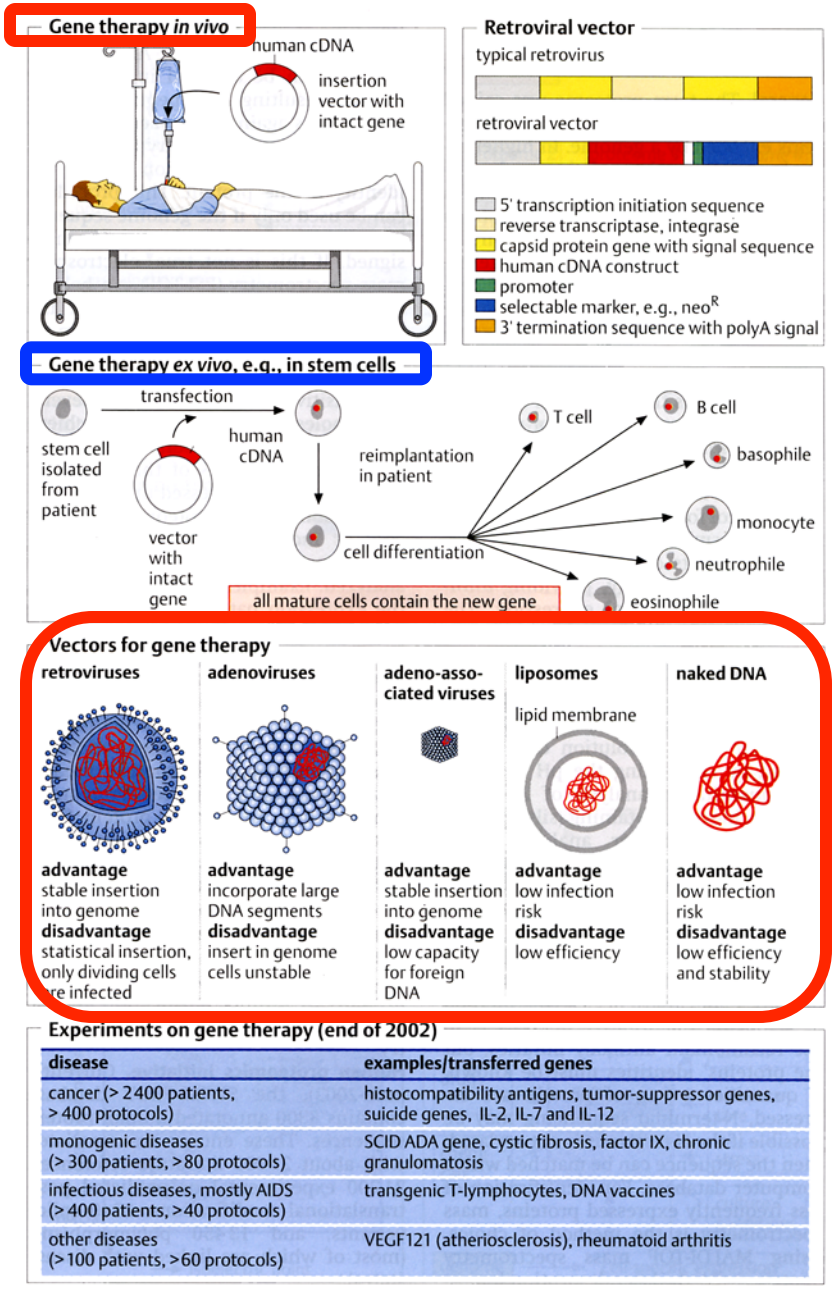


In Vivo Gene Therapy

In vivo gene therapy involves introduction of therapeutic DNA directly into the patient's body. The DNA is introduced by cell-specific direct injection into tissue in need. DNA in the form of a plasmid vector is introduced by a dermal vaccination. Modified liposomes are not currently used for gene therapy, but they will likely be the next advancement in therapeutic gene delivery as cell-specific receptor-mediated DNA carriers. Once inside the body and in contact with the specifically targeted cells, the inserted DNA is incorporated into the tissue's cells where it encodes the production of the needed protein.



Ex Vivo vs In Vivo Somatic Cell Gene Therapy



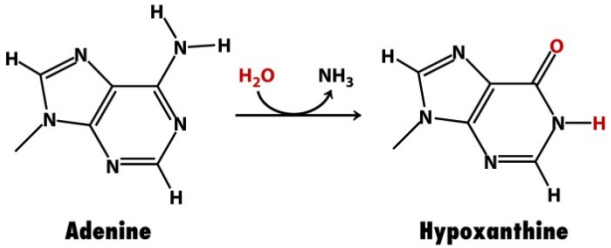
Experiments on gene therapy (end of 2002)

disease	examples/transferred genes
cancer (> 2400 patients, > 400 protocols)	histocompatibility antigens, tumor-suppressor genes, suicide genes, IL-2, IL-7 and IL-12
monogenic diseases (> 300 patients, > 80 protocols)	SCID ADA gene, cystic fibrosis, factor IX, chronic granulomatosis
infectious diseases, mostly AIDS (> 400 patients, > 40 protocols)	transgenic T-lymphocytes, DNA vaccines
other diseases (> 100 patients, > 60 protocols)	VEGF121 (atheriosclerosis), rheumatoid arthritis

Ex Vivo Gene Therapy Example

Adenosine Deaminase Gene (*ADA*) Deficiency and Severe Combined Immunodeficiency (SCID) Disease

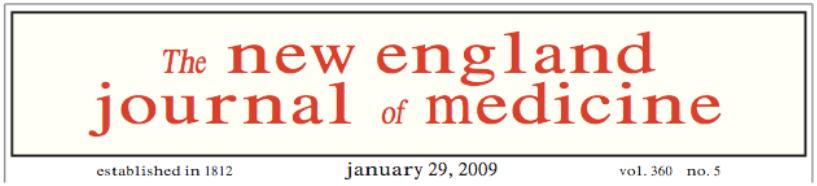
32,213 kb Gene
Chromosome 20
12 Exons
1,092 kb mRNA
323 aa protein



Degradation of Purine

David Vetter-Died at Age 12

- ADA deficiency results in elevated adenosine and deoxyadenosine levels
- Abnormal levels impair lymphocyte development and function
- **The immune system is severely compromised or completely defective**



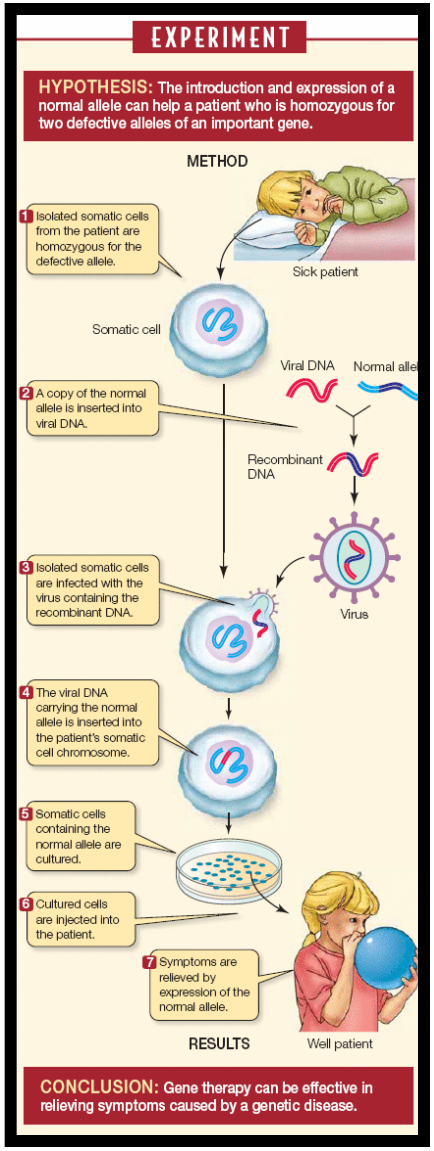
Gene Therapy for Immunodeficiency Due to Adenosine Deaminase Deficiency

Gene therapy cures 'bubble boy disease'

31 Jan 2009, 1128 hrs IST, AP

Humans Have Been Genetically Engineered To Cure a Lethal Genetic Disease (SCID)

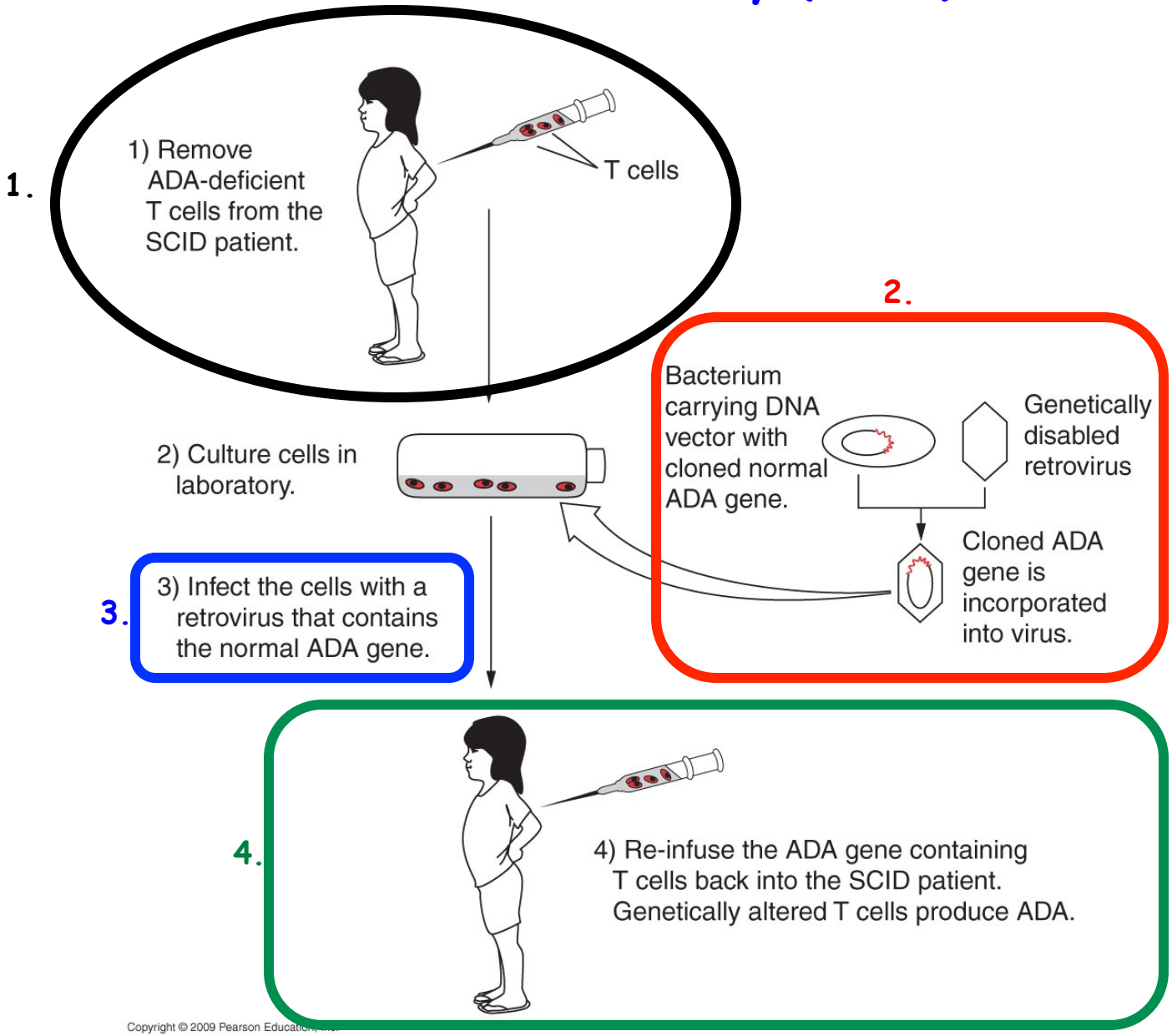
The Age of Human Genetic Engineering Began More Than Twenty Years Ago – SCID Treated With Normal ADA Gene!!!



Several Teenagers Are Alive Because They Have Been Engineered With an ADA Gene That They Were Not Born With!!!

Adenosine Deaminase Gene (ADA)

Ex Vivo Gene Therapy for Severe Combined Immunodeficiency (SCID)



Animal Viruses are Used as Vectors to Deliver Genes for Gene Therapy

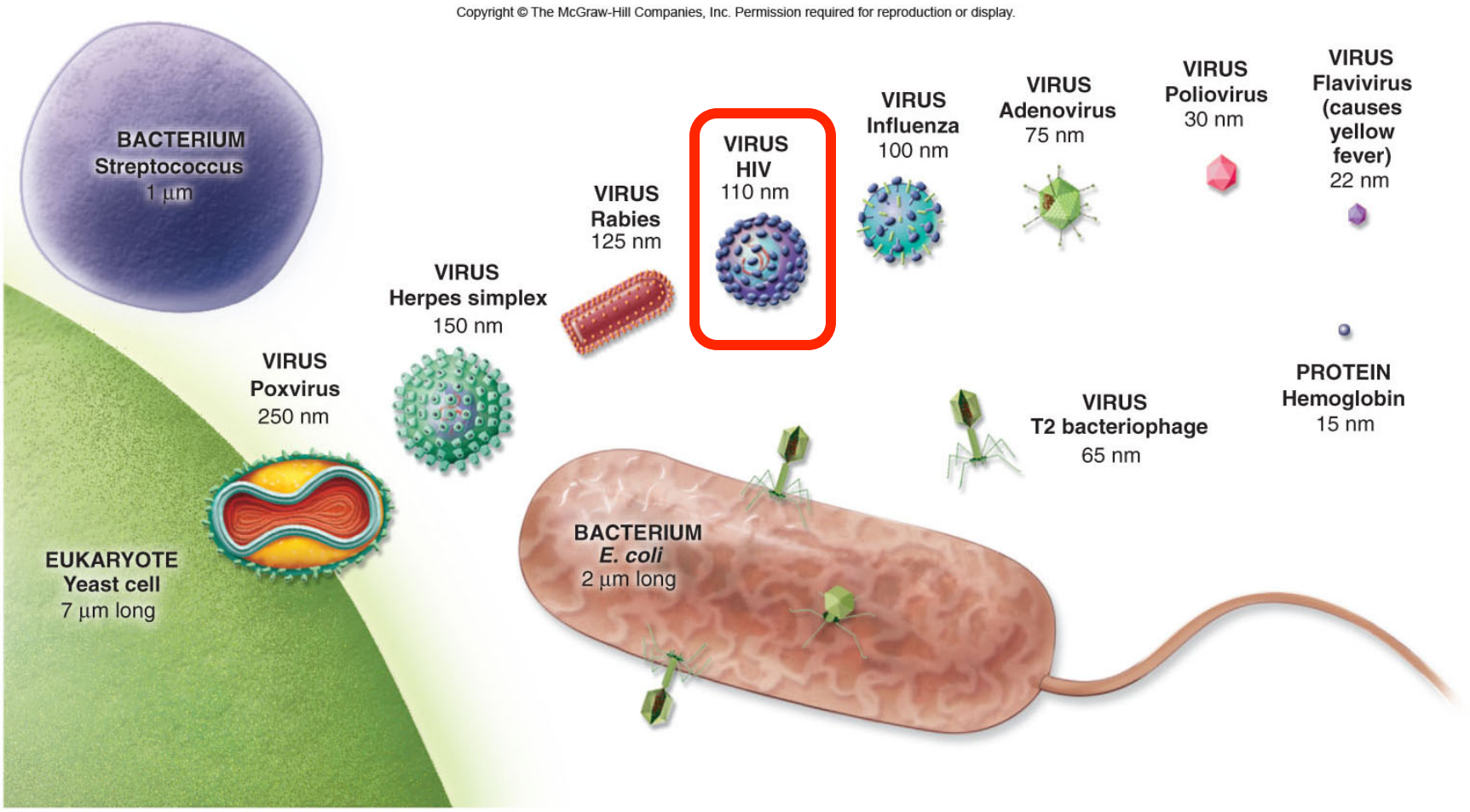
Table 19.3 Vectors used in gene therapy

Vector	Advantages	Disadvantages
Retrovirus	Efficient transfer	Transfers DNA only to dividing cells, inserts randomly; risk of producing wild-type viruses
Adenovirus	Transfers to nondividing cells	Causes immune reaction
Adeno-associated virus	Does not cause immune reaction	Holds small amount of DNA; hard to produce
Herpes virus	Can insert into cells of nervous system; does not cause immune reaction	Hard to produce in large quantities
Lentivirus	Can accommodate large genes	Safety concerns
Liposomes and other lipid-coated vectors	No replication; does not stimulate immune reaction	Low efficiency
Direct injection	No replication; directed toward specific tissues	Low efficiency; does not work well within some tissues
Pressure treatment	Safe, because tissues are treated outside the body and then transplanted into the patient	Most efficient with small DNA molecules
Gene gun (DNA coated on small gold particles and shot into tissue)	No vector required	Low efficiency

Source: After E. Marshall, Gene therapy's growing pains, *Science* 269:1050–1055, 1995.

