Why can’t we cure more diseases ?

杜建達　Location

陳珩昌　Delivery vehicles

朱彧緯　Gene therapy

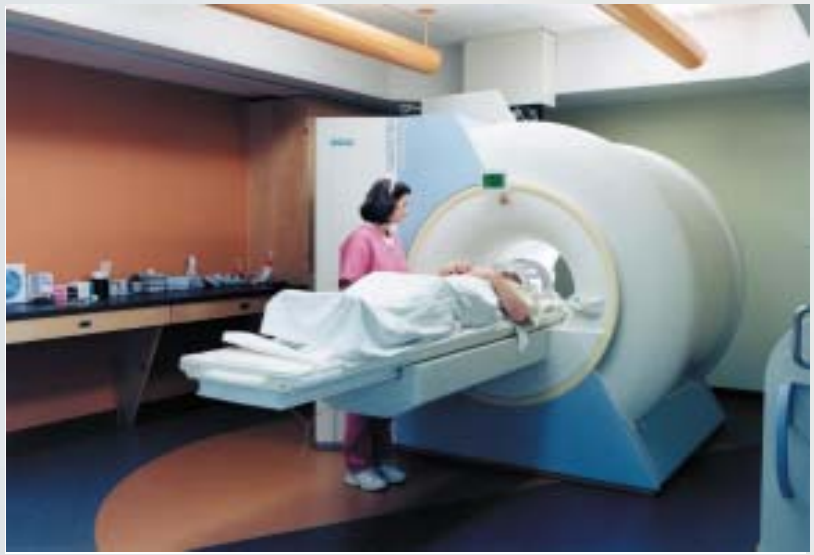
葉明泓　Pharmacogenetics

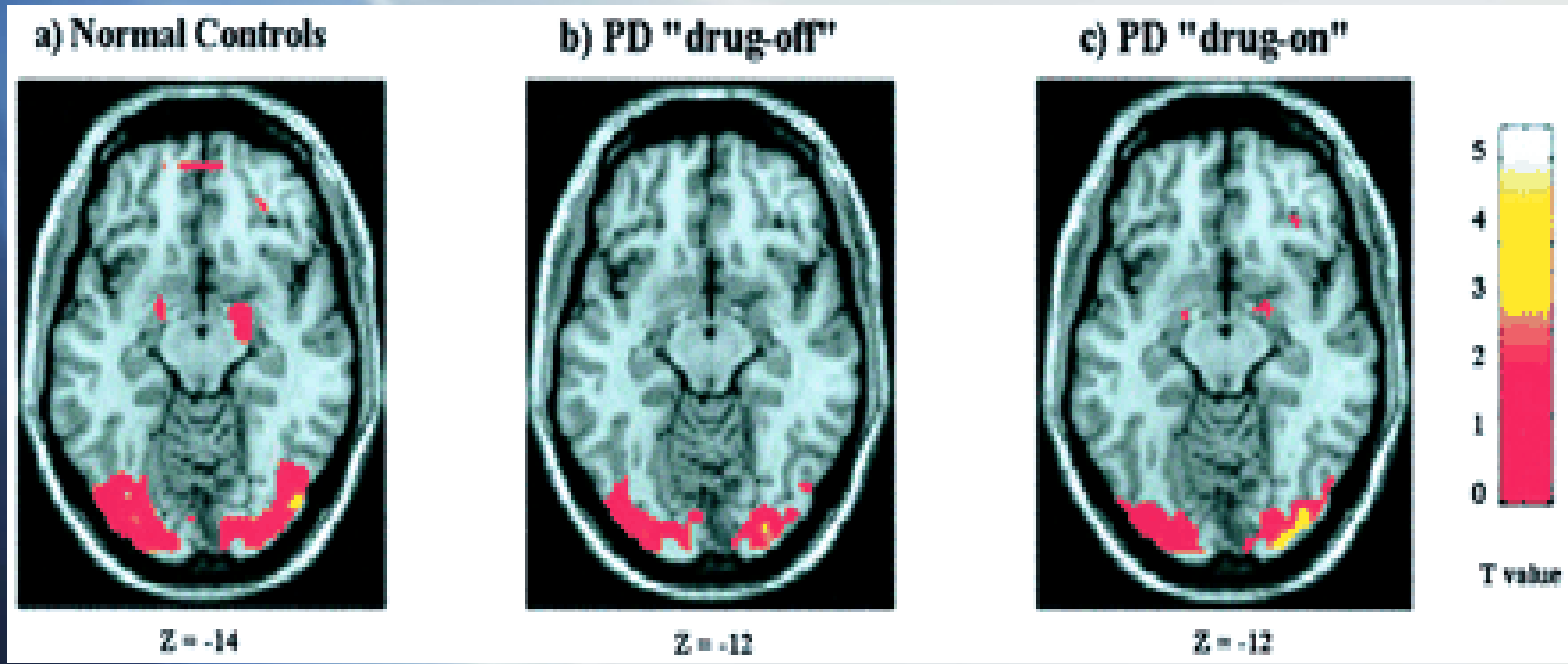
謝崇斌　Drug design

Location

Magnetic resonance imaging (MRI)

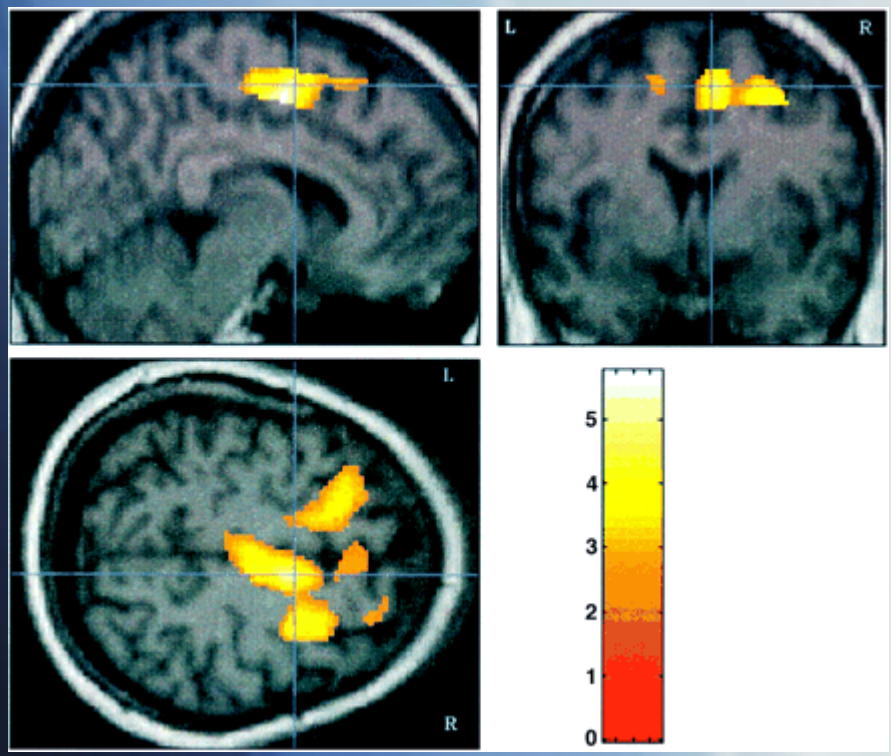
- 磁場原理
- no ionization radiation is used
- the optimal method for non-invasive evaluation
- The image represents a sectional view
- The physical scanner does not have any moving parts
- it have not yet been possible to establish any damaging effect on the human body
- 高對比的解像力





- Statistical parametric maps illustrating the difference in the BOLD response of the amygdala across the three groups ($p < 0.05$, corrected).

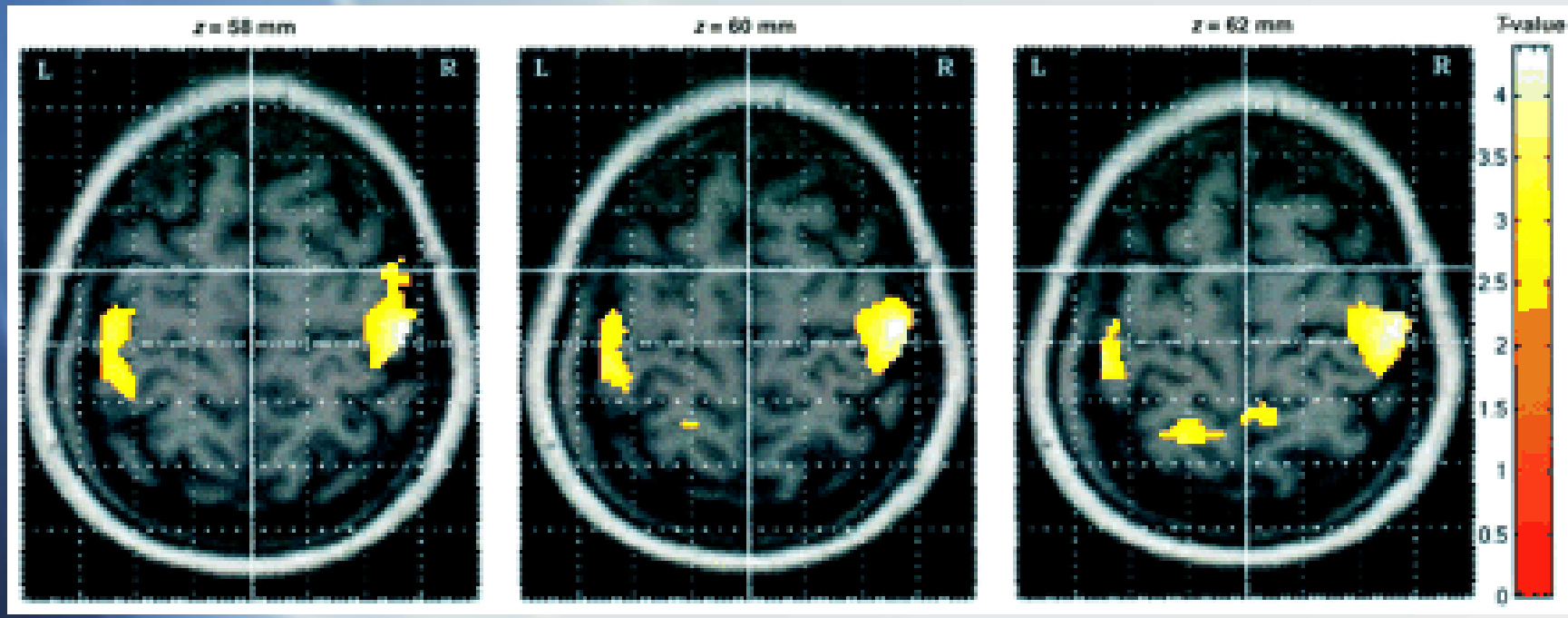
The Journal of Neuroscience, October 15, 2002, 22(20):9099-9103



- Cortical areas showing significant inceases of movement-related activation following levodopa medication in Parkinson's disease patients ($P < 0.01$ uncorrected, extent threshold 10 voxels).