Table 19-3
Genetics: A Conceptual Approach, Third Edition

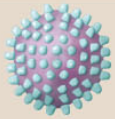
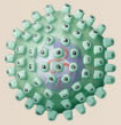
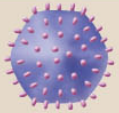
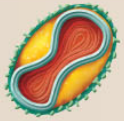
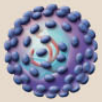
Comparison of Virus and Cell Sizes



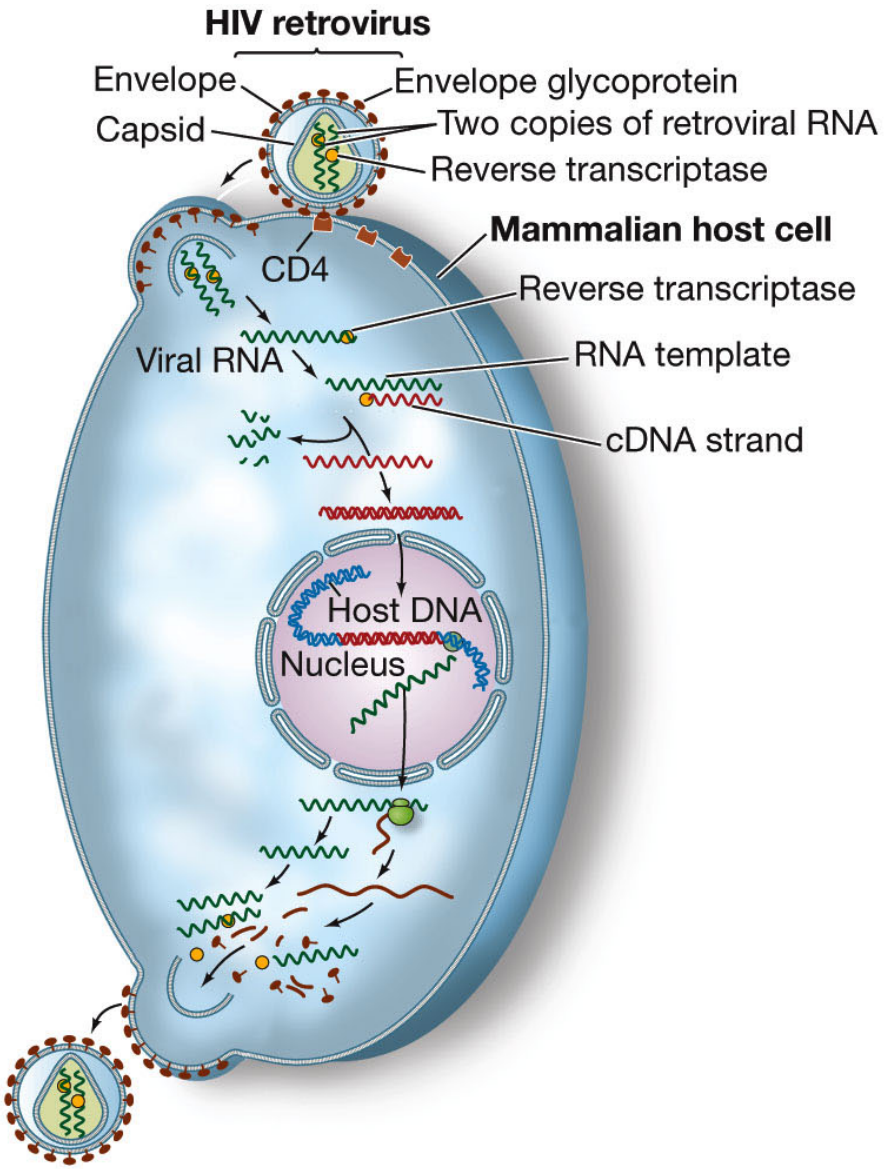
Note: 1 nm = 10^{-9} m

Human Retroviruses Are Used As Gene Therapy Vectors

TABLE 27.1 Important Human Viral Diseases

Disease	Pathogen	Genome	Vector/Epidemiology
Chicken pox	Varicella zoster 	Double-stranded DNA	Spread through contact with infected individuals. No cure. Rarely fatal. Vaccine approved in U.S. in early 1995.
Hepatitis B (viral)	Hepadnavirus 	Double-stranded DNA	Highly infectious through contact with infected body fluids. Approximately 1% of U.S. population infected. Vaccine available. No cure. Can be fatal.
Herpes	Herpes simplex virus 	Double-stranded DNA	Blisters; spread primarily through skin-to-skin contact with cold sores/blisters. Very prevalent worldwide. No cure. Exhibits latency—the disease can be dormant for several years.
Mononucleosis	Epstein–Barr virus 	Double-stranded DNA	Spread through contact with infected saliva. May last several weeks; common in young adults. No cure. Rarely fatal.
Smallpox	Variola virus 	Double-stranded DNA	Historically a major killer; the last recorded case of smallpox was in 1977. A worldwide vaccination campaign wiped out the disease completely.
AIDS	HIV 	(+) Single-stranded RNA (two copies)	Destroys immune defenses, resulting in death by infection or cancer. As of 2005, WHO estimated that 40 million people are living with AIDS; 4.1 million new HIV infections were predicted and 2.8 million deaths were expected. More than 25 million have died from AIDS since 1981.
Polio	Enterovirus 	(+) Single-stranded RNA	Acute viral infection of the CNS that can lead to paralysis and is often fatal. Prior to the development of Salk's vaccine in 1954, 60,000 people a year contracted the disease in the U.S. alone.

HIV is a Retrovirus



T-Cell

LIFE 8e, Figure 13.6

Discovery of Retroviruses

The Retrovirus Genome Encodes Reverse Transcriptase

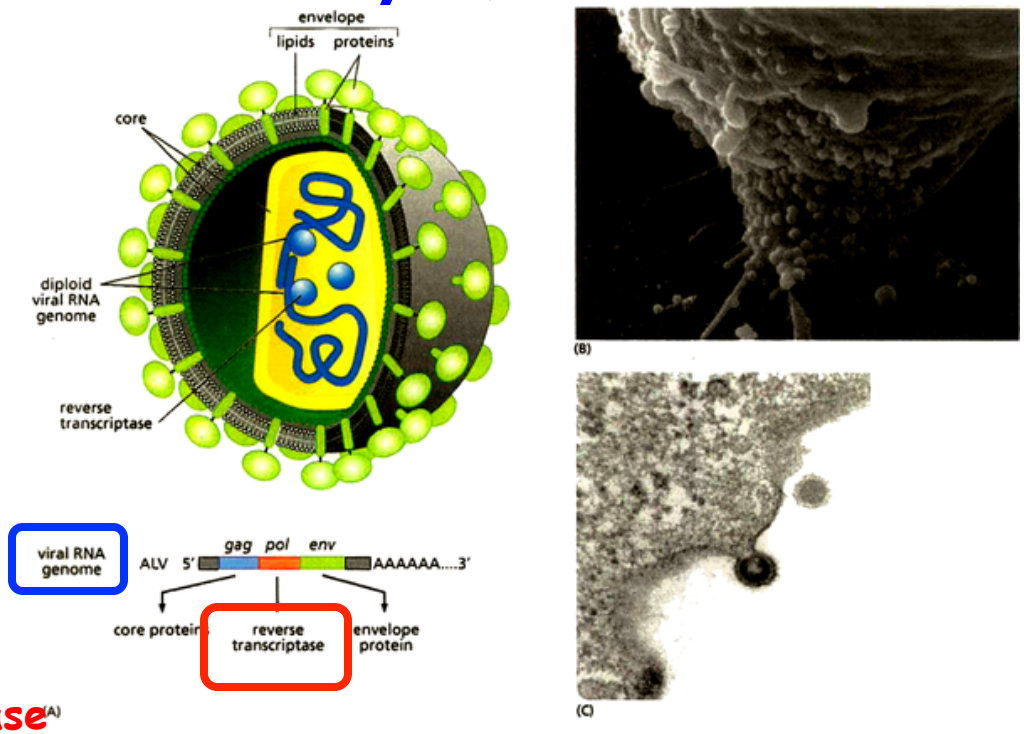


Figure 3.4 The virion of RSV and related viruses (A) This schematic drawing of the structure of a *retrovirus* virion, such as that of Rous sarcoma virus, indicates three major types of viral proteins. The glycoprotein spikes (encoded by the viral *env* gene) protrude from the lipid bilayer that surrounds the virion; these spikes enable the virion to *adsorb* (attach) to the surface of a cell and to introduce the internal contents of the virion into its cytoplasm. These include a complex protein coat formed by the several core proteins encoded by the viral *gag* gene. Within this protein shell are found two identical copies of the viral genomic RNA and a number of enzyme molecules specified by the viral *pol* gene. (B) Scanning electron micrograph and (C) transmission electron micrograph showing murine leukemia virus (MLV) particles budding from the surface of an infected cell. As the nucleocapsids (containing the gag proteins, the virion RNA, and the reverse transcriptase) leave the cell, they wrap themselves with a patch of lipid bilayer taken from the plasma membrane of the infected cell. (A, adapted from H. Fan et al., The Biology of AIDS. Boston, MA: Jones and Bartlett Publishers, 1989; B, courtesy of Albert Einstein College of Medicine; C, courtesy of Laboratoire de Biologie Moleculaire.)

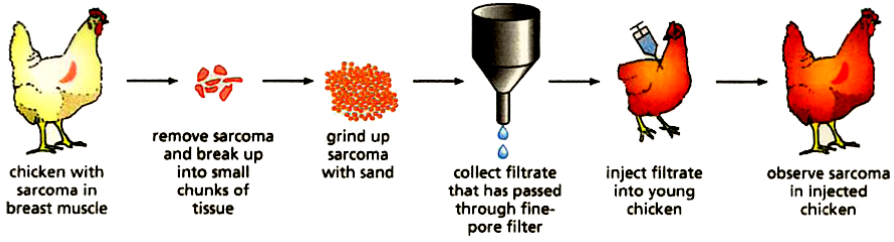
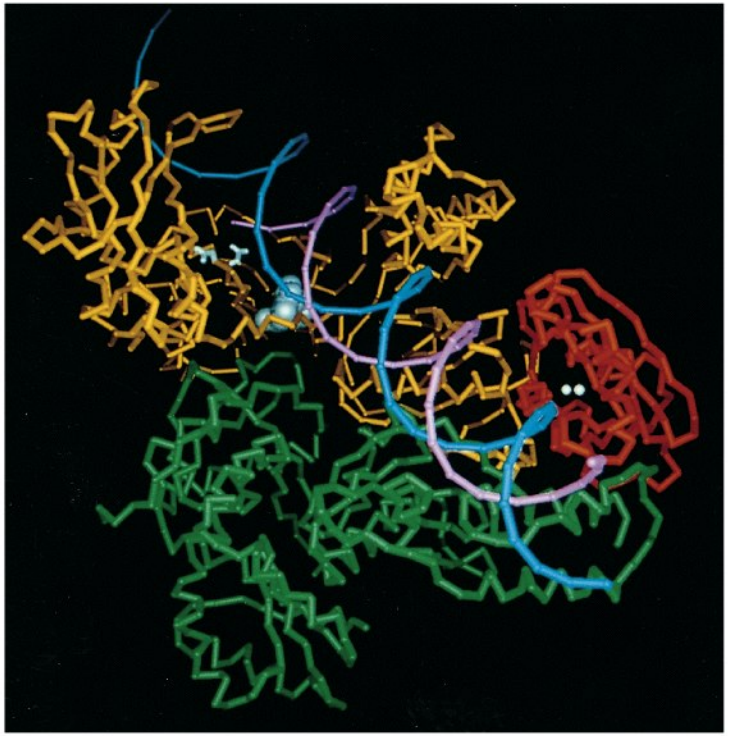


Figure 3.2 Rous's protocol for inducing sarcomas in chickens Rous removed a sarcoma from the breast muscle of a chicken, ground it with sand, and passed the resulting homogenate through a fine-pore filter. He then injected the filtrate (the liquid that passed through the filter) into the wing web of a young chicken and observed the development of a sarcoma many weeks later. He then ground up this new sarcoma and repeated the cycle of homogenization, filtration, and injection, once again observing a tumor in another young chicken. These cycles could be repeated indefinitely; after repeated serial passaging, the virus was able to produce sarcomas far more rapidly than the original viral isolate.

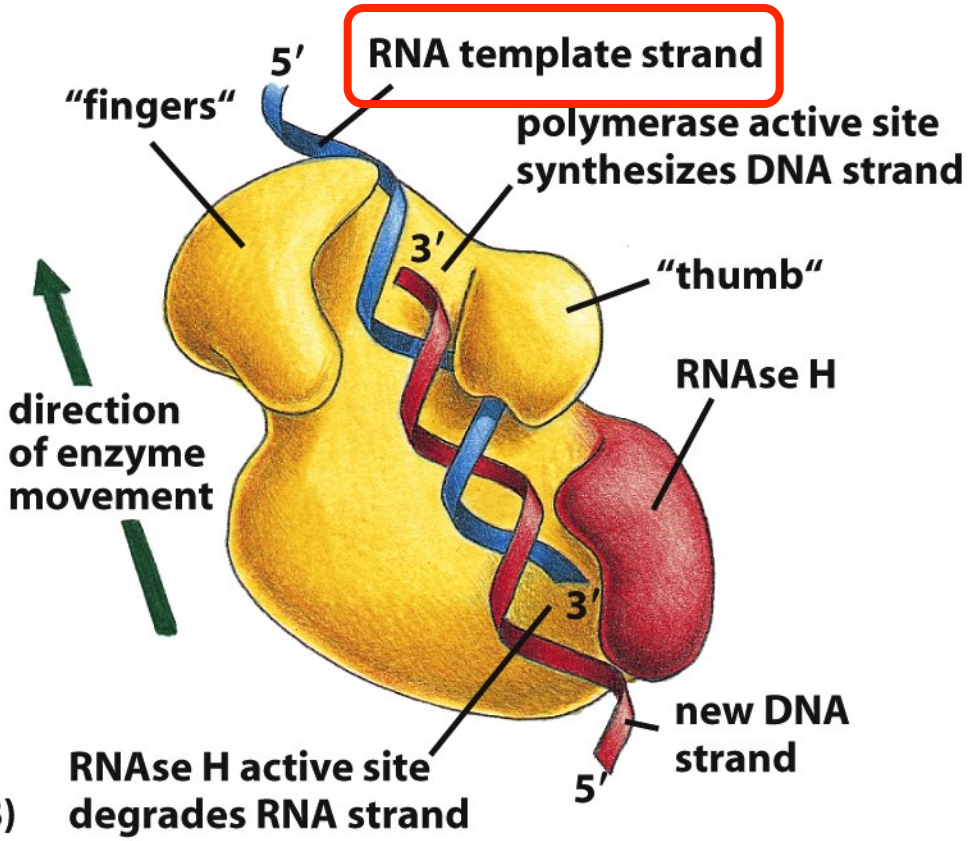
Rous Sarcoma Virus is a Retrovirus That Cases Cancer and Contains Oncogenes in its Genome

Francis Peyton Rous
Nobel Prize, 1966

Reverse Transcriptase is Encoded by a Retrovirus Genome and Converts the RNA Genome into a Double-Stranded DNA Genome That is Integrated Into a Host Cell



(A)

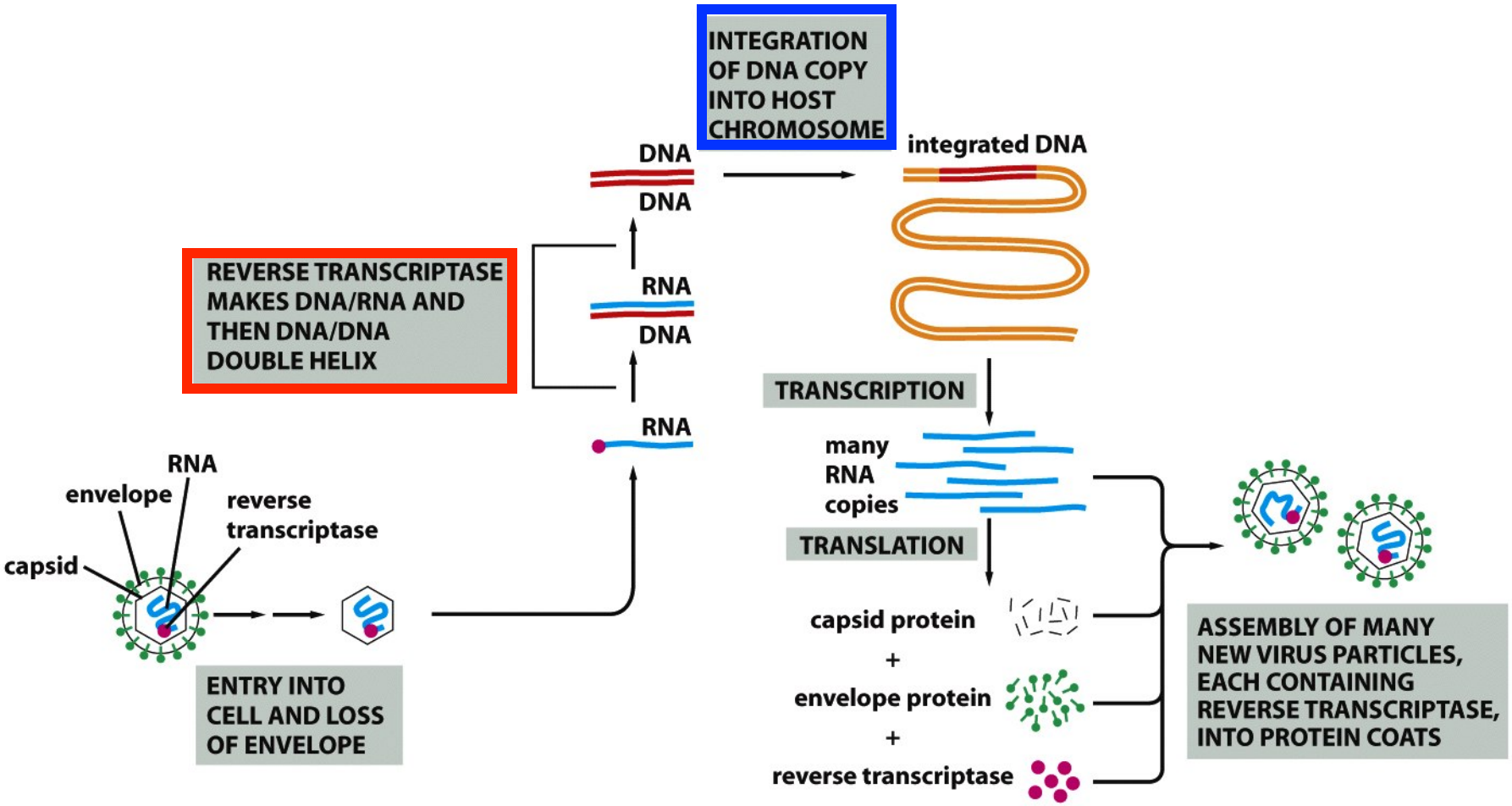


(B)

Reverse Transcriptase

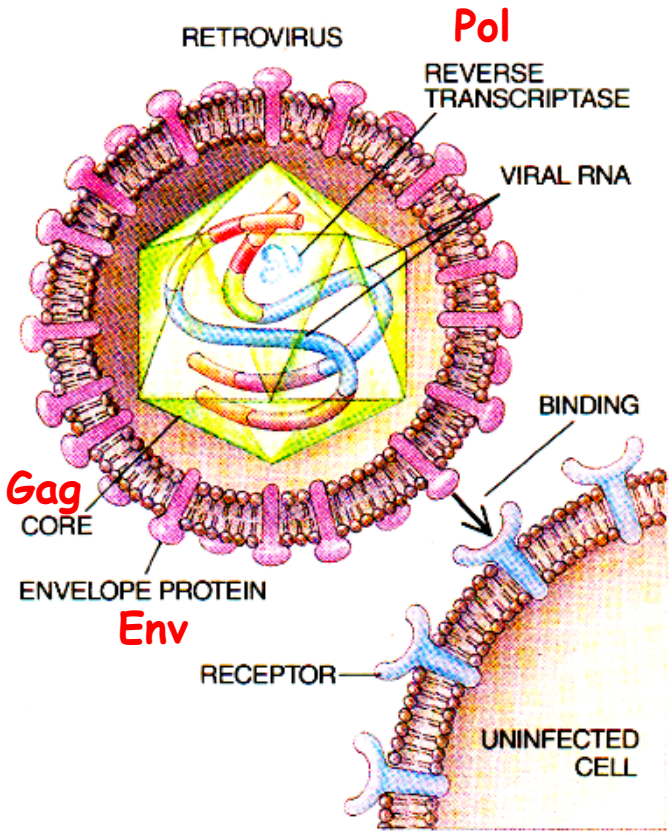
Figure 5-72 *Molecular Biology of the Cell* (© Garland Science 2008)

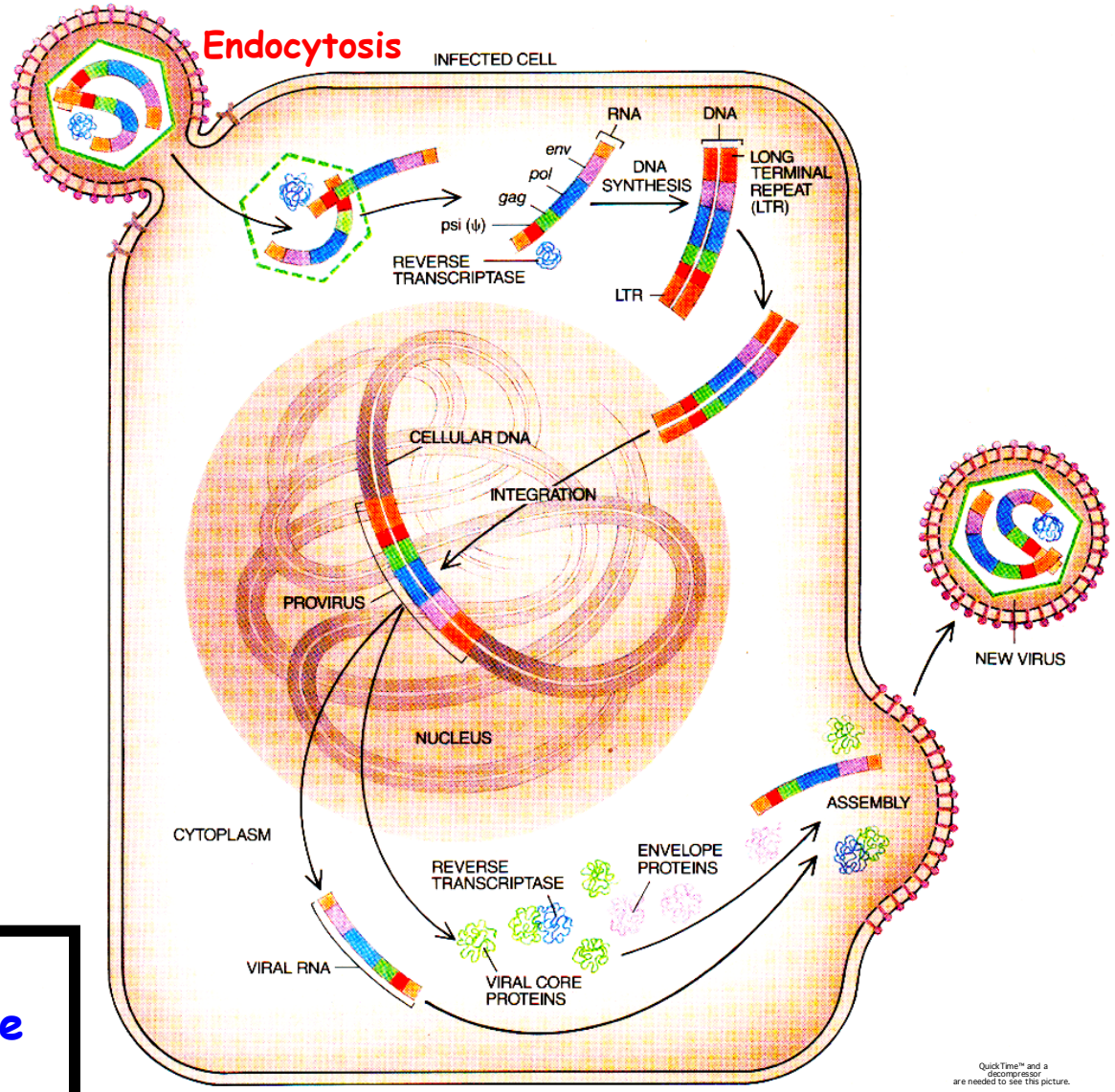
Retrovirus Life Cycle



Retroviruses Replicate Using Reverse Transcriptase
David Baltimore & Howard Temin-Nobel Prize 1975
Modified the Central Dogma of Molecular Biology
Use For Genetic Engineering?

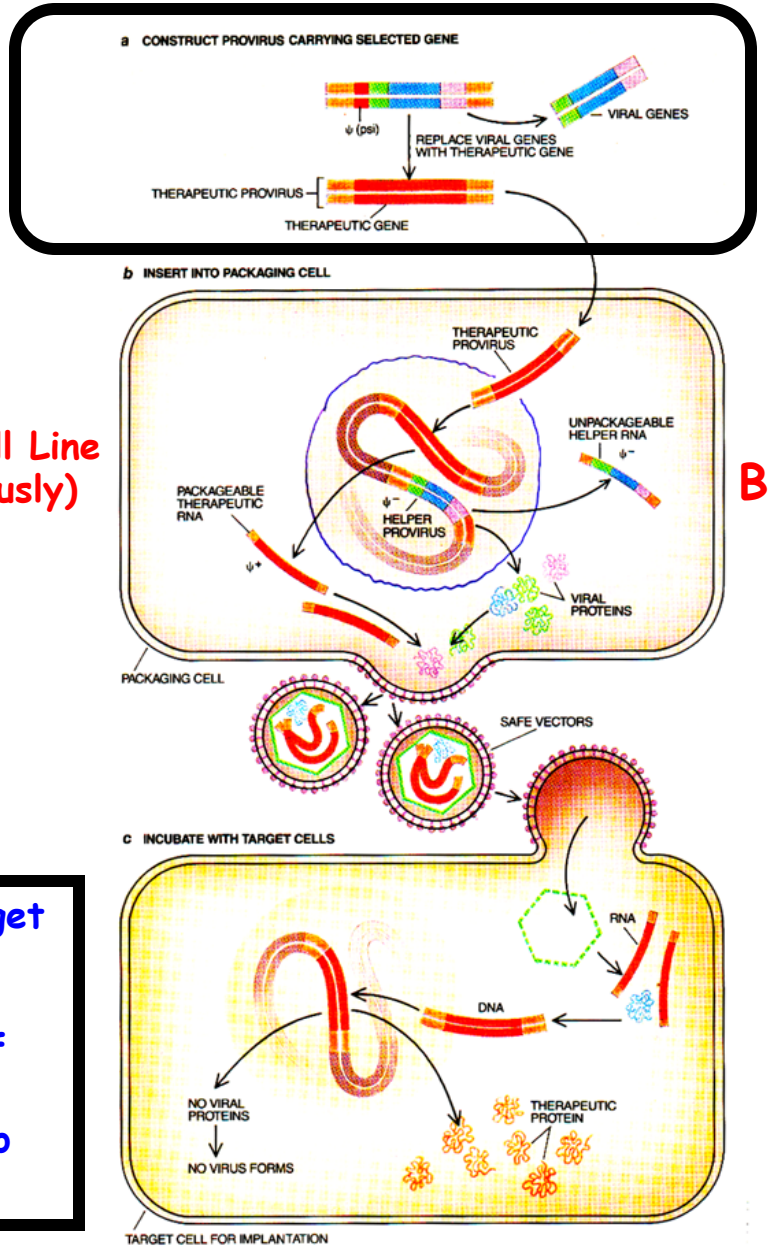
Using a Retrovirus as a Vector For Human Ex Vivo Gene Therapy





- Gag = Capid Protein
- Pol = Reverse Transcriptase
- Env = Envelope Protein
- Ψ (Psi) = Packaging Sequence

Using Retroviruses for Ex Vivo Gene Therapy



A.

1. Cloning in Bacteria
2. DNA Transformation into Packaging Cell

Packaging Cell Line (Made Previously)

B.

1. Packaging Cells Makes Viral Proteins
2. Cannot Package (Ψ-Minus)
3. Packages Therapeutic Transcript (Ψ-Plus)

C.

1. Infect Target Cells
2. Check For Presence of Gene
3. Transfer To Patient

RETROVIRAL VECTORS are assembled, or packaged, in cells designed to release only safe vectors. Investigators substitute a therapeutic gene for viral genes in a provirus (*a*) and insert that provirus into a packaging cell (*b*). The viral DNA directs the synthesis of viral RNA but, lacking viral genes, cannot give rise to the proteins needed to package the RNA into particles for delivery to other cells. The missing proteins are supplied by a "helper" provirus from which the psi region has been deleted. Psi is crucial to the inclusion of RNA in viral particles; without it, no virus carrying helper RNA can form. The particles that escape the cell, then, carry therapeutic RNA and no viral genes. They can enter other cells (*c*) and splice the therapeutic gene into cellular DNA, but they cannot reproduce.

Did it Work?

Science
AAAS

T Lymphocyte–Directed Gene Therapy for ADA⁻ SCID: Initial Trial Results After 4 Years

R. Michael Blaese,* Kenneth W. Culver, A. Dusty Miller, Charles S. Carter, Thomas Fleisher, Mario Clerici,† Gene Shearer, Lauren Chang, Yawen Chiang, Paul Tolstoshev, Jay J. Greenblatt, Steven A. Rosenberg, Harvey Klein, Melvin Berger, Craig A. Mullen,‡ W. Jay Ramsey, Linda Muul, Richard A. Morgan, W. French Anderson§

In 1990, a clinical trial was started using retroviral-mediated transfer of the adenosine deaminase (ADA) gene into the T cells of two children with severe combined immunodeficiency (ADA⁻ SCID). The number of blood T cells normalized as did many cellular and humoral immune responses. Gene treatment ended after 2 years, but integrated vector and ADA gene expression in T cells persisted. Although many components remain to be perfected, it is concluded here that gene therapy can be a safe and effective addition to treatment for some patients with this severe immunodeficiency disease.

- ADA gene expression in T cells persisted after four years
- Patients remained on ADA enzyme replacement therapy throughout the gene therapy treatment

Ashanthi DeSilva

Some Problems With Human Gene Therapy

- Delivery Systems To Target Cells
- Gene Expression Levels
- Adverse Immune Reactions to Vector
- Insertional Mutagenesis-Causing Other Diseases (e.g., leukemia)
- Human Error-Failure To Adhere To Strict NIH and IRB Procedures (Experimental Therapies)

The New York Times

Death Leads to Concerns For Future of Gene Therapy

By NICHOLAS WADE
Published: September 30, 1999

1999

BBC NEWS

Gene therapy 'caused leukaemia'

2003

A Recent Comeback for Gene Therapy

The new england journal of medicine
established in 1812 january 29, 2009 vol. 360 no. 5

Gene Therapy for Immunodeficiency Due to Adenosine Deaminase Deficiency

November 3, 2009

Giving Sight by Therapy With Genes

Science News

Gene Therapy for Metastatic Melanoma in Mice Produces Complete Remission

ScienceDaily (Nov. 18, 2010) — A potent anti-tumor gene introduced into mice with metastatic melanoma has resulted in permanent immune reconfiguration and produced a complete remission of their cancer, according to an article to be published in the December 2010 issue of the *Journal of Clinical Investigation*.

nature Vol 461|8 October 2009|doi:10.1038/nature08401

LETTERS

Gene therapy for red–green colour blindness in adult primates

Katherine Mancuso[1], William W. Hauswirth[2], Qiuhong Li[2], Thomas B. Connor[3], James A. Kuchenbecker[1], Matthew C. Mauck[3], Jay Neitz[1] & Maureen Neitz[1]

Science AAAS
A Comeback for Gene Therapy
Luigi Naldini
Science **326**, 805 (2009);
DOI: 10.1126/science.1181937

Science News

New Anti-HIV Gene Therapy Makes T-Cells Resistant to HIV Infection

ScienceDaily (Jan. 26, 2011) — An innovative genetic strategy for rendering T-cells resistant to HIV infection without affecting their normal growth and activity is described in a paper published in *Human Gene Therapy*, a peer-reviewed journal published by Mary Ann Liebert, Inc.

Vol 467|16 September 2010 nature

NEWS & VIEWS

GENE THERAPY

Targeting β-thalassaemia

Derek A. Persons

Patients with disorders of the blood protein haemoglobin often depend on lifelong blood transfusions. That could change, given the success of gene therapy in a patient with one such disorder.

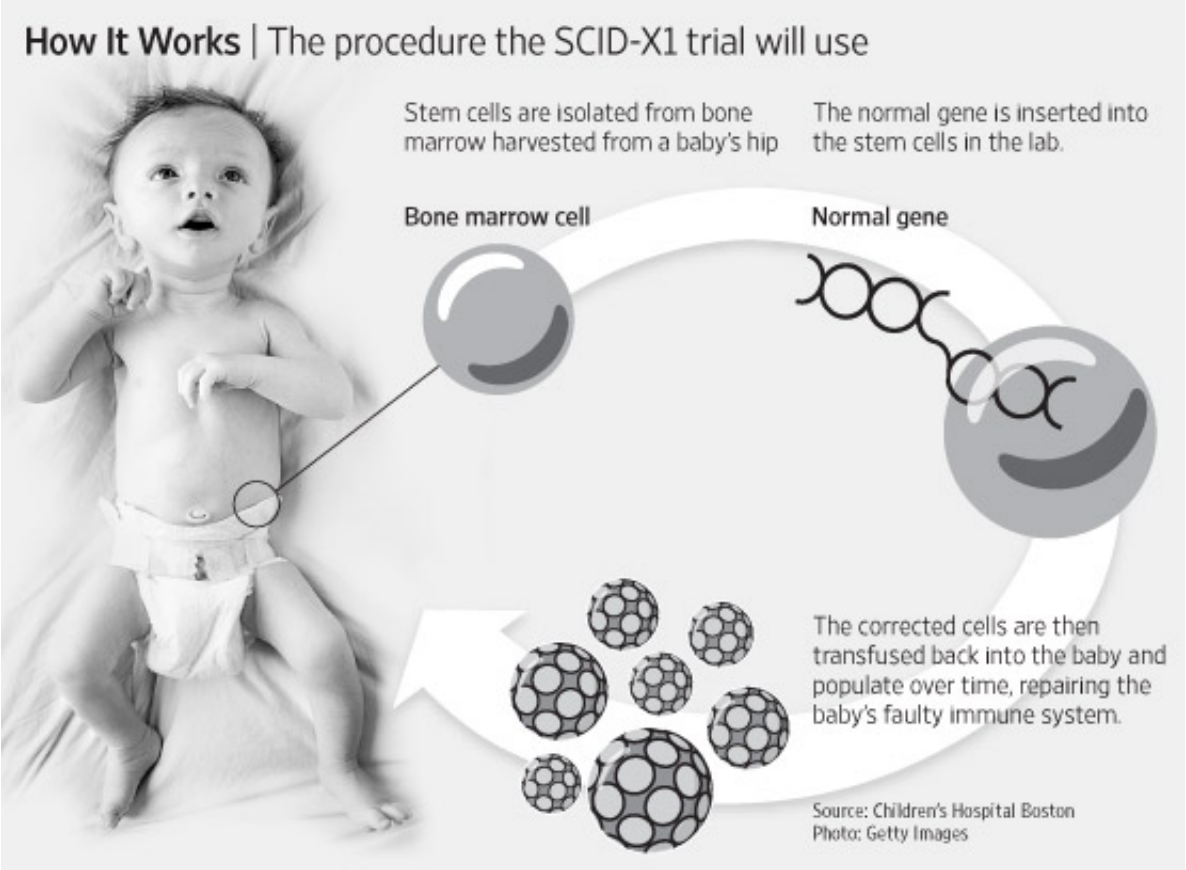
Science News

Virus-Based Gene Therapy for Metastatic Kidney Cancer Developed

ScienceDaily (Dec. 19, 2010) — Researchers at Virginia Commonwealth University Massey Cancer Center and the VCU Institute of Molecular Medicine (VIMM) have developed a novel virus-based gene therapy for renal cell carcinoma that has been shown to kill cancer cells not only at the primary tumor site but also in distant tumors not directly infected by the virus. Renal cell carcinoma is the most common form of kidney cancer in adults and currently there is no effective treatment for the disease once it has spread outside of the kidney.

Updated Ex-Vivo Gene Therapy for ADA-SCID & SCID-X1

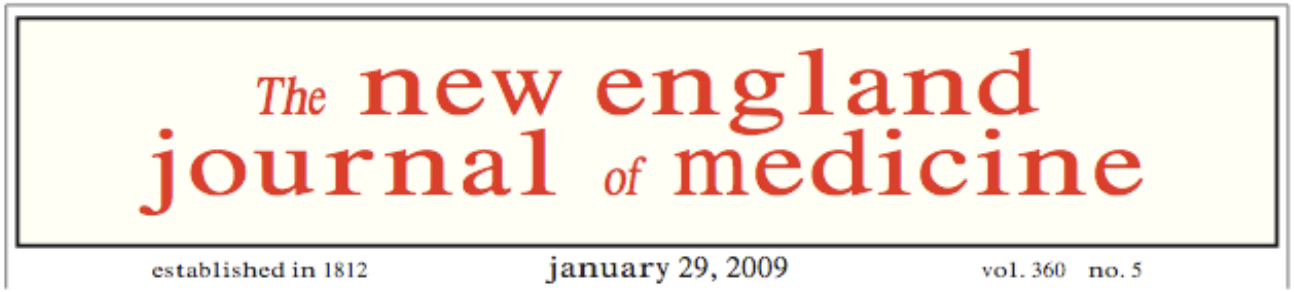
- **SCID-X1**
  - Most common form of SCID
  - Results from mutations in the common gamma chain gene required for interleukin receptors
  - Patients are immune deficient
- **Gene Therapy Improvements**
  - Used hematopoietic stem cells
  - Improved retroviral vectors with higher titers



It Works!