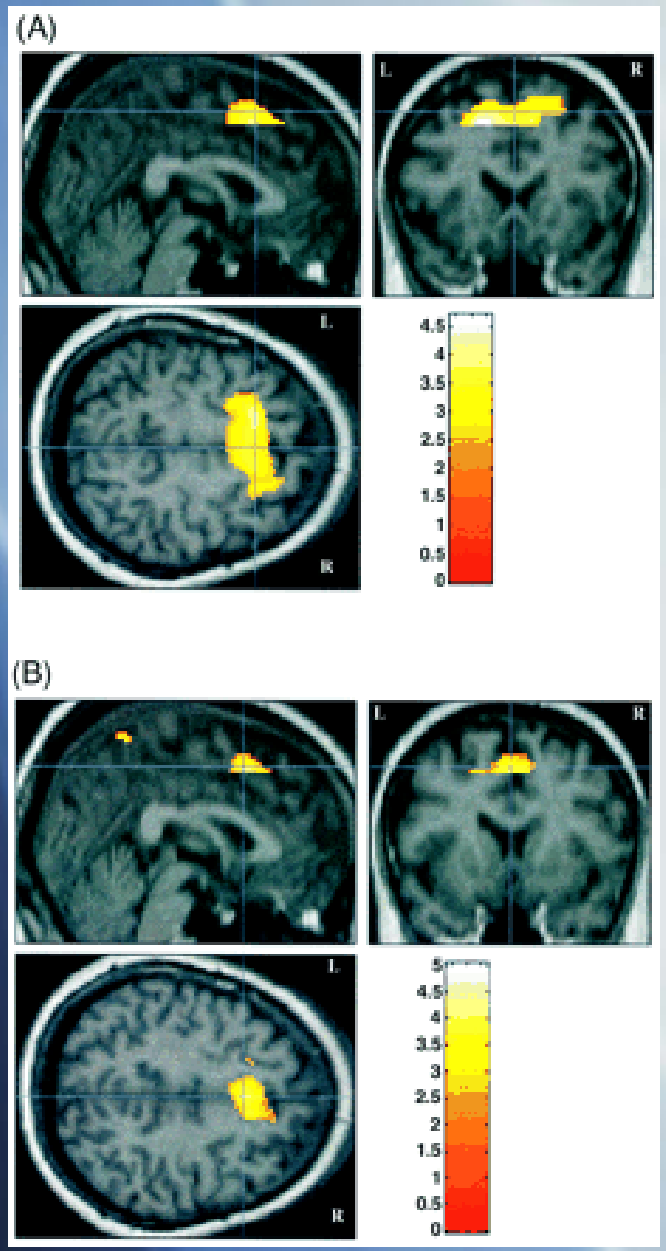
- Activation is displayed onto three orthogonal sections of a normalized T1-weighted anatomical magnetic resonance image. R = right; L = left.

Brain, Vol. 124, No. 3, 558-570, March 2001



- Cortical areas with significant signal increases off versus on levodopa in Parkinson's disease ($P < 0.01$ uncorrected, extent threshold 10 voxels). BOLD signal increases are overlaid onto three consecutive axial slices of a normalized T1-weighted anatomical magnetic resonance image. R = right; L = left.

Brain, Vol. 124, No. 3, 558-570, March 2001

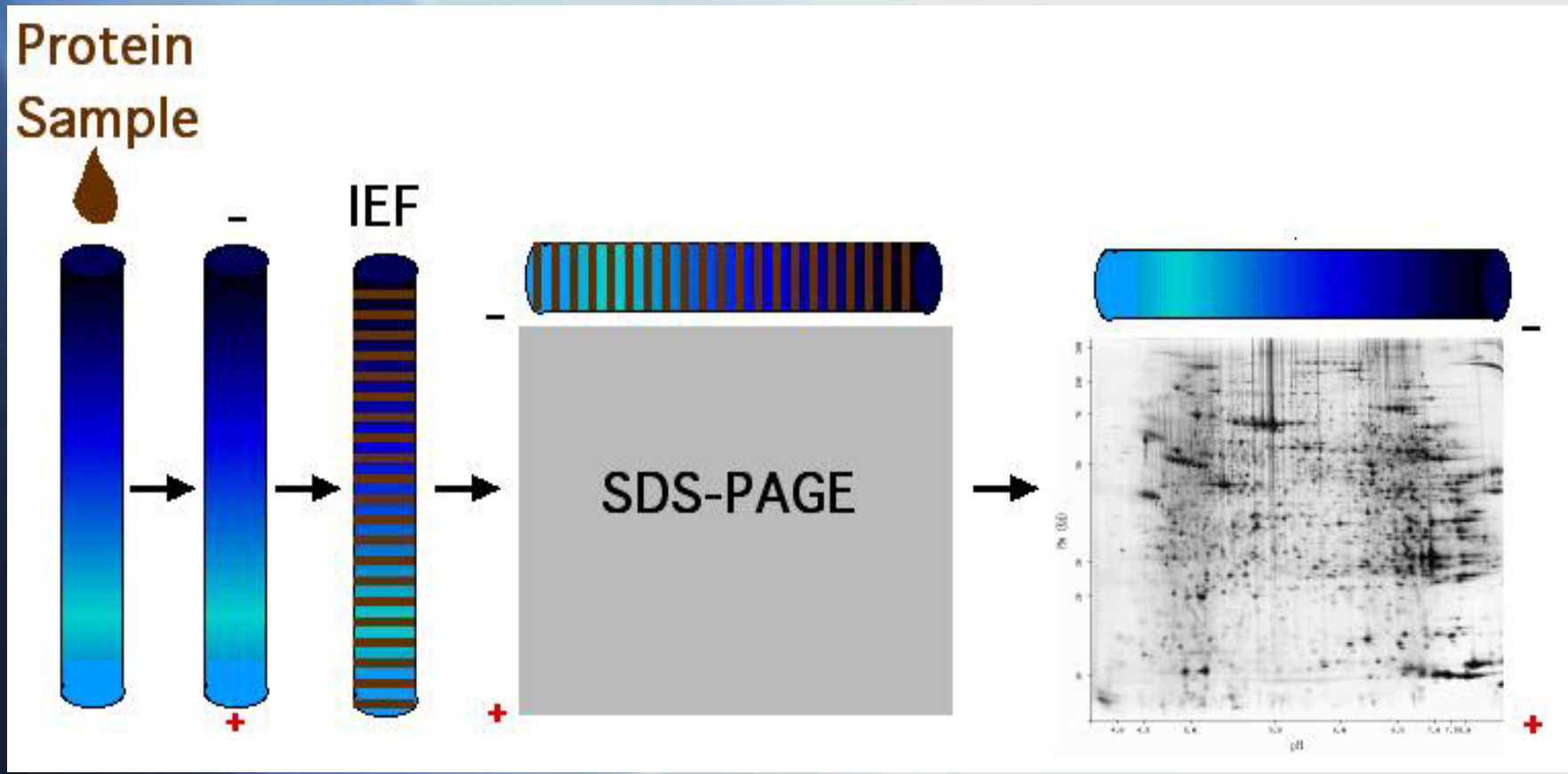


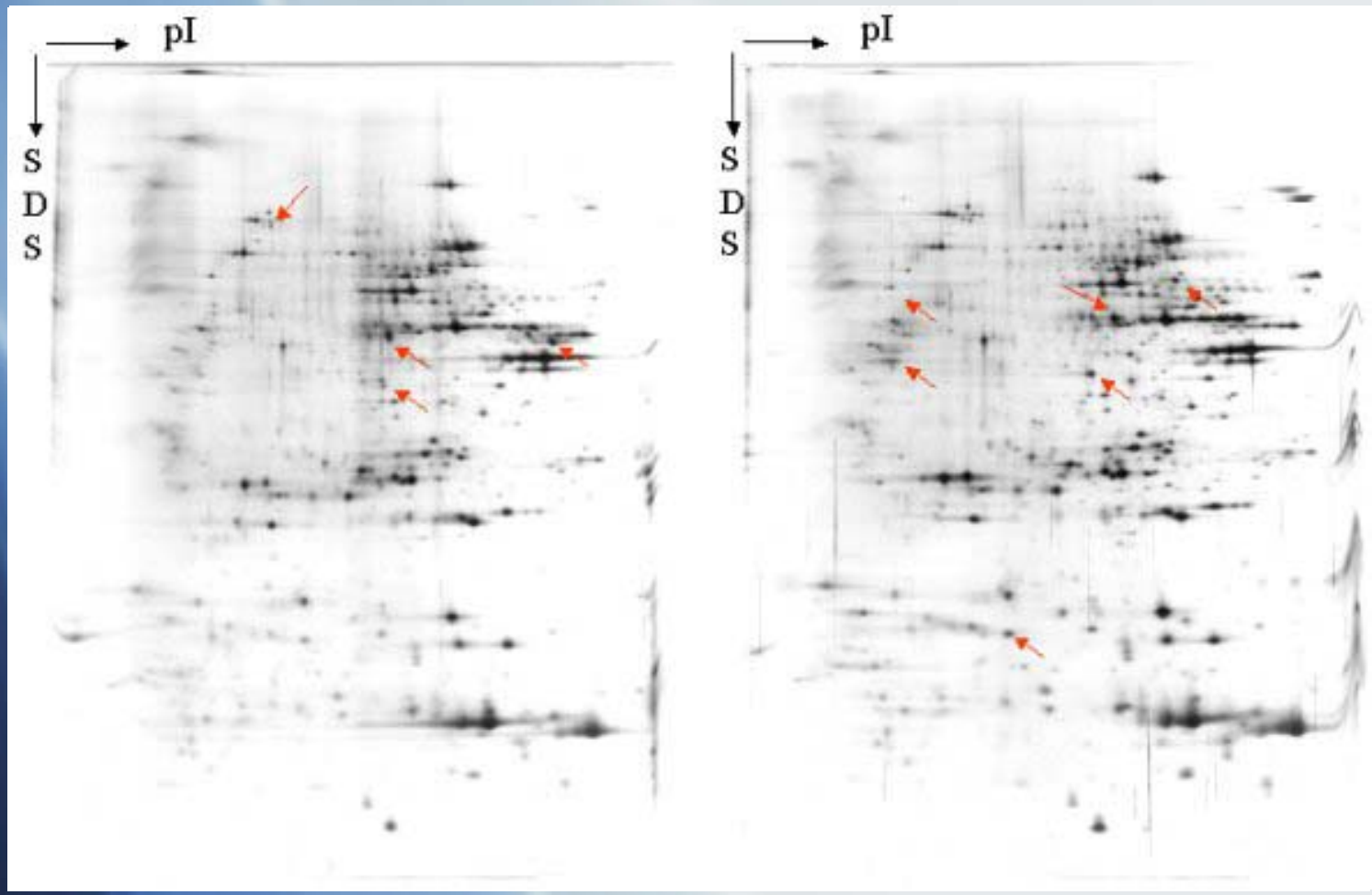
- Between–group analysis showing cortical areas with significant underactivity in Parkinson's disease patients off (**A**) and on (**B**) levodopa compared with healthy volunteers ($P < 0.01$ uncorrected, extent threshold 10 voxels).