Gene therapy cures 'bubble boy disease'

31 Jan 2009, 1128 hrs IST, AP



Gene Therapy for Immunodeficiency Due to Adenosine Deaminase Deficiency

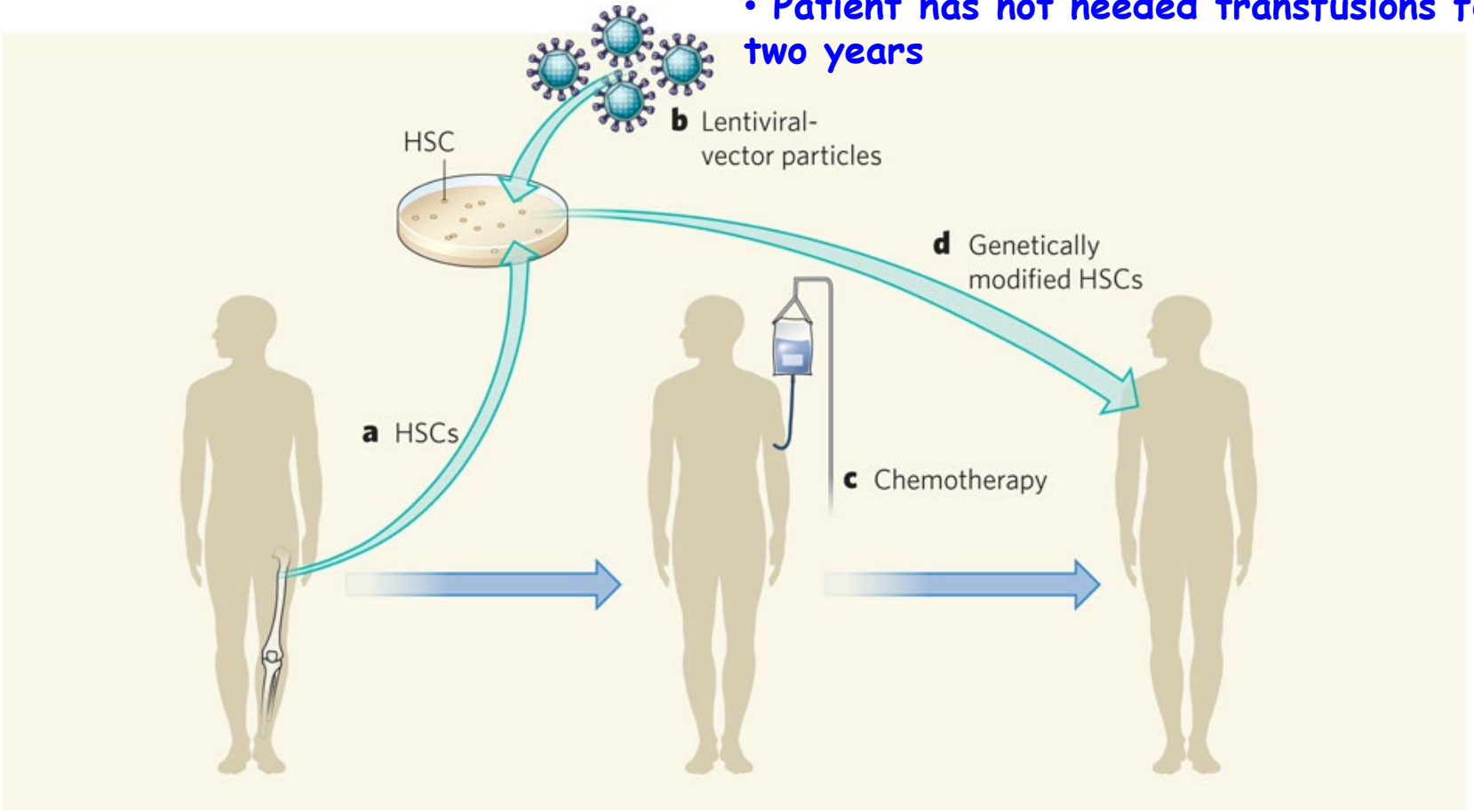
Results after 10 years

- ADA-SCID – 4 of 6 children experienced immune reconstitution
- SCID-X1 – 9 of 10 children experienced normal T-cell number
- In another study, 5 of 20 SCID-X1 subjects experienced leukemia-like T lymphoproliferation

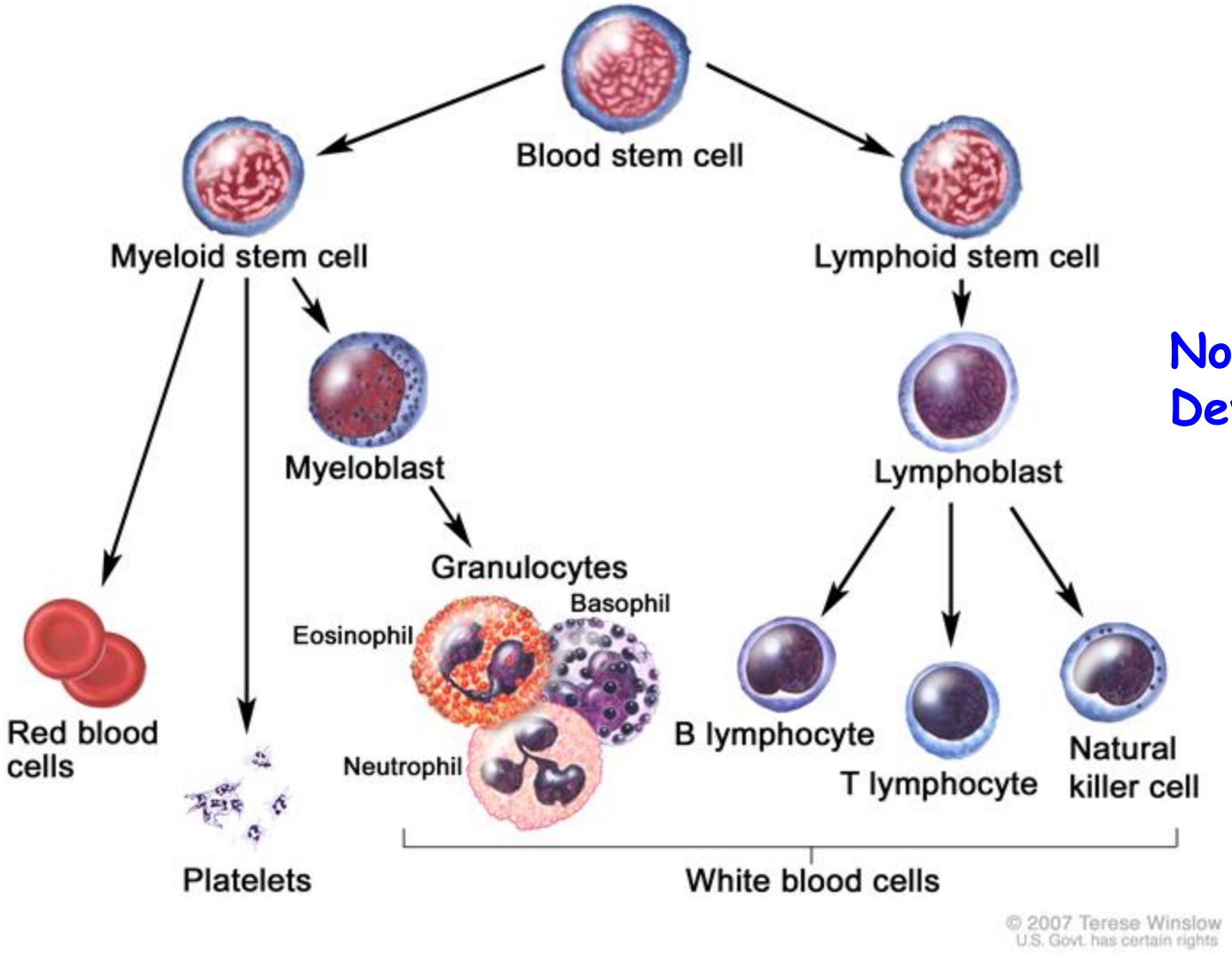
Ex-vivo Gene Therapy for β-Thalassemia

- Recessive mutation in β-globin gene causes reduced rates of synthesis and formation of abnormal hemoglobin and anemia
- Disease is treated with regular blood transfusions

- Gene therapy – transduced hematopoietic stem cells (HSC) with lentivirus (HIV) engineered with β-globin gene
- Transplanted therapeutic HSCs into patient following chemotherapy to destroy diseased HSCs
- Patient has not needed transfusions for two years



Leukemia

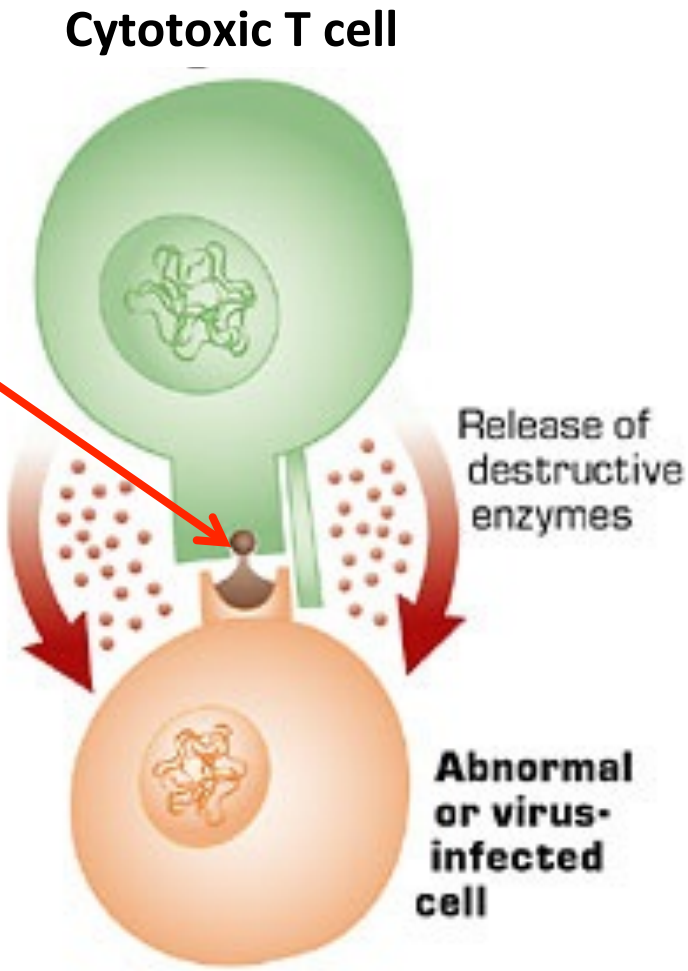


Normal Blood Cell Development

In leukemia, blood stem cells develop into immature white blood cells that are abnormal

Ex-vivo Gene Therapy for Chronic Lymphocytic Leukemia

- **Protocol**
  - Removed T cells from patients
  - Transferred Chimeric Antigen Receptor (CAR) genes into T cells that allow them to target chronic lymphocytic leukemia cells (B cells)
  - Infused CAR T cells back into patients
- **Results**
  - CAR T cells expanded more than 1,000 fold and persisted more than six months
  - Estimated that each CAR T cell killed more than 1,000 cancer cells
  - 2 of 3 patients had complete remission of leukemia



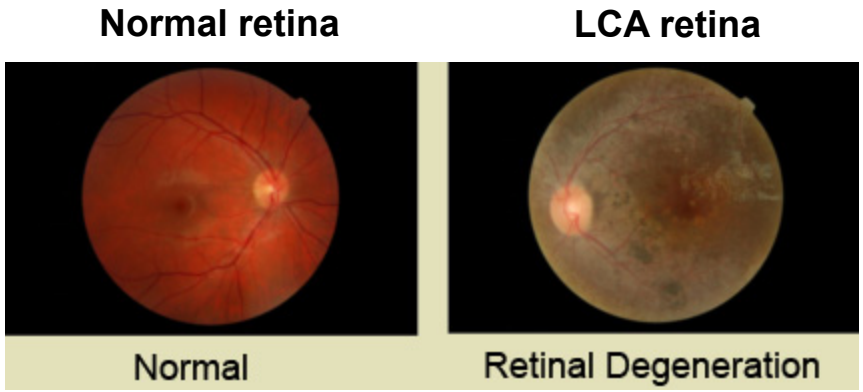
Science Translational Medicine AAAS

2011

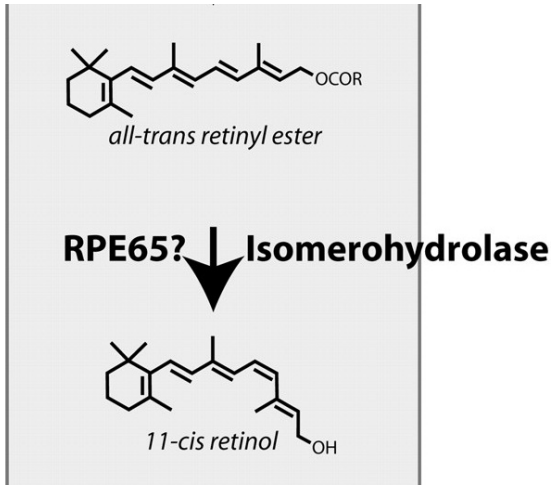
In Vivo Gene Therapy Examples

Leber Congenital Amaurosis (LCA)

- Degenerative diseases of the retina
- The most common cause of congenital blindness in children

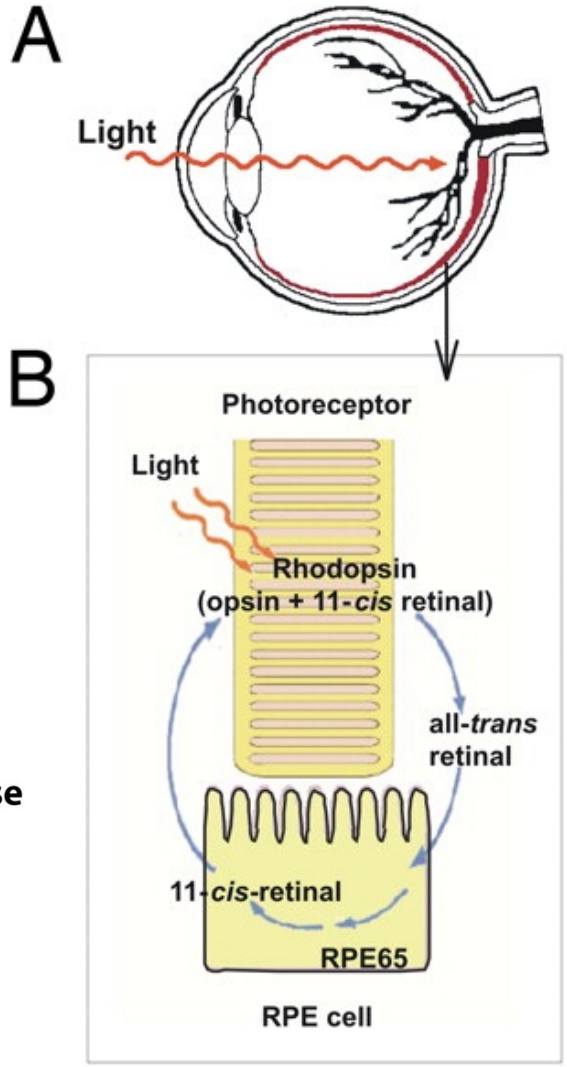


Type 2 LCA is caused by recessive mutations in the RPE65 isomerase gene



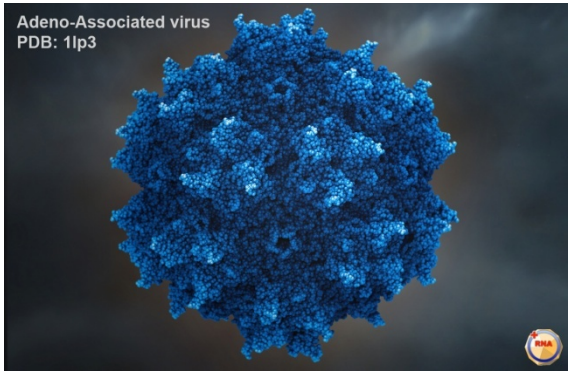
Moiseyev G et al. PNAS 2005;102:12413-12418

How We See



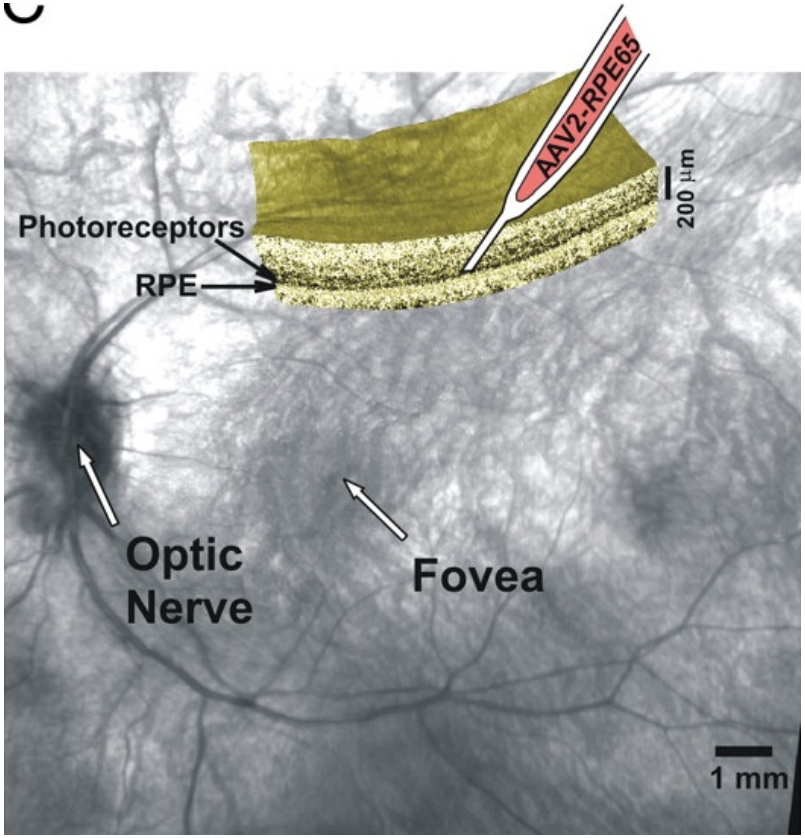
Cideciyan et al. PNAS 2008;105:15112

LCA Gene Therapy Using RPE65 & AAV



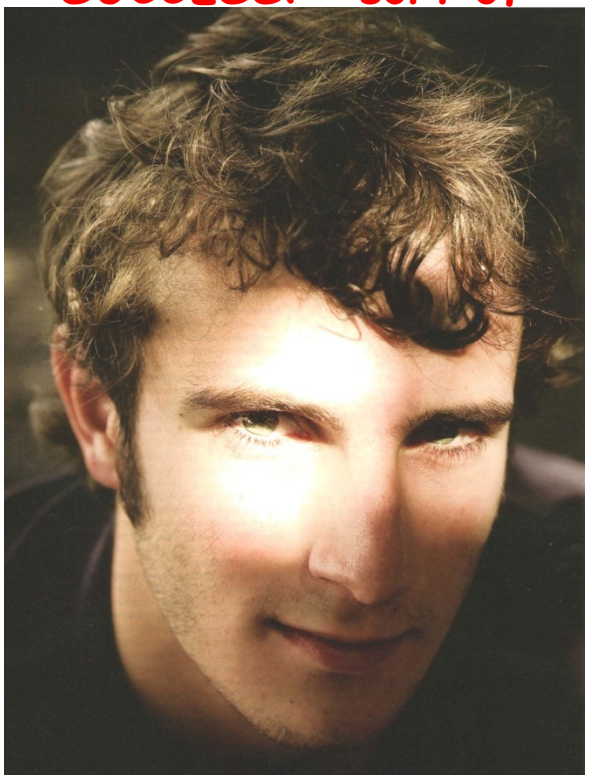
Adeno-associated viruses (AAV)

- Does not generally provoke antibody formation
- Infects nondividing cells of many different tissues
- Little or no integration of viral DNA into the host genome
- Has a small genome and can carry only short segments of DNA



Cideciyan et al. PNAS 2008;105:15112

SUCCESS! – sort of



ALESSANDRO CANNATA

Are Two Eyes Better than One?