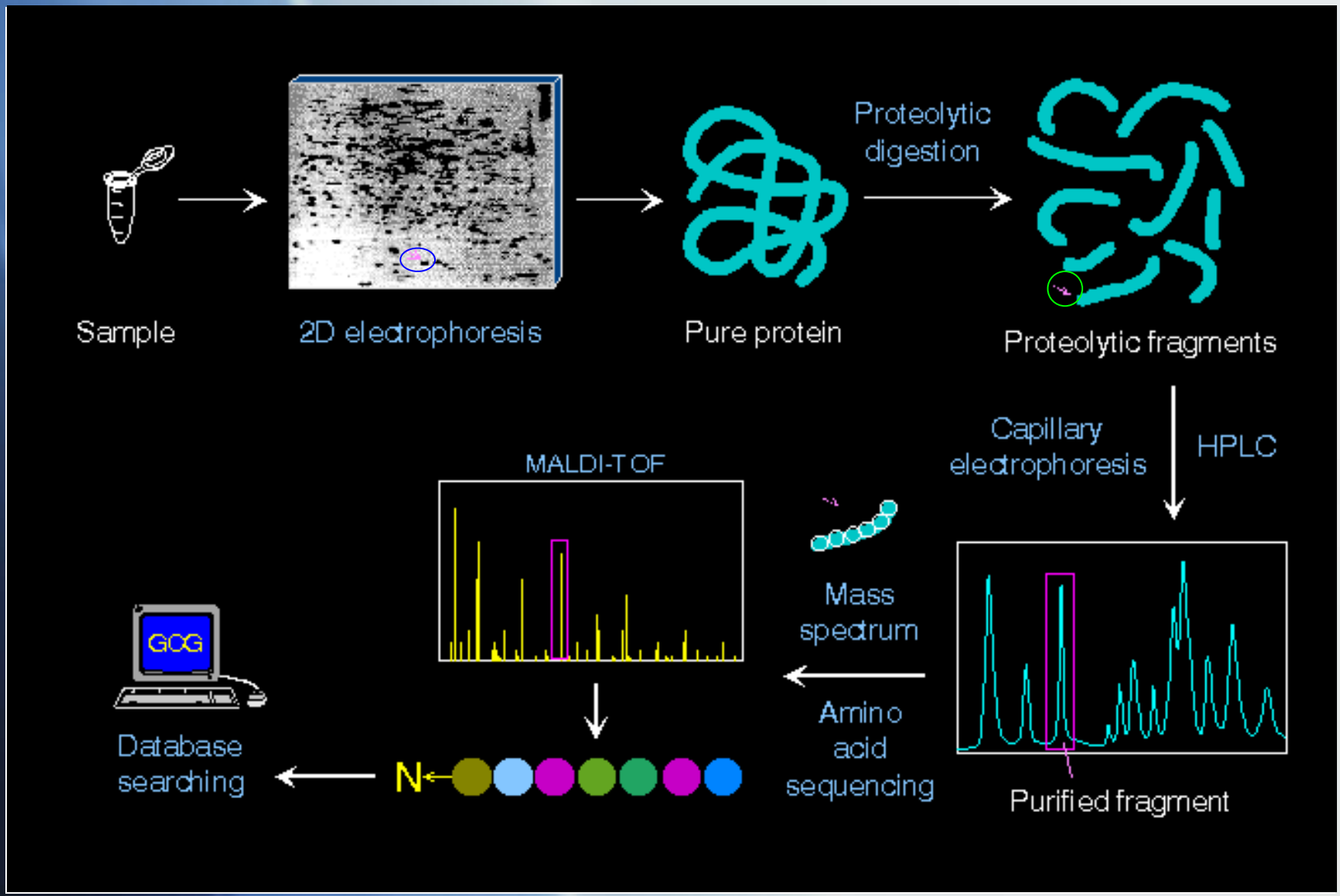
- Activation is displayed onto three orthogonal sections of a normalized T1-weighted anatomical magnetic resonance image. R = right; L = left.

Brain, Vol. 124, No. 3, 558-570, March 2001

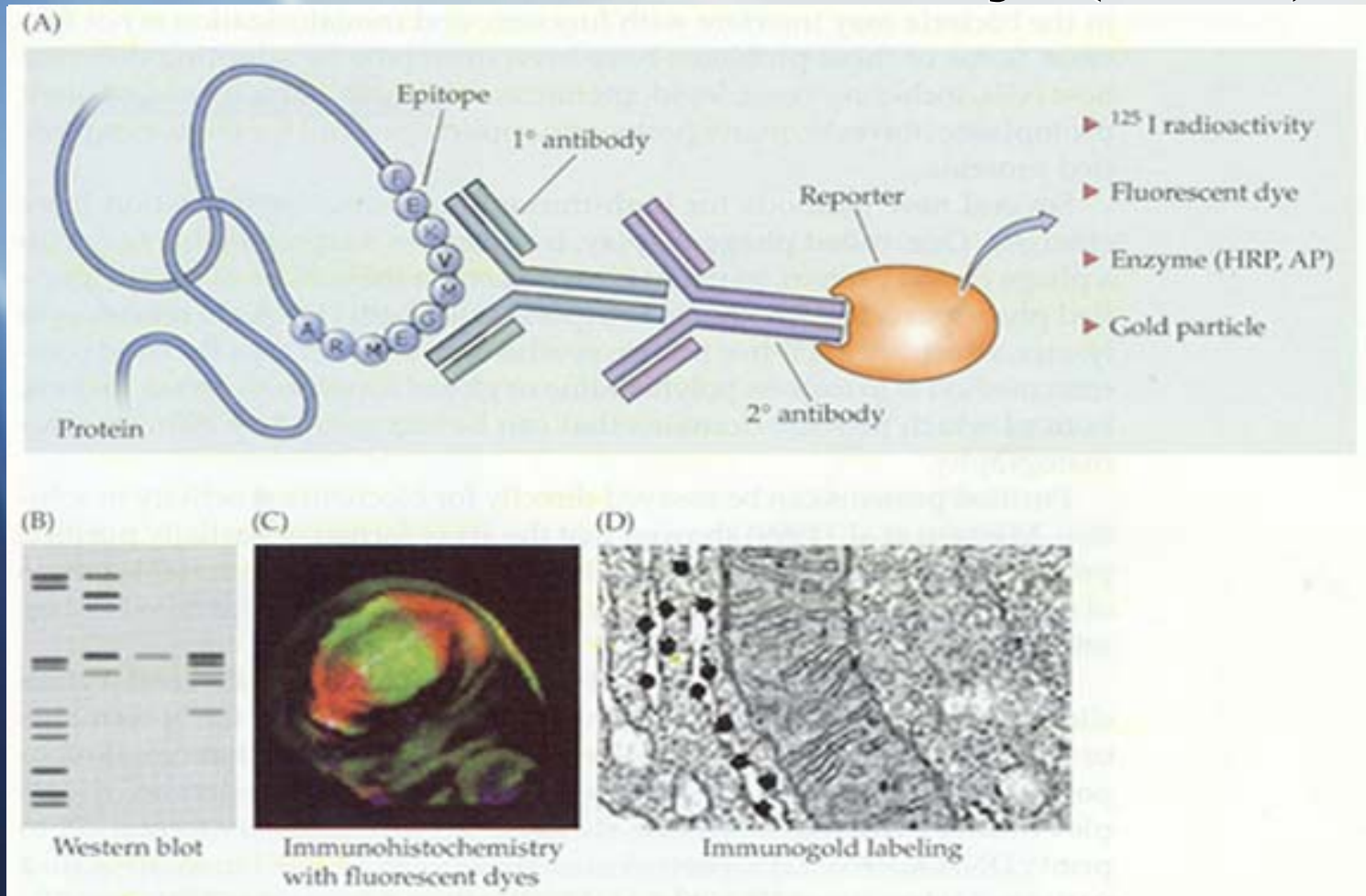
2D gel electrophoresis

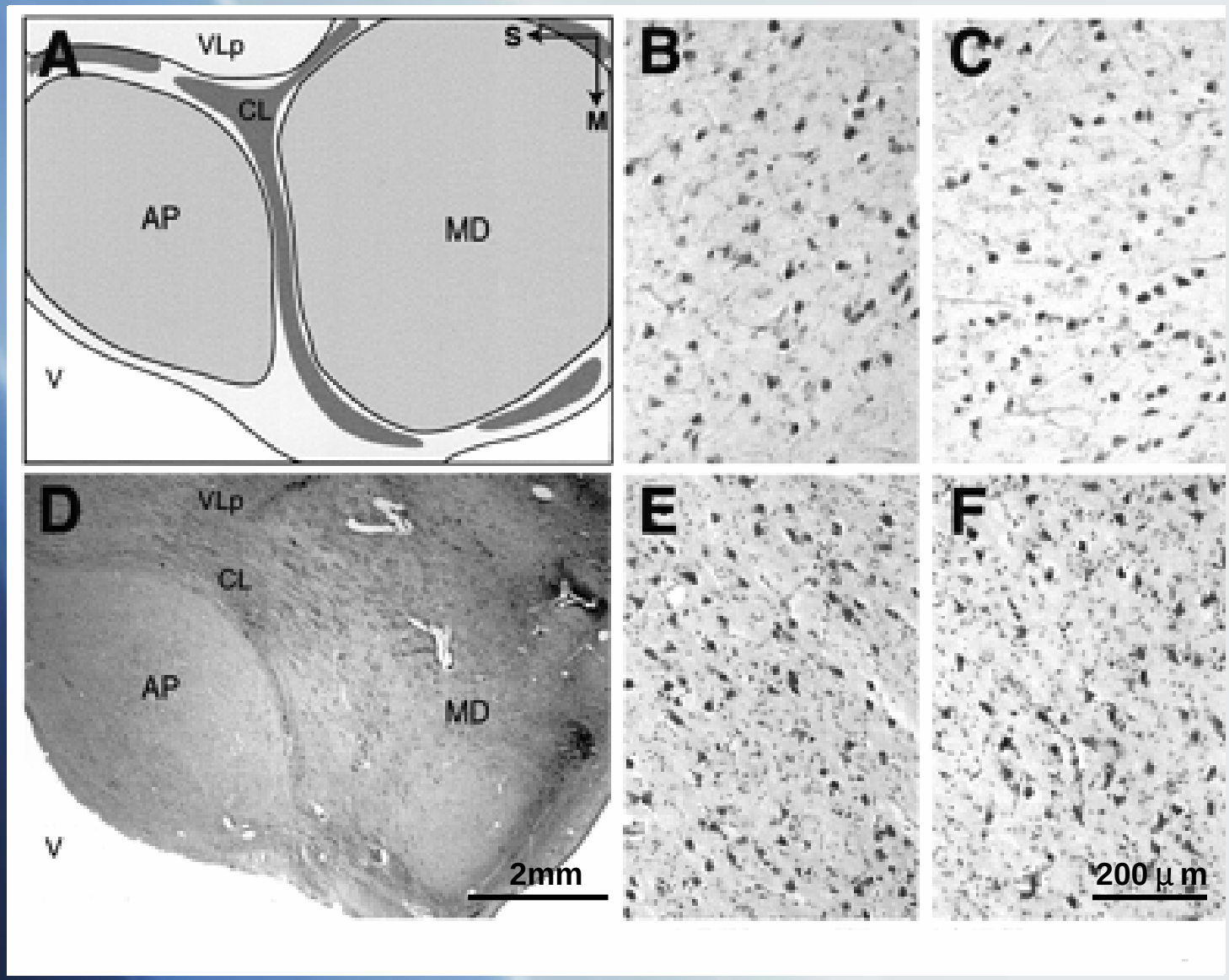



pI
SDS
pI
SDS


Sample
2D electrophoresis
Pure protein
Proteolytic digestion
Proteolytic fragments
Capillary electrophoresis
HPLC
Purified fragment
Amino acid sequencing
Mass spectrum
MALDI-TOF
N
Database searching
GCG

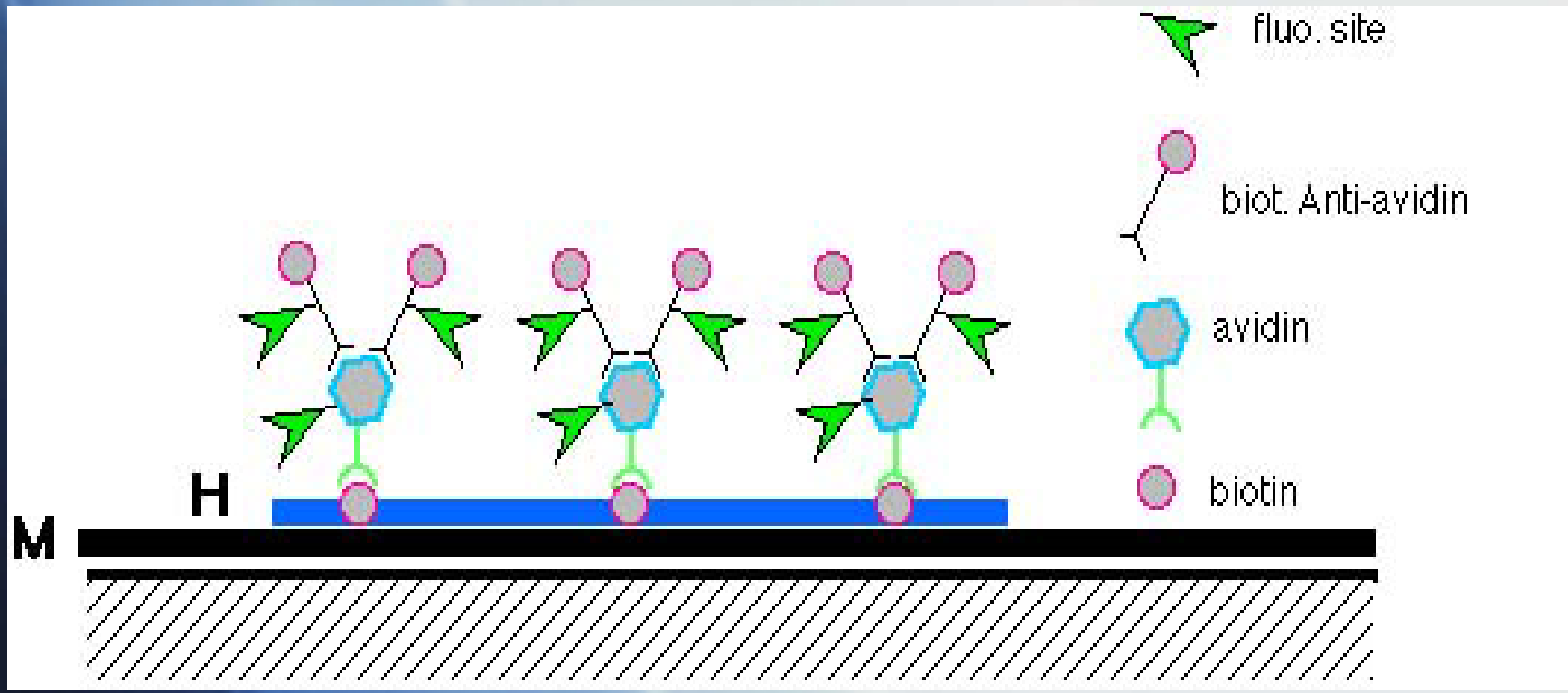
Immunohistochemistry (IHC)