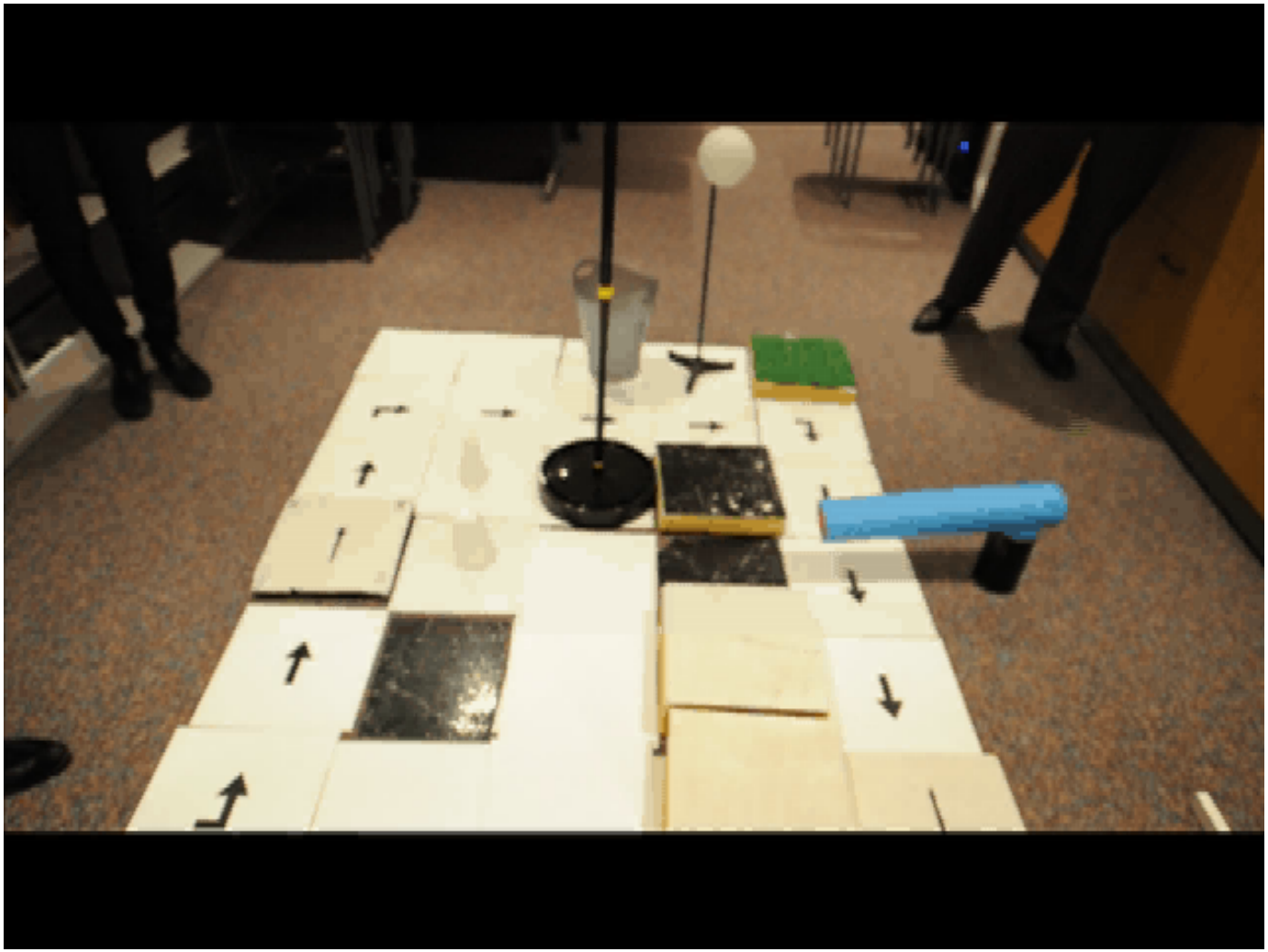
- **Question**
  - Can the second eye of LCA patients who had previously undergone RPE65 gene therapy be treated?
- **Protocol**
  - RPE65 gene administered with the AAV vector to 3 of the original 12 patients 1.7 to 3.3 years after initial treatment
- **Results**
  - Second treatment was safe and effective.

Science Translational Medicine AAAS

AAV2 Gene Therapy Readministration in Three Adults with Congenital Blindness
Jean Bennett, *et al.*
Sci Transl Med **4**, 120ra15 (2012);
DOI: 10.1126/scitranslmed.3002865

Are Two Eyes Better than One?

Before



Are Two Eyes Better than One?

After

Mutations in Factor IX Gene Cause Hemophilia B

TABLE 13.2 Some Important Genetic Disorders

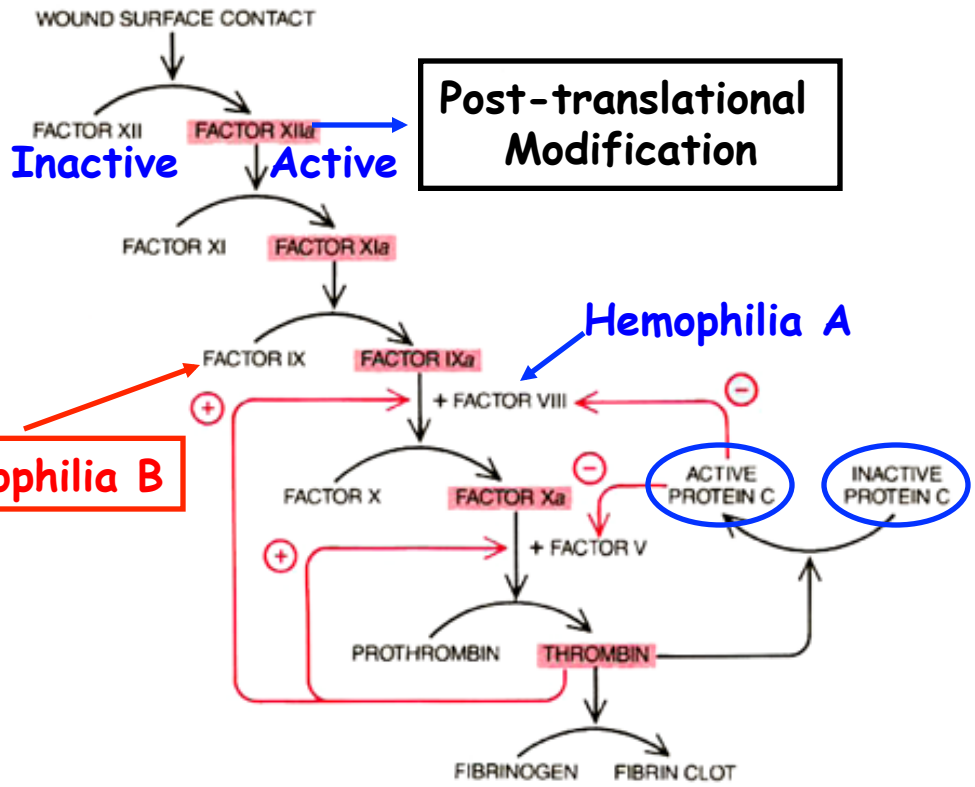
Disorder	Symptom	Defect	Dominant/ Recessive	Frequency Among Human Births
Cystic fibrosis	Mucus clogs lungs, liver, and pancreas	Failure of chloride ion transport mechanism	Recessive	1/2500 (Caucasians)
Sickle cell anemia	Blood circulation is poor	Abnormal hemoglobin molecules	Recessive	1/600 (African Americans)
Tay–Sachs disease	Central nervous system deteriorates in infancy	Defective enzyme (hexosaminidase A)	Recessive	1/3500 (Ashkenazi Jews)
Phenylketonuria	Brain fails to develop in infancy	Defective enzyme (phenylalanine hydroxylase)	Recessive	1/12,000
Hemophilia	Blood fails to clot	Defective blood-clotting factor VIII	X-linked recessive	1/10,000 (Caucasian males)
Huntington disease	Brain tissue gradually deteriorates in middle age	Production of an inhibitor of brain cell metabolism	Dominant	1/24,000
Muscular dystrophy (Duchenne)	Muscles waste away	Degradation of myelin coating of nerves stimulating muscles	X-linked recessive	1/3700 (males)
Hypercholesterolemia	Excessive cholesterol levels in blood lead to heart disease	Abnormal form of cholesterol cell surface receptor	Dominant	1/500

18,000 People in US Have Hemophilia & 400 Babies/Year Are Born With Disorder
Prior to 1960s – Average Life Span Was 11 Years

Hemophilia A	Defective Factor VIII Gene	1/10,000 males	80%
Hemophilia B	Defective Factor IX Gene	1/30,000 males	20%
Henophilia C	Defective Factor XI Gene	Autosomal	<1%

Both Factor VIII & IX Genes on X-Chromosome (♀→♂'s)

How Does Blood Clot After Wounding?



Post-translational Modification

Hemophilia A

Hemophilia B

CLOTTING CASCADE begins when cell damage at a wound somehow activates the enzyme factor XII; it ends with the conversion of fibrinogen into fibrin by thrombin. At each step an inactive protein is converted into a protease, or protein-cutting enzyme (*color*), which activates the next protein. Some steps require cofactors such as factors VIII and V. The cascade includes positive- and negative-feedback loops (*colored arrows*). Thrombin activates factors VIII and V; it also deactivates them (by activating protein *C*), which helps to halt clotting. Some 85 percent of hemophiliacs lack factor VIII. The rest lack factor IX.

Eight Proteins/Genes Required:

1. Factor VII
2. Factor XI
3. Factor IX
4. Factor VIII
5. Factor X
6. Protein C
7. Prothrombin
8. Fibrinogen

What Happens If Any Of These Proteins Or Genes Are Mutated?

↓

No Blood Clot!

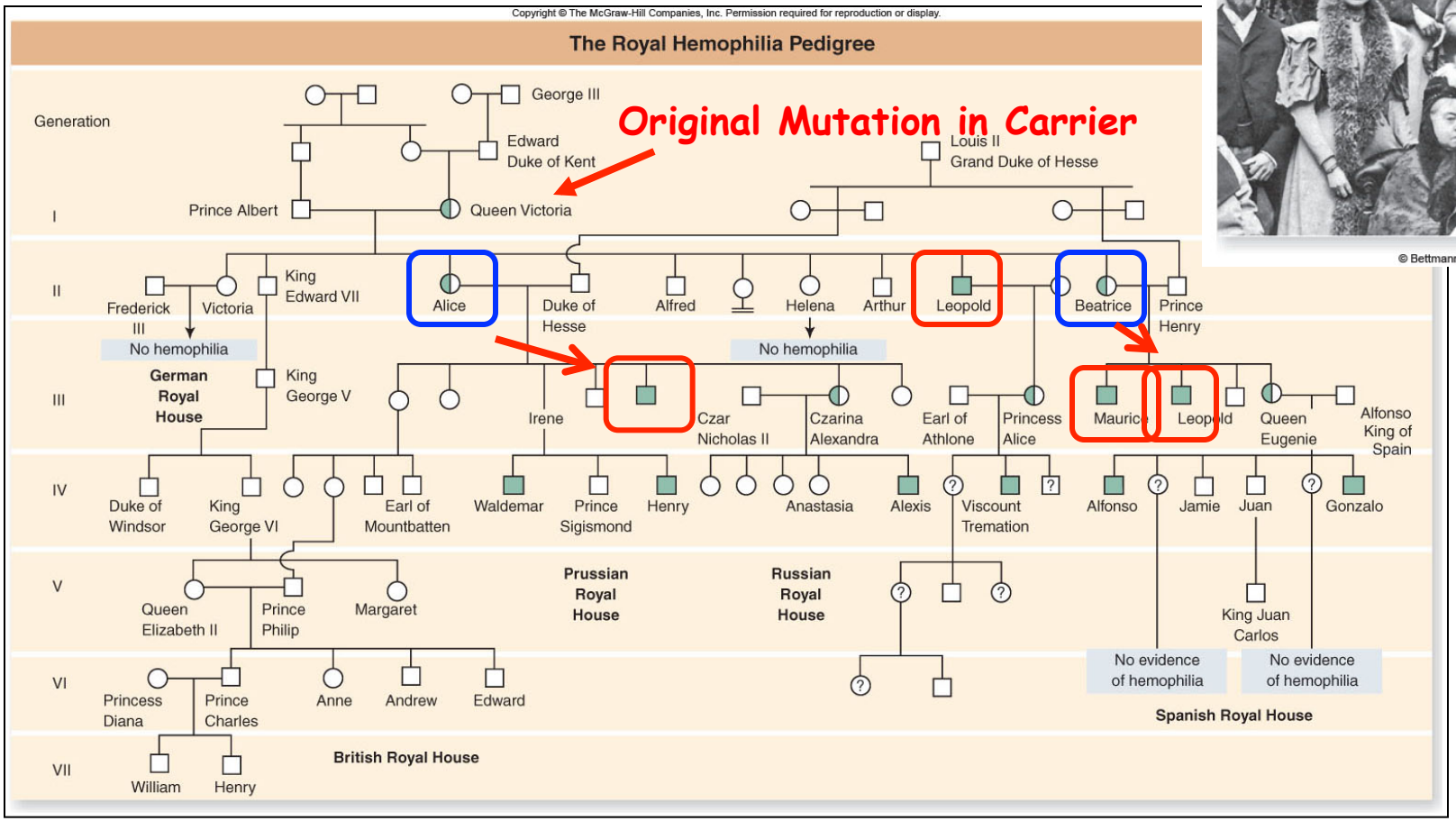
ATryn® 2009

Anti-Thrombin?? → Anti-Thrombin Deficiency (At-III) genetic disease

Cascade

Hemophilia A and B Genes (Traits) Are Sex Linked

© Bettmann/Corbis



Note: 1. Males Obtain Detective Gene From Mothers

2. 50% of Sons Of A Maternal Carrier Have The Defective Gene

December 10, 2011

The New York Times

Treatment for Blood Disease Is Gene Therapy Landmark 2011

- **Protocol**
  - Transferred Human Factor IX gene into adenovirus-associated virus vector that targets liver cells
  - Infused AAV vector into six participant with severe hemophilia B (FIX <1% of normal)
  - Participants monitored for 6 -16 months
- **Results**
  - AAV-mediated expression of FIX at 2 to 11% of normal levels
  - Four of six discontinued FIX prophylaxis; in the other two, the interval between prophylactic injections was increased

Adenovirus-Associated Virus Vector–Mediated Gene Transfer in Hemophilia B

In Vivo Suicide Gene Therapy for Brain Cancer

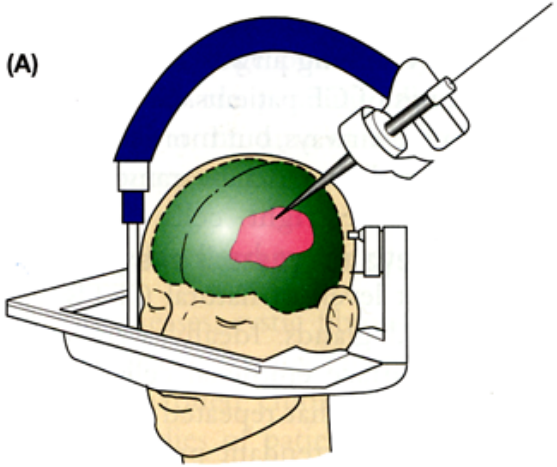
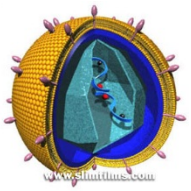
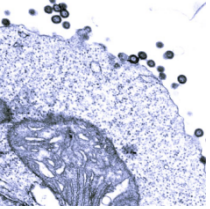


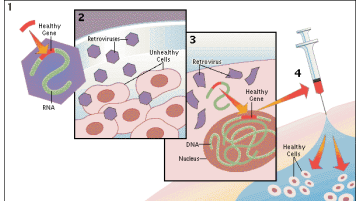
Figure 21.12: *In vivo* gene therapy for brain tumors.

A retrovirus is engineered to produce the herpes simplex virus thymidine kinase (HSV-TK). Vector-producing cells (VPC; blue) are injected into the brain tumor. Because retroviruses infect only dividing cells, they infect the tumor cells (pink) but not the surrounding normal brain tissue (green). The nontoxic prodrug gancyclovir (gcv) is given intravenously. In TK^+ cells gcv is converted to the highly toxic gcv-triphosphate and the cell is killed.



Retrovirus Vector for *Hs-tk* Gene





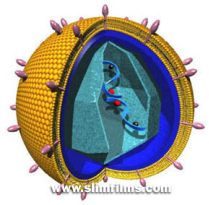
How Suicide Gene Therapy Works

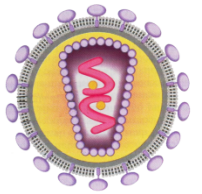
1. The retrovirus carrying the therapeutic gene is incorporated into the genome of the tumor cells and expresses a protein encoded by the new gene [herpes simplex virus *thymidine kinase* gene-(*HS-tk*)]

2. The protein (the herpes simplex virus enzyme thymidine kinase, HS-tk) encoded by the *HS-tk* gene sensitizes the tumor cells to an antiviral drug (ganciclovir, GCV) which is a substrate for HS-tk. Human tk is not affected by GCV (i.e., normal cells surrounding a tumor remain healthy).

3. The enzymatic process induced by GCV leads to death of the cell expressing the herpes TK activity, i.e., death of the tumor cells.

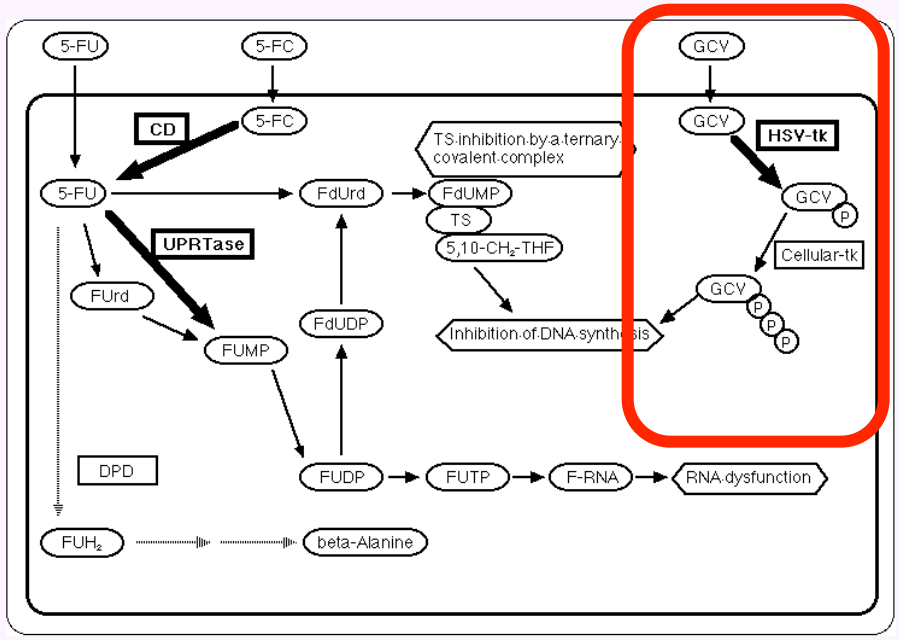
4. Because the human HS-tk enzyme has very low affinity for GCV, systemic toxicity related to this mechanism is not observed.





Clinical Trial Using Suicide Gene Therapy

Gene Set Bank - Suicide gene therapy



Brain Tumor Cell

Neurosurgical Focus

Treatment of progressive or recurrent pediatric malignant supratentorial brain tumors with herpes simplex virus thymidine kinase gene vector--producer cells followed by intravenous ganciclovir administration

Roger J. Packer, M.D., Cory Raffel, M.D., Ph.D., Judith G. Villablanca, M.D., Jörg-Christian Tonn, M.D., Stefan E. Burdach, M.D., Klaus Burger, M.D., Ph.D., Deborah LaFond, P.N.P., J. Gordon McComb, M.D., Philip H. Cogen, M.D., Ph.D., Gilbert Vezina, M.D., and Leonard P. Kapcala, M.D.