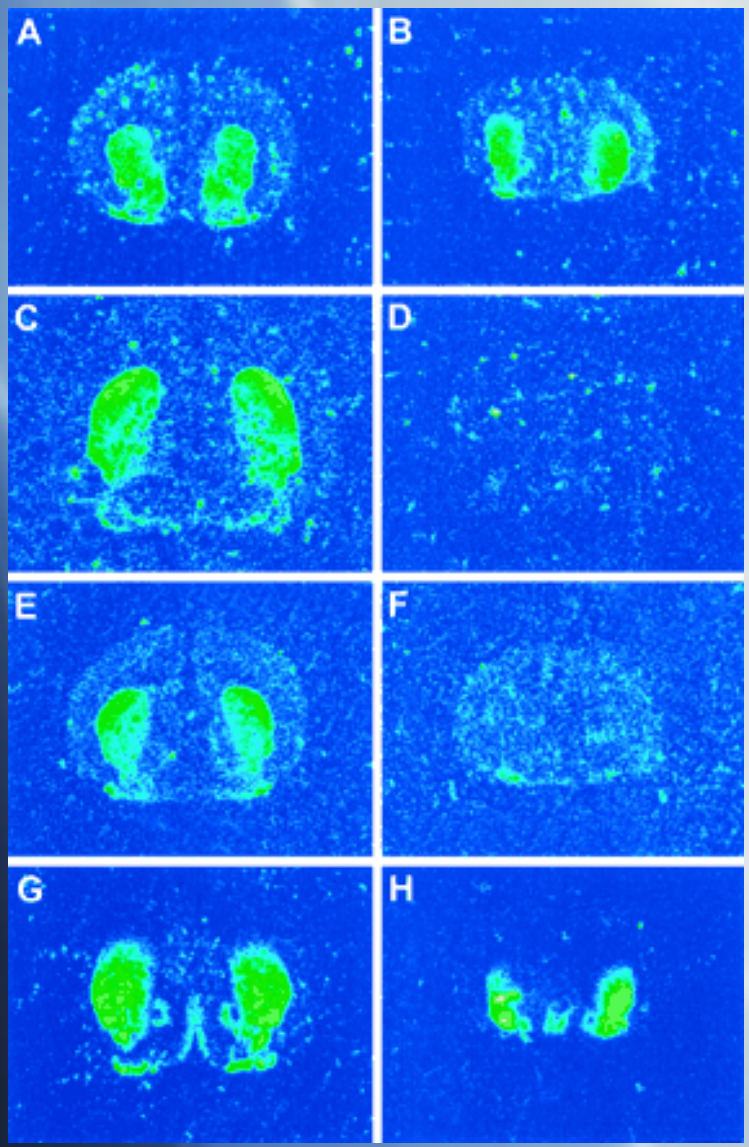




Brain, Vol. 123, No. 7, 1410-1421, July 2000

Fluorescent *in situ* Hybridization (FISH)





In situ hybridization for

- D_2R (*A*, *B*) ,
- dynorphin (*C*, *D*) ,
- substance P (*E*, *F*) ,
- and enkephalin (*G*, *H*)

in P3/P4 normal (*A*, *C*, *E*, *G*) and mutant (*B*, *D*, *F*, *H*) pups.