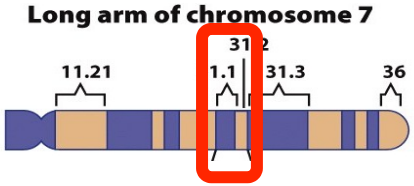
Departments of Neurology, Pediatrics, Hematology/Oncology, Neurosurgery, and Diagnostic Imaging, Children's National Medical Center, Washington, D.C.; The George Washington University Hospital, Washington, D.C.; Department of Neurosurgery, Mayo Clinic, Rochester, Minnesota; Departments of Pediatrics and Neurosurgery, Children's Hospital Los Angeles and University of Southern California, Los Angeles, California; Kinderklinik, Würzburg, Germany; Universitäts-Kinderklinik, Düsseldorf, Germany; Department of Pediatrics, Martin-Luther Universität Halle--Wittenberg, Halle, Germany; Novartis Pharma GmbH, Nuremberg, Germany; and Genetic Therapy, Inc., Bethesda, Maryland

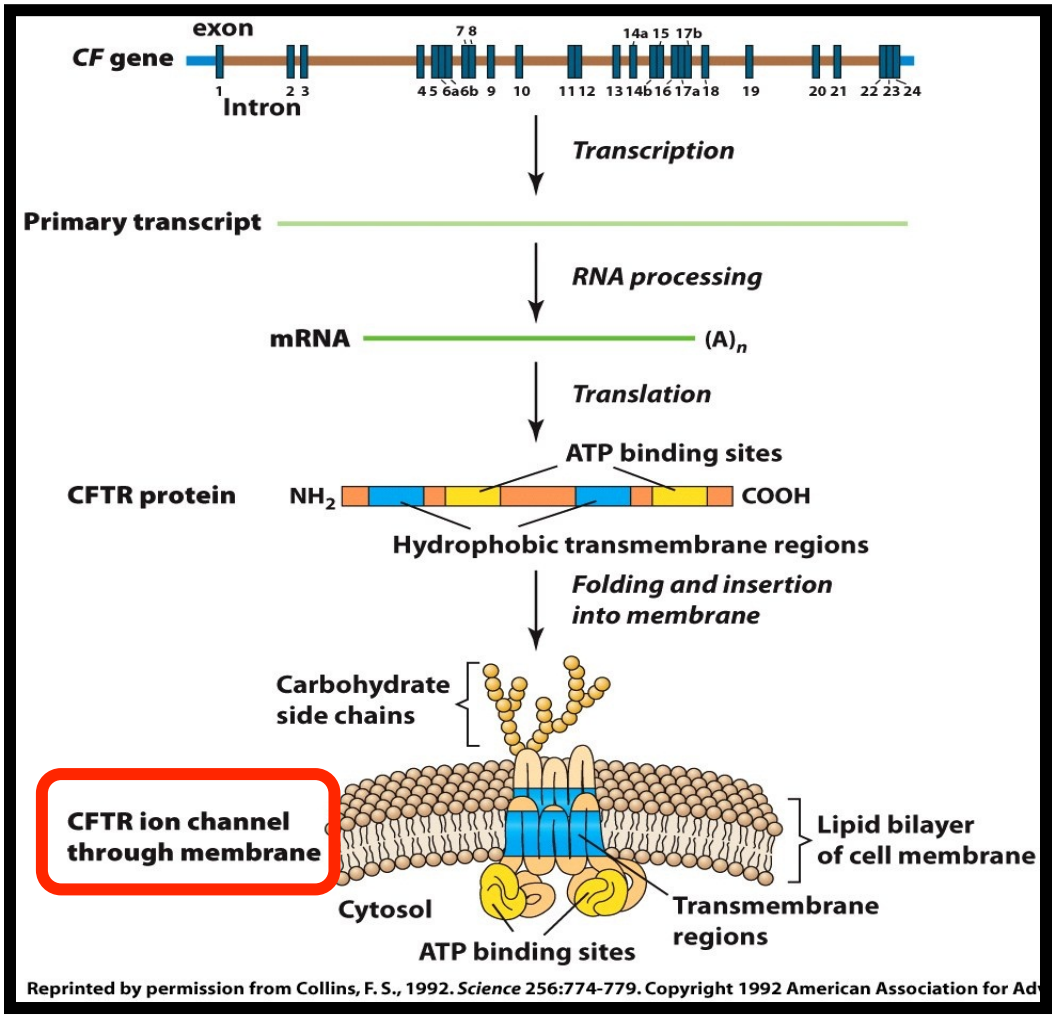
Object. The outcome for children with recurrent malignant brain tumors is poor. The majority of patients die of progressive disease within months of relapse, and other therapeutic options are needed. The goal of this Phase I study was to evaluate the safety of in vivo suicide gene therapy in 12 children with recurrent, malignant, supratentorial brain tumors.

Methods. After optimal repeated tumor resection, multiple injections of murine vector--producing cells shedding murine replication--defective retroviral vectors coding the **herpes simplex virus thymidine kinase type 1 (HSV-Tk1)** gene were made into the rim of the resection cavity. Fourteen days after the vector-producing cells were injected, ganciclovir was administered for 14 days. The retroviral vector that was used only integrated and expressed **HSV-Tk1** in proliferating cells, which are killed after a series of metabolic events lead to cell death. The median age of the patients was 11 years (range 2--15 years). Treated brain tumors included seven malignant gliomas, two ependymomas, and three primitive neuroectodermal tumors. The patients were treated with one of three escalating dose concentrations of vector-producer cells. Four transient central nervous system adverse effects were considered possibly related to the vector-producing cells. In no child did permanent neurological worsening or ventricular irritation develop, and tests for replication-competent retroviruses yielded negative findings.

Conclusions. This Phase I study demonstrates that in vivo gene therapy in which a replication-defective retroviral vector in murine vector--producing cells is delivered by brain injections can be performed with satisfactory safety in a select group of children with localized supratentorial brain tumors.

Cystic Fibrosis



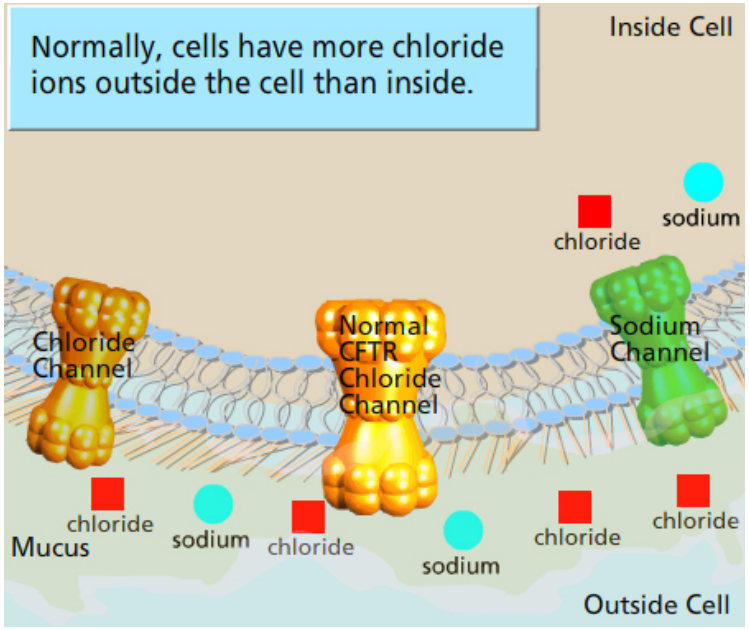


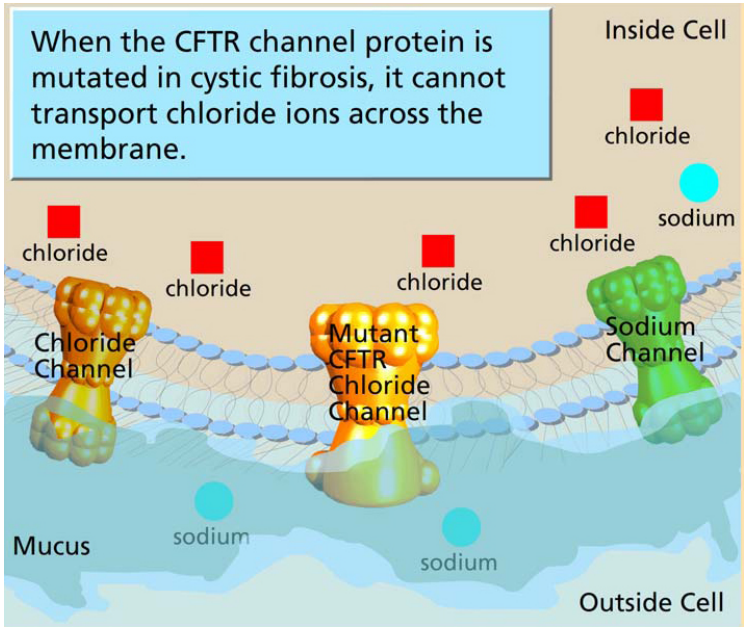
200 kb gene!

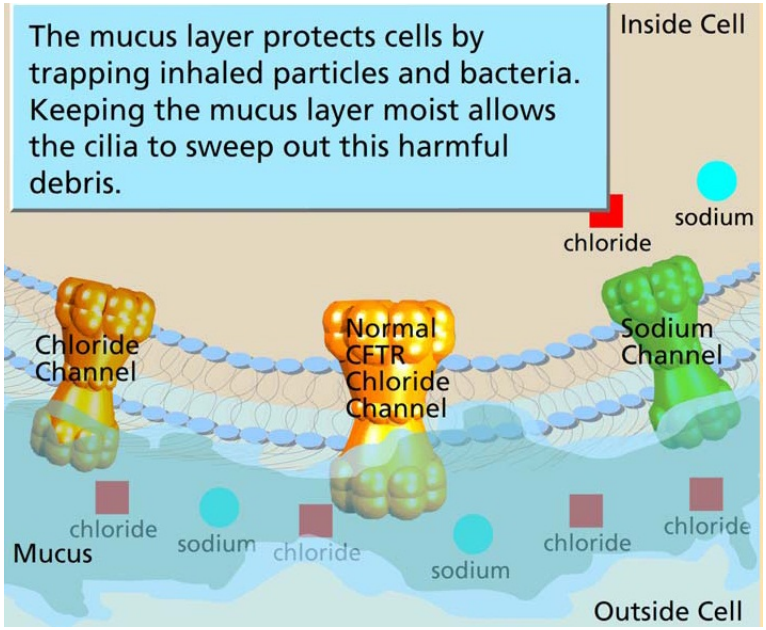
Physiological Consequences of Cystic Fibrosis

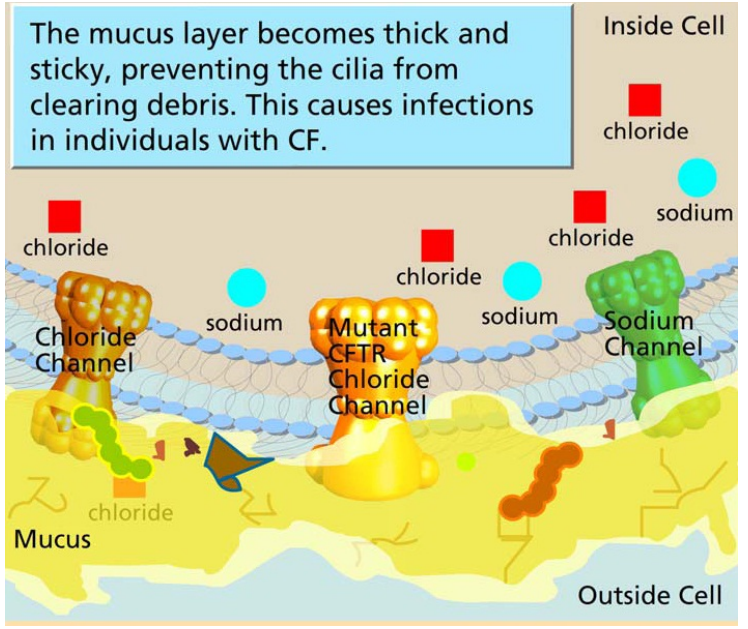
Normal

Diseased



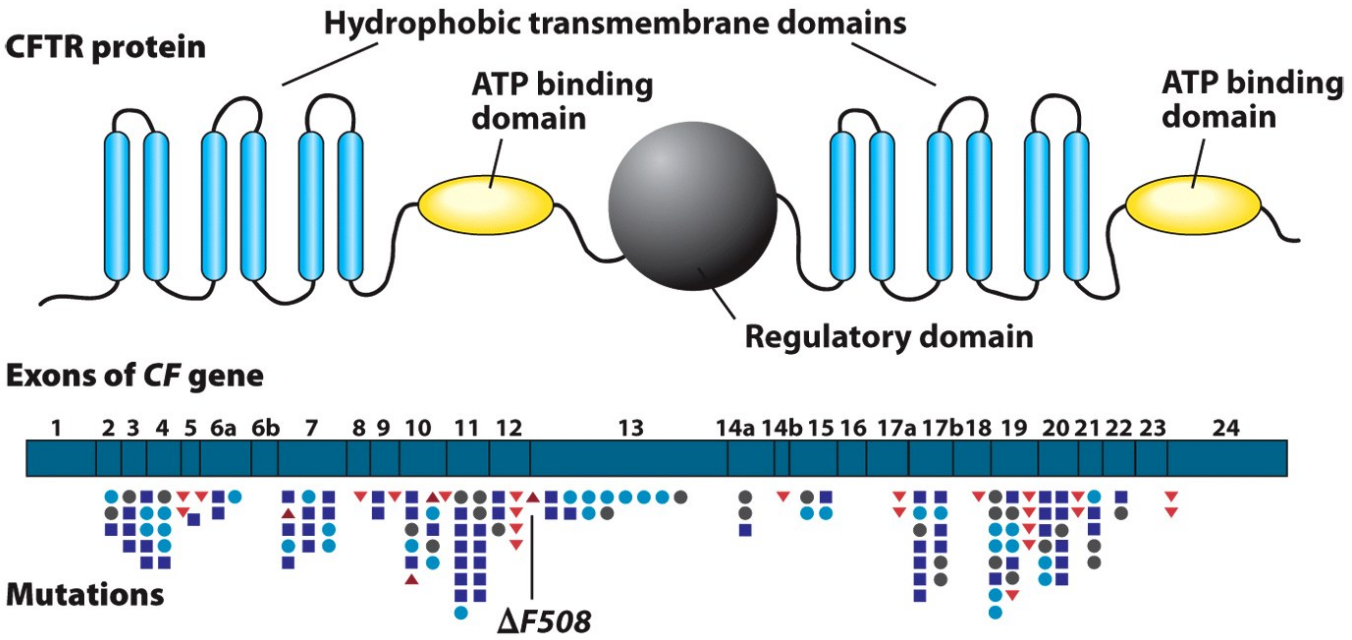






http://learn.genetics.utah.edu/content/tech/genetherapy/cysticfibrosis/index.html

Mutant Cystic Fibrosis Genes
[Recessive (Loss-Of-Function) Mutations]

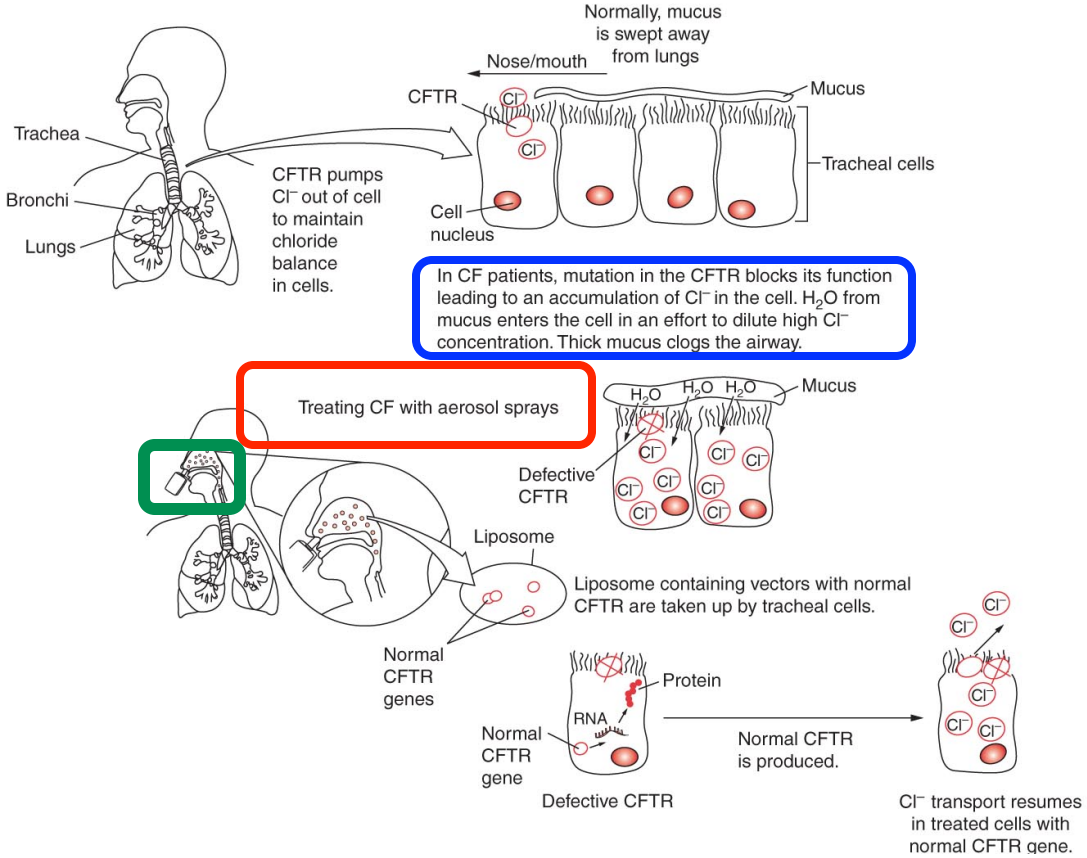


- Mostly N. European Ancestry
- 1/2500 CF Babies Born in US/Year
- 1/20 Americans are CF Carriers
- 30 Year Life Span
- Abnormality of Mucus/Sweat Glands
- Cannot Regulate Salt (Chlorides)

70% of Families Have ΔF508 Deletion-What Are Consequences For CF Testing? How Can it be in 70% of CF Families?

Key:
- In-frame deletion
- Missense mutation
- Nonsense mutation
- Frame-shift mutation
- Splicing mutation

In Vivo Cystic Fibrosis Gene Therapy



Somatic Cell Gene Therapy

Gene Therapy Research Offers Promise of a Cure for Cystic Fibrosis

Gene therapy offers great promise for life-saving treatment for CF patients since it targets the cause of CF rather than just treating symptoms. Gene therapy for CF had its start in 1990, when scientists successfully corrected faulty CFTR genes by adding normal copies of the gene to laboratory cell cultures.

In 1993, the first experimental gene therapy treatment was given to a patient with CF. Researchers modified a common cold virus to act as a delivery vehicle - or "vector"- carrying the normal genes to the CFTR cells in the airways of the lung.

Subsequent studies have tested other methods of gene delivery, such as fat capsules, synthetic vectors, nose drops or drizzling cells down a flexible tube to CFTR cells lining the airways of lungs. Researchers are now testing aerosol delivery using nebulizers.

But finding the best delivery system for transporting normal CFTR genes is only one problem that scientists must solve to develop an effective treatment for CF. Scientists must also determine the life span of affected lung cells, identify the "parent cells" that produce CFTR cells, find out how long treatment should last and how often it needs to be repeated.

The first cystic fibrosis gene therapy experiments have involved lung cells because these cells are readily accessible and because lung damage is the most common, life-threatening problem in CF patients. But scientists hope that the technologies being developed for lung cells will be adapted to treat other organs affected by CF.

Approved Gene Therapy Trials

Table 1 Selected gene therapy clinical trials

Company (location)	Therapy	Indication	Phase of development
Retrovirus			
San Raffaele	ADA-SCID GT: CD34+ cells transduced with Moloney murine leukemia virus carrying ADA gene	Primary immunodeficiencies	Phase 1/2
Ribozyme (Boulder, CO, USA)	CD34+ cells transduced with retrovirus vector with multiple ribozymes	Non-Hodgkin's lymphoma, HIV/AIDS	Phase 2
Tocagen (San Diego)	Toca-511: replication-competent retrovirus with prodrug activator cytosine deamidase gene injected into tumor	Glioma	Phase 1/2
Lentivirus			
Bluebird Bio	LentiGlobin: introduces globin gene into patient hematopoietic stem cells	β-thalassemia and sickle cell anemia	Phase 1/2
Lentigen	LG-740: T cells treated *ex vivo* with lentivirus with chimeric T-cell receptor gene	B-cell leukemia and lymphoma	Phase 1
Oxford BioMedica	ProSavin: lentivirus with three genes required for dopamine biosynthesis injected into striatum of brain	Parkinson's disease	Phase 1/2
Adenovirus			
Advantagene (Auburndale, MA, USA)	ADV-tk: replication-deficient adenovirus with herpes simplex thymidine kinase gene injected into tumor during biopsy	Glioma Pancreatic cancer	Phase 1 Phase 1
Aventis (Paris)	Ad5CMV-p53	Head and neck cancer	Phase 2
Biogen	Adenoviral mediated interferon-β	Pleural mesothelioma Colon cancer, glioma	Phase 1 Phase 1/2
GenVec	TNFerade: replication deficient adenovirus with TNF-a controlled by radiation induced promoter	Esophageal cancer	Phase 2
Shenzhen SiBiono GeneTech	rAd-p53: replication deficient adenovirus encoding hu recombinant p53	Advanced thyroid tumors, oral, maxillofacial tumors	Phase 4
AAV			
Applied Genetic Technologies (Alachua, FL, USA)	rAAV1-CB-hAAT: AAV with α-1-antitrypsin gene	α-1-antitrypsin deficiency	Phase 2
	rAAV2-CB-human retinal pigment epithelium-specific 65-dalton protein (RPE65)	Congenital amaurosis (blindness with mutation in RPE gene)	Phase 1/2
Amsterdam Molecular	AMT-101: AAV with human lipoprotein lipase (LPL) gene	LPL deficiencey	Phase 3?
Ceregene (San Diego)	CERE-120: AAV with neurotrophic factor, neuturin	Parkinson's disease	Phase 1/2
	CERE-110: AAV with gene for nerve growth factor	Alzheimer's disease	Phase 1/2
Celladon (La Jolla, CA, USA)	SERCA-2a: sarcoplasmic reticulum Ca_2 plus ATPase gene with AAV vector	Congestive heart failure	Phase 1/2
Genzyme	AAV2-sFLT01: AAV with anti-VEGF	Wet macular degeneration	Phase 1
Neurologix	NLX-P101: GAD in virus injected into subthalmic nucleus of the brain	Parkinson's disease	Phase 2 completed
Targeted Genetics (Seattle)	tgAAG76: AAV with hRPE65	Congenital amaurosis (blindness with mutation in RPE gene)	Phase 1/2
	tgAAC94: AAV2 with TNF-α-IgG1 fusion gene	Arthritis	Phase 2 completed
Plasmid			
AnGes (Tokyo)	Hepatocyte growth factor plasmid	Arterial disease	Phase 2
Genexine (Seoul, Korea)	GX-12: plasmid plus IL-12 mutant, given with HAART	HIV-AIDS	Phase 1
ScanCell (Nottingham, UK)	SCIB1: plasmid with gene for tyrosine related protein	Melanoma	Phase 1/2
Vical (San Diego)	Allovectin-7: plasmid with HLA-B7 and β-2 microglobulin genes, injected into tumors	Melanoma	Phase 3
ViroMed (Minnetonka, MN, USA)	VM202: plasmid with two isoforms of hepatocyte growth factor, HGF728 and HGF 723	Limb ischemia Myocardial ischemia	Phase 2 Phase 1/2
Other			
Diamyd Medical (Stockholm)	Nerve-targeting drug delivery system: HSV vector with enkaphalin administered intradermally	Pain	Phase 1
Epeius Biotechnologies (San Marino, CA, USA)	Rexin-G: nanoparticle delivering cyclin-G1 gene	Advanced pancreatic metastatic breast cancer, osteosarcoma, and soft tissue sarcoma	Phase 1/2
MultiGene Vascular Systems (Nesher, Israel)	Patient cells modified with four angiogenic genes	Peripheral artery disease	Phase 1/2

AAV, adeno-associated virus; HSV, herpes simplex virus

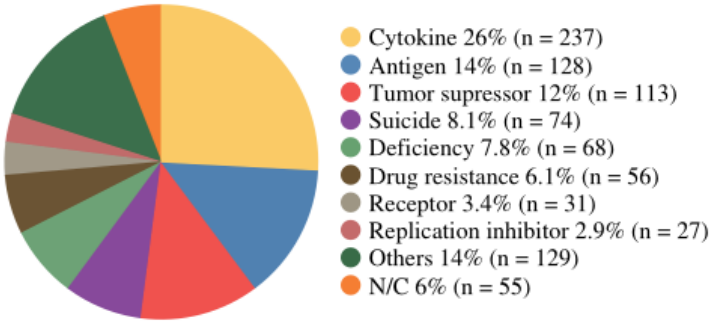


Figure 5. Distribution of gene therapy clinical trials by gene. N/C = not communicated

Nature Biotechnology, February 2011

Approved Gene Therapy Trials By Disease and Vector

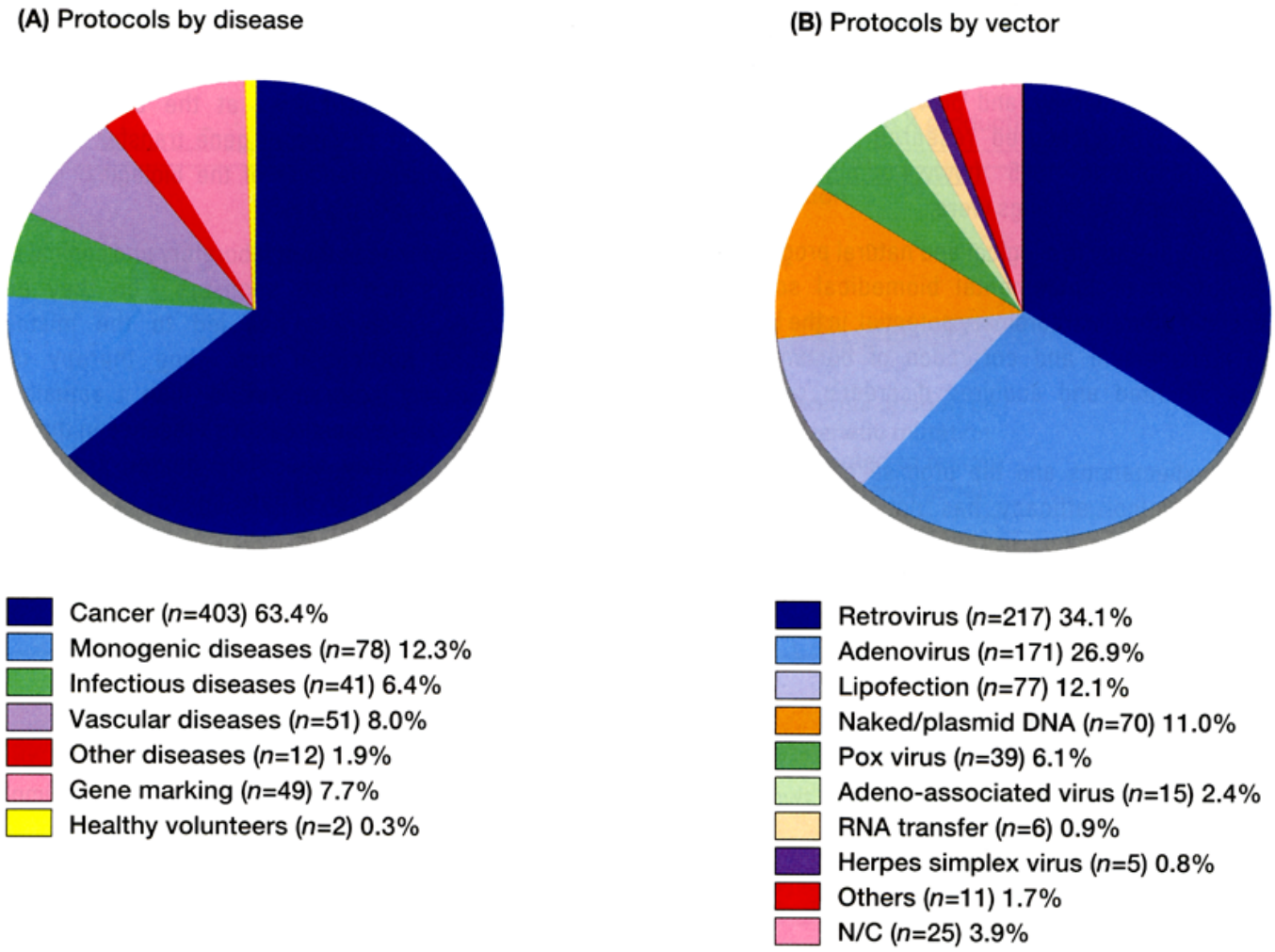


Figure 21.5: Gene therapy trial protocols.

(A) Distribution by disease. **(B)** Distribution by vector. The figures include all approved protocols for completed, ongoing or pending trials listed in December 2002. Reproduced from www.wiley.co.uk/genetherapy/clinical with permission.

Types of Human Gene Therapy Clinical Trials

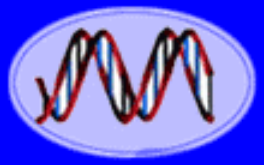
Table 12.4 GENE THERAPIES BEING STUDIED IN CANCER PATIENTS THAT MAY RECEIVE PATENTS AND REGULATORY APPROVAL

Approach	Number of U.S. Trials Approved since 1988 or Awaiting Federal Approval
Antisense therapy (to block synthesis of proteins encoded by deleterious genes)	4
Chemoprotection (to add proteins to normal cells to protect them from chemotherapies)	7
Immunotherapy (to enhance the body's immune defenses against cancer)	58
Pro-drug, or suicide gene, therapy (to render cancer cells highly sensitive to selected drugs)	21
Tumor suppressor genes (to replace a lost or damaged cancer-blocking gene)	6
Antibody genes (to interfere with the activity of cancer-related proteins in tumor cells)	2
Oncogene down-regulation (to shut off genes that favor uncontrolled growth and spread of tumor cells)	2

Source: Fiattman, G. I., and Kaplan, J. M. (2001). "Patenting Expressed Sequence Tags and Single Nucleotide Polymorphisms," *Nature Biotechnology,* 19: 683.

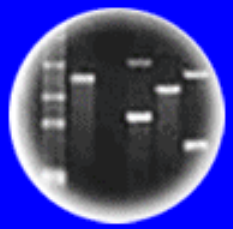
Some Issues With Human Gene Therapy

- Regulation
- Consent
- Risks
- Enhancement
- Eugenics (Germ Line)
- Availability To Everyone



DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria



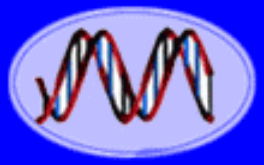
DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

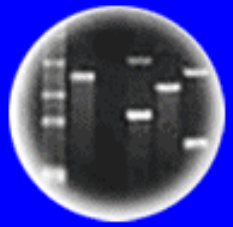
Would you alter the germ line of your child for the trait(s) of "your choice" using germ-line gene therapy if the procedure was 100% "safe?"

a. Yes
b. No



DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria



DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

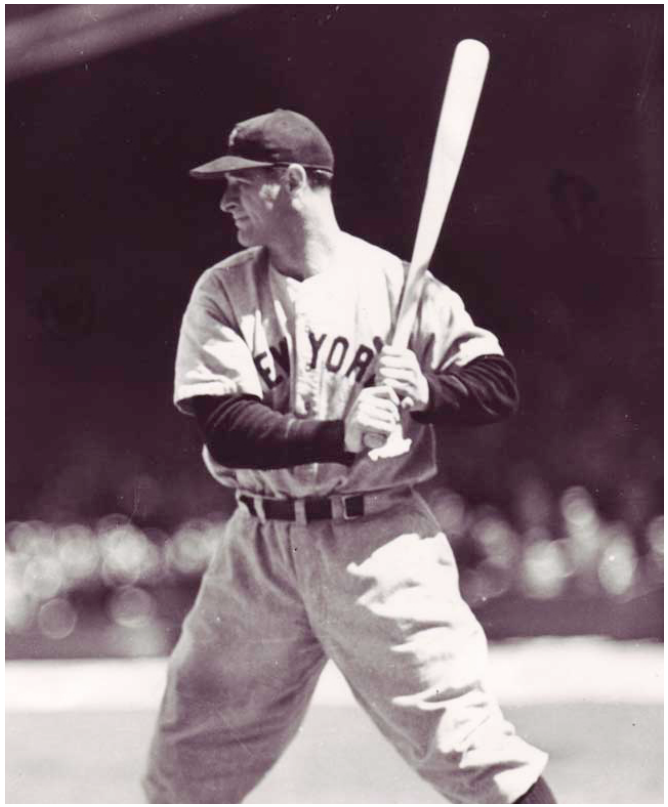
Would you alter a somatic cell of your child for the trait(s) of "your choice" using somatic cell gene therapy if the procedure was 100% "safe?" (For example, correcting a genetic defect in a stem cell line, producing a therapeutic clone, or correcting the defect with a genetically engineered stem cell implant)

a. Yes
b. No

Future Human Gene Therapy Examples
The Frontiers of Medicine

- Therapeutic Cloning + Gene Therapy
- Anti-Sense and RNAi "Drugs"
- Ribozyme "Drugs"

Can Gene Therapy be Used for Dominant Mutations?
A "Molecular Drug" To Shut Off Genes-RNAi
(e.g., Disease Genes, Viral Genes)



Lou Gehrig's Disease
Amyotrophic Lateral Sclerosis
(ALS)

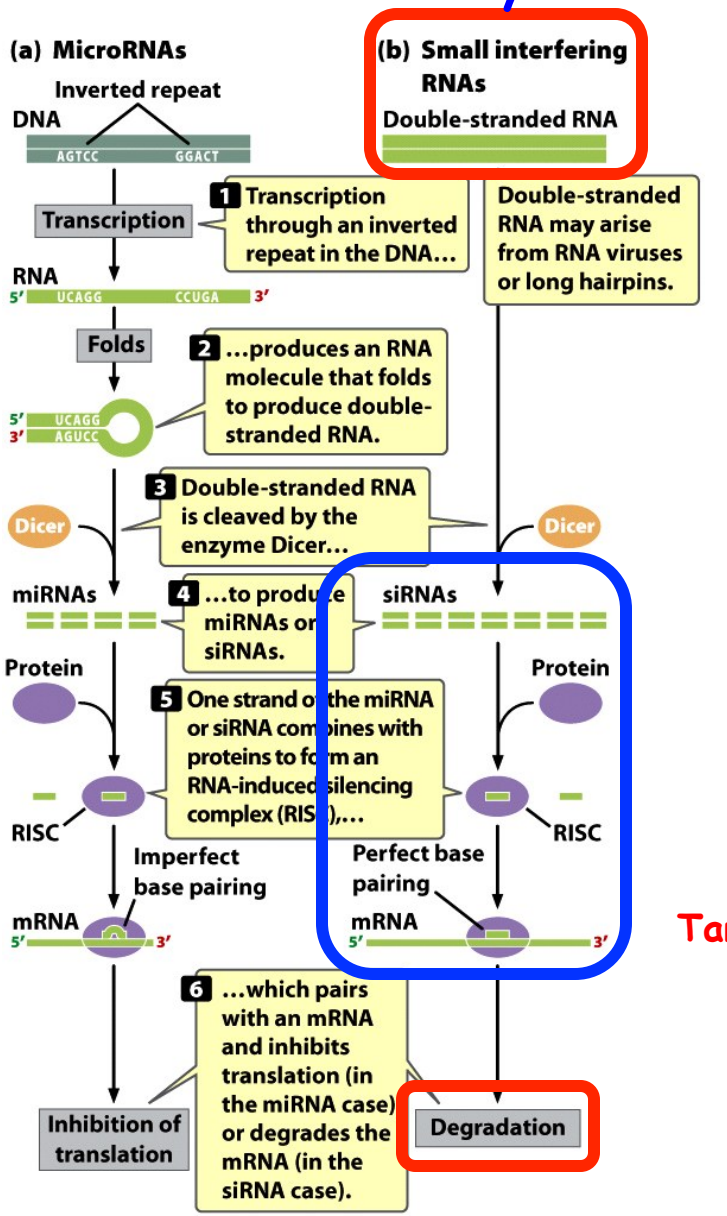
One Cause - Dominant Mutations
in the Coding Region of the
Superoxide Dismutase (SOD1) Gene
(SOD is an Anti-Oxidant)

Mutant SOD1 Protein is Toxic
to Motor Neurons

If Mutant Gene Could Be Shut Off With a "Molecular Drug,"
Disease Might Not Develop

Small RNAs Target Specific mRNAs For Degradation and/or Protein Synthesis Inhibition

RNAi is Considered to be the Genome's "Immune System" Protecting Against RNA Viruses & Transposable Element Movement

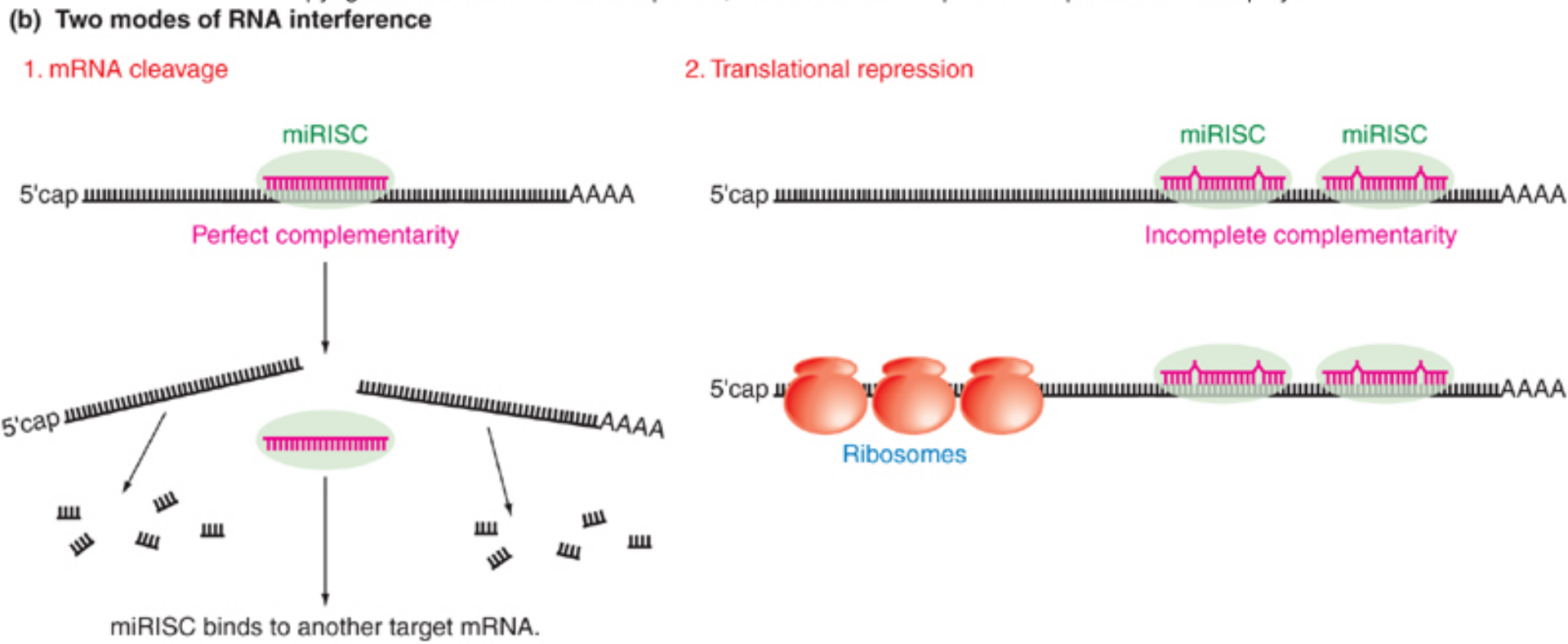


Target mRNA

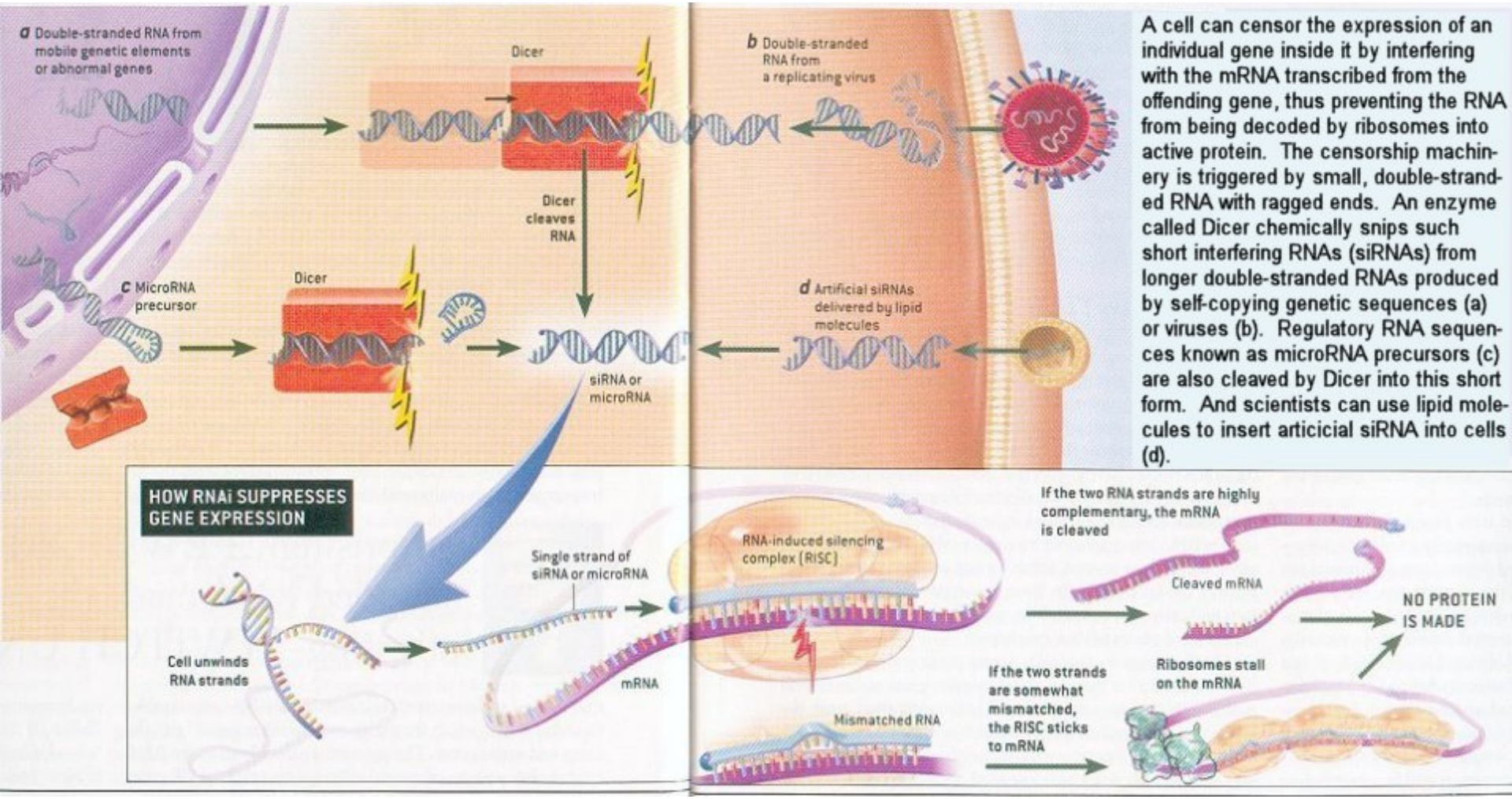
Figure 14-22
Genetics: A Conceptual Approach, Third Edition

Andrew Fire & Craig Mello Nobel Prize-2006

RNA Interference (RNAi) Specifically Inhibits the Accumulation of Targeted Proteins

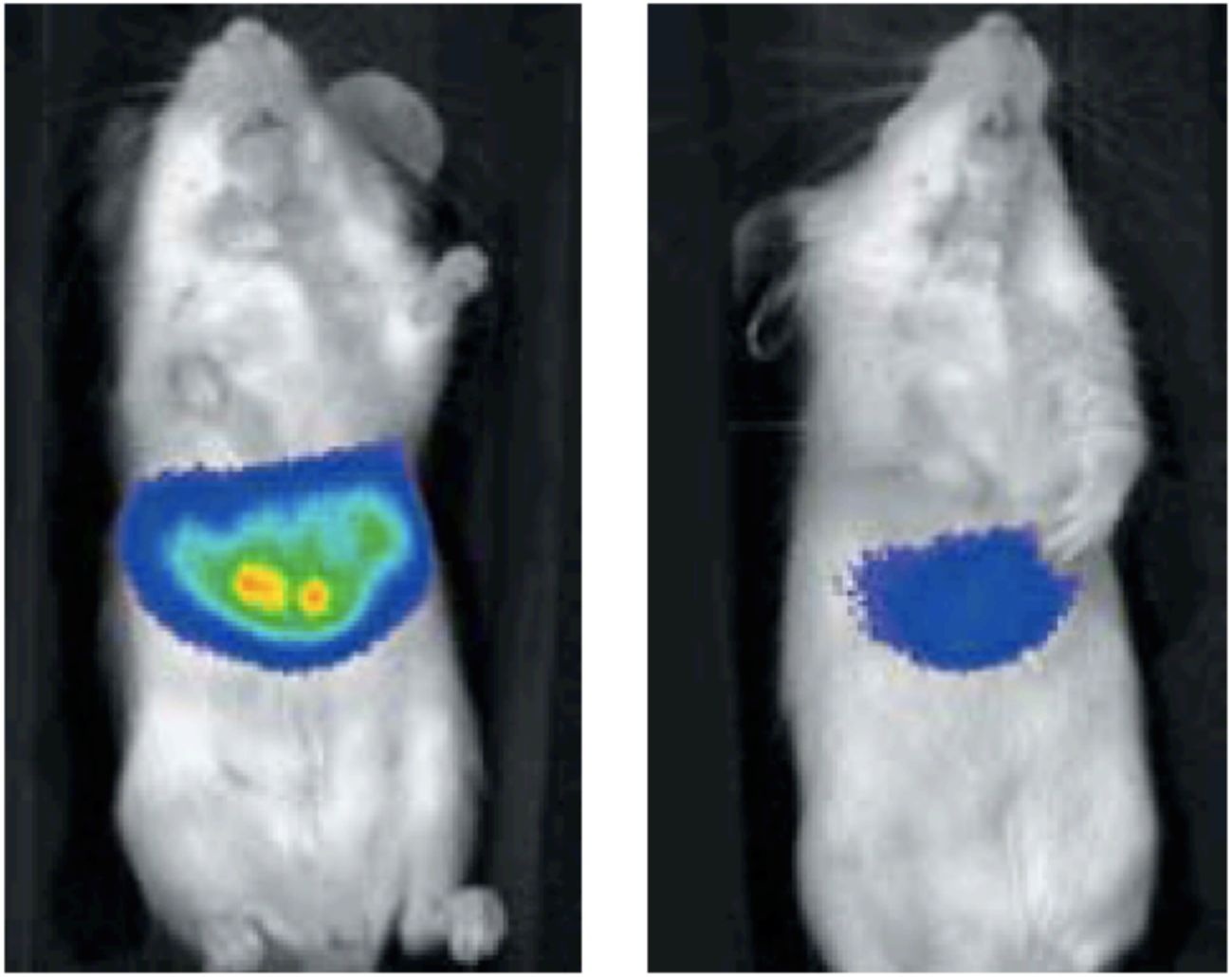


RNAi Can be Used in Gene Therapy Strategies to Suppress Expression of Targeted Genes



A cell can censor the expression of an individual gene inside it by interfering with the mRNA transcribed from the offending gene, thus preventing the RNA from being decoded by ribosomes into active protein. The censorship machinery is triggered by small, double-stranded RNA with ragged ends. An enzyme called Dicer chemically snips such short interfering RNAs (siRNAs) from longer double-stranded RNAs produced by self-copying genetic sequences (a) or viruses (b). Regulatory RNA sequences known as microRNA precursors (c) are also cleaved by Dicer into this short form. And scientists can use lipid molecules to insert articicial siRNA into cells (d).

Using RNAi To Inhibit Gene Activity



MICE LIGHT UP when injected with DNA containing the luciferase gene (*left*). But scientists took the shine off the mice by also injecting siRNAs that match the gene (*right*), thus demonstrating one way to exploit RNAi in mammals.

RNAi is One of the Most-Exciting New Fields For Combating Human Diseases (e.g., Cancer & Pathogens)

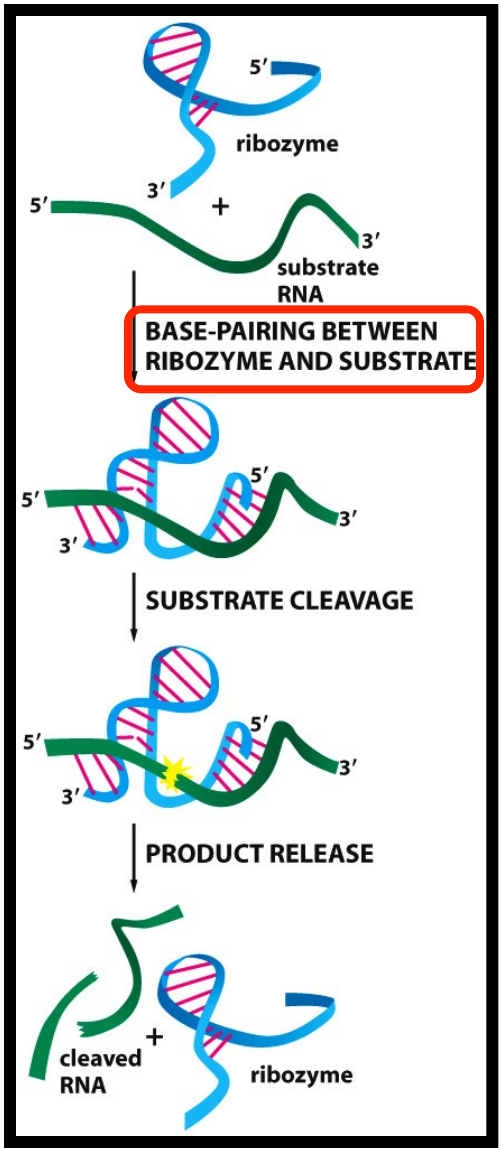
ffforts to Apply RNA Interference to Medicine

THE MACHINERY for RNA interference was discovered to operate in mammals just two years ago. Yet about 10 companies, including the sampling below, have already begun testing ways to exploit gene censoring to treat or prevent human disease. —*The Editors*

COMPANY	PROJECTS	STATUS
Alnylam Pharmaceuticals Cambridge, Mass.	Researching therapeutic applications of RNAi, but specific disease targets not yet announced	Founded in 2002 by Bartel, Tuschl, Sharp and Zamore, the firm has secured initial funding and several patents
Cenix Biosciences Dresden, Germany	Investigating the use of RNAi-based therapies for cancer and viral diseases	With Texas-based Ambion, Cenix is creating a library of siRNAs to cover the entire human genome
Ribopharma Kulmbach, Germany	Attempting to chemically modify siRNAs to make drugs for glioblastoma, pancreatic cancer and hepatitis C	Clinical trials in brain cancer patients are expected to begin this year
Sirna Therapeutics Boulder, Colo.	Testing a catalytic RNA medicine for advanced colon cancer in clinical trials; development of RNAi-based therapeutics is still in early stages	Changed name from Ribozyme Pharmaceuticals in April; recently secured $48 million in venture capital

Major Challenge: Delivery Systems

Using Target-Specific Ribozyme Gene Therapy To Destroy Specific mRNAs



Ribozymes
Are
RNA
Enzymes!

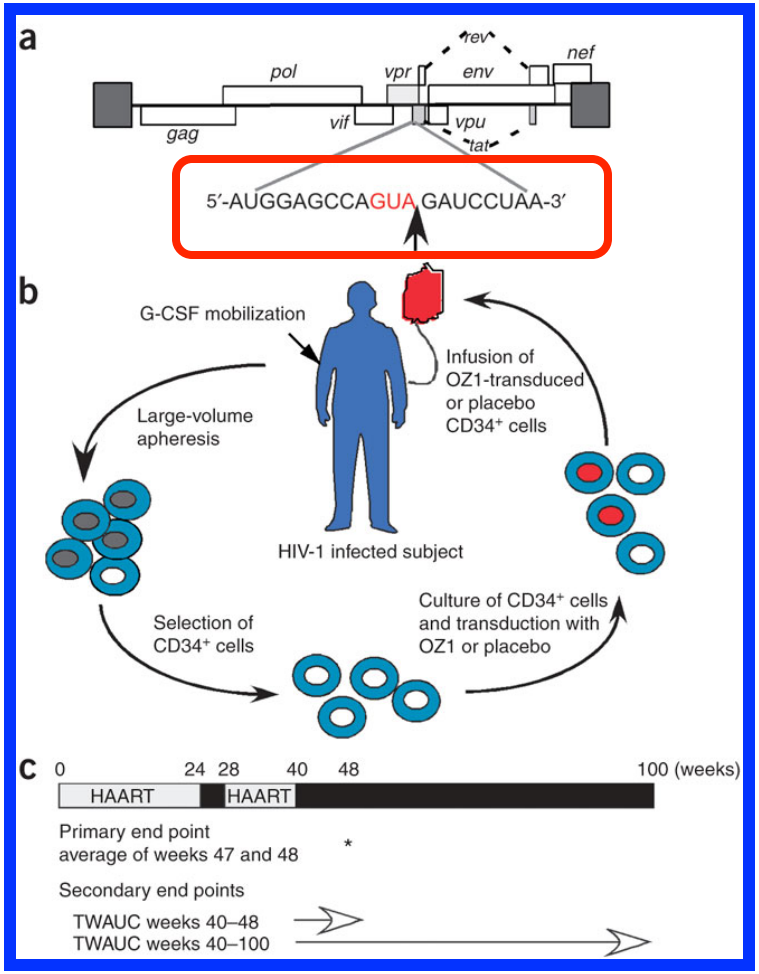
They Can
Be Engineered
And Transformed
Into a Cell to
Degrade Specific
mRNAs!!

Figure 6-103 *Molecular Biology of the Cell* (© Garland Science 2008)

Using Ribozymes To Treat Human Diseases

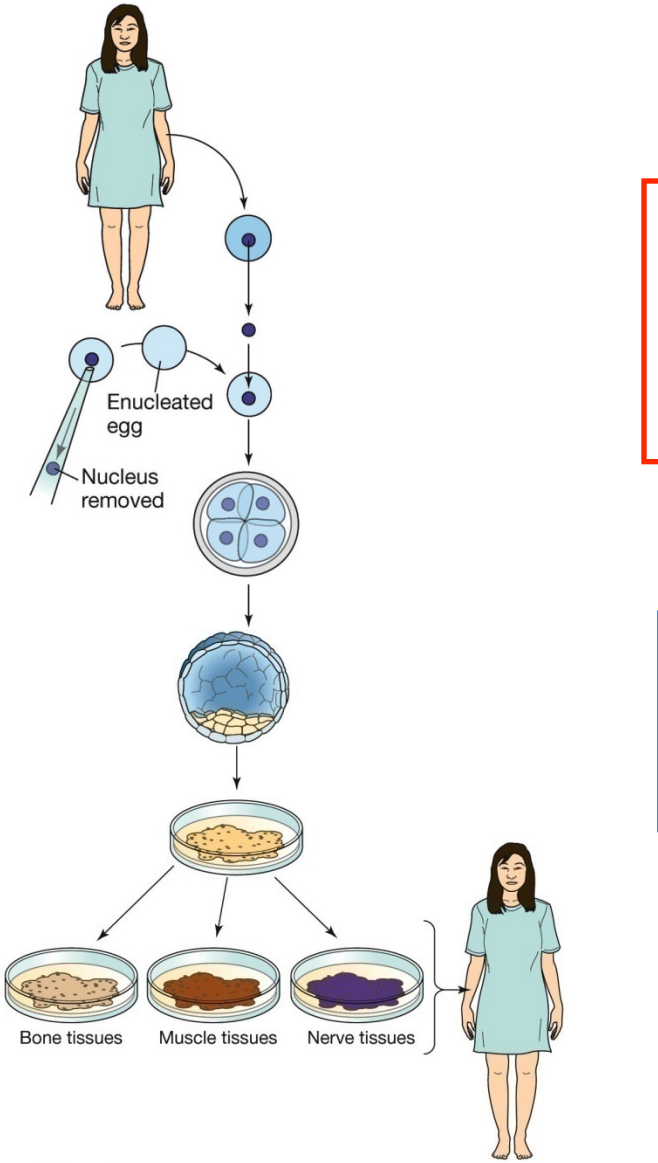
Phase 2 gene therapy trial of an anti-HIV ribozyme in autologous $CD34^+$ cells **Nature Medicine January, 2009**

Ribozyme Specific For an HIV mRNA



Highly Active Antiretroviral Therapy, or HAART

Combining Gene Therapy With Stem Cells & Therapeutic Cloning in the Future

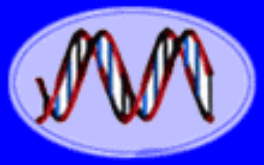


LIFE 8e, Figure 19.8 (I

LIFE 8e, Figure 19.8 (Part 2)

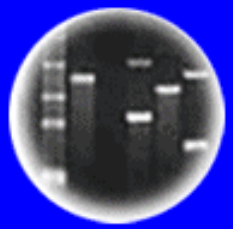
Genetic Engineer Cells Before Nuclear or Cell Transfer

Example Defective Insulin Gene in Pancreas



DNA
Genetic Code of Life

Entire Genetic Code of a Bacteria



DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

The End!!

HC70A/SAS70A Lectures on the History, Science, and Applications of Genomics & Genetic Engineering