The Journal of Neuroscience, December 1, 1998, 18(23):9845-9857

Delivery Vehicles

05/13/03

Speaker: Heng-Chang Chen

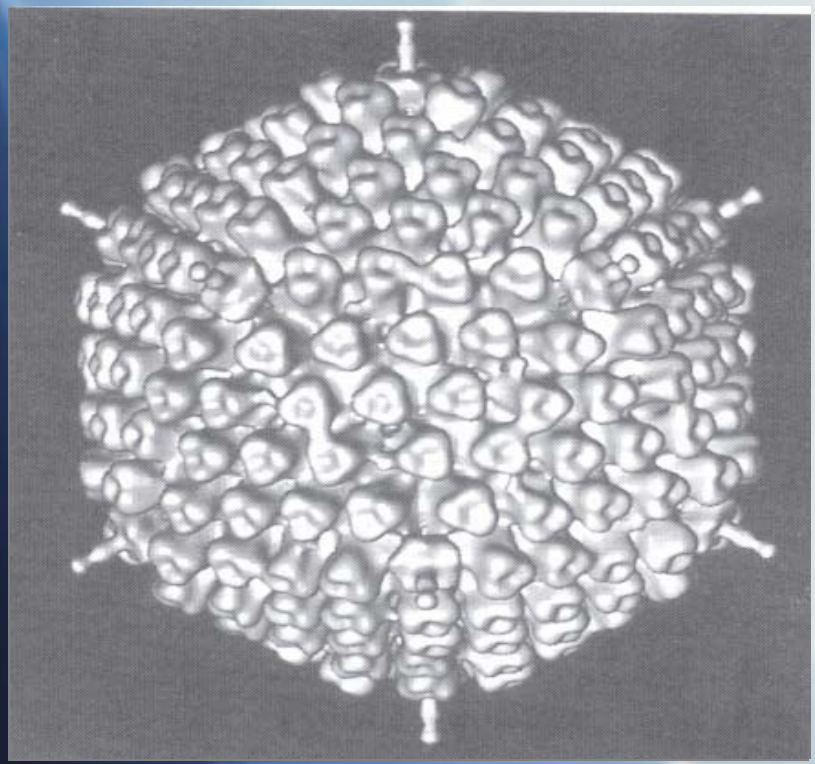
3 Main Delivery Vehicles

- Viral Vehicles
- Protein Carrier
- Nucleic Acids

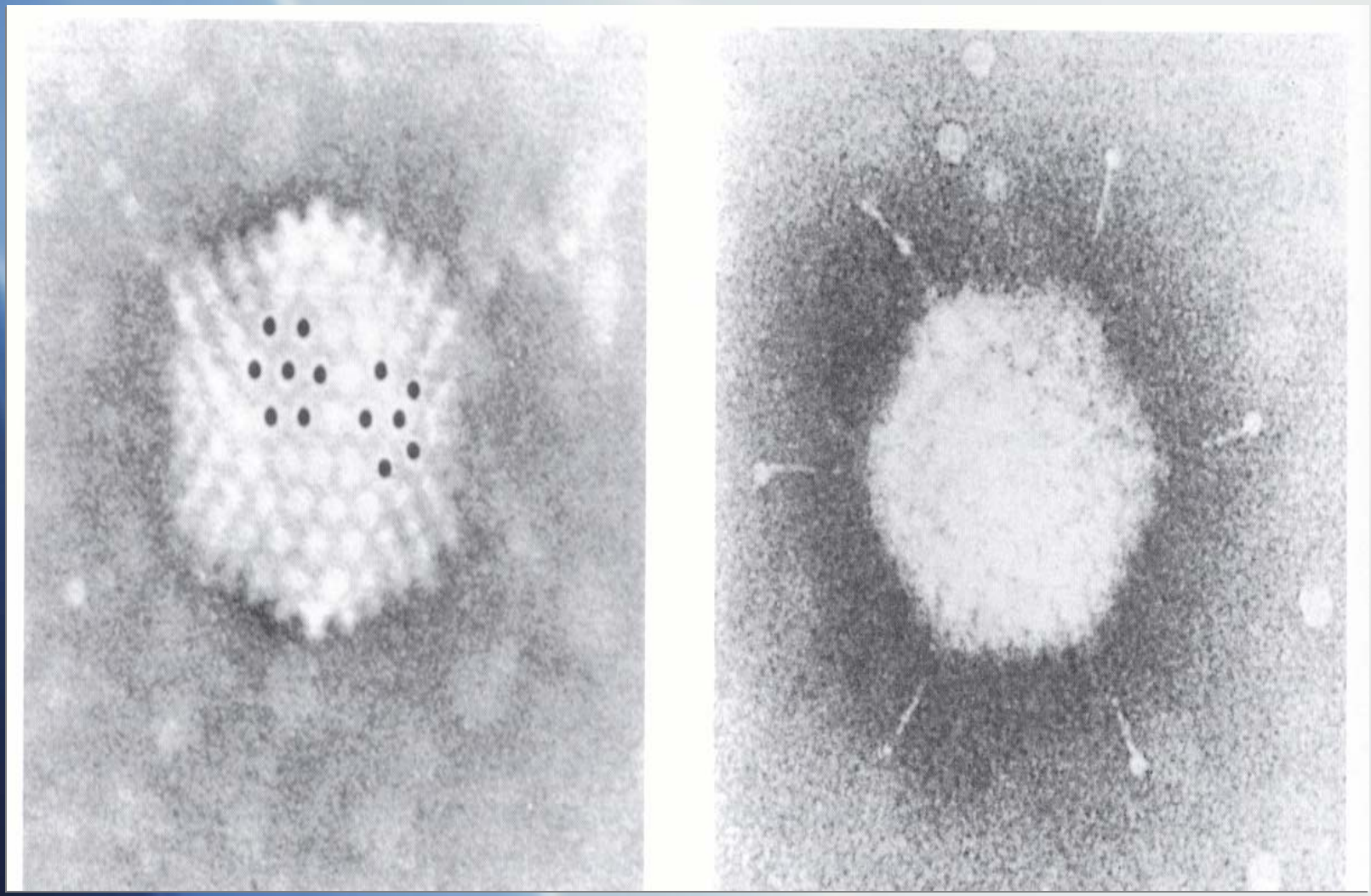
Viral Vehicles

- Adenovirus
- Adeno-associated virus
- Lentivirus
- Herpesvirus
- Retrovirus

ADENOVIRUS



- Adenoviridae
- Linear, dsDNA
- Be divided into 6 groups(A to F)



- Advantages:

  High level expression.

  Efficient infection on Non-dividing cells.
- Disadvantages:

  High immunogenecity.

  Complexity of its genome.

  Cannot maintain stable expression pattern.

Commentary

Future of adenoviruses in the gene therapy of arthritis

Christopher H Evans*, Steven C Ghivizzani*, Thomas A Oligino† and Paul D Robbins†

*Center for Molecular Orthopaedics, Harvard Medical School, Boston, MA, USA

†Department of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

- Widespread application both in preclinical and clinical studies.
- So far, 86 clinical trails, over 20% of all human gene-therapy protocols, have made use of adenoviruses for gene delivery.
- The death in 1999 of a patient who received a large dose of recombinant adenovirus.

In preclinical studies…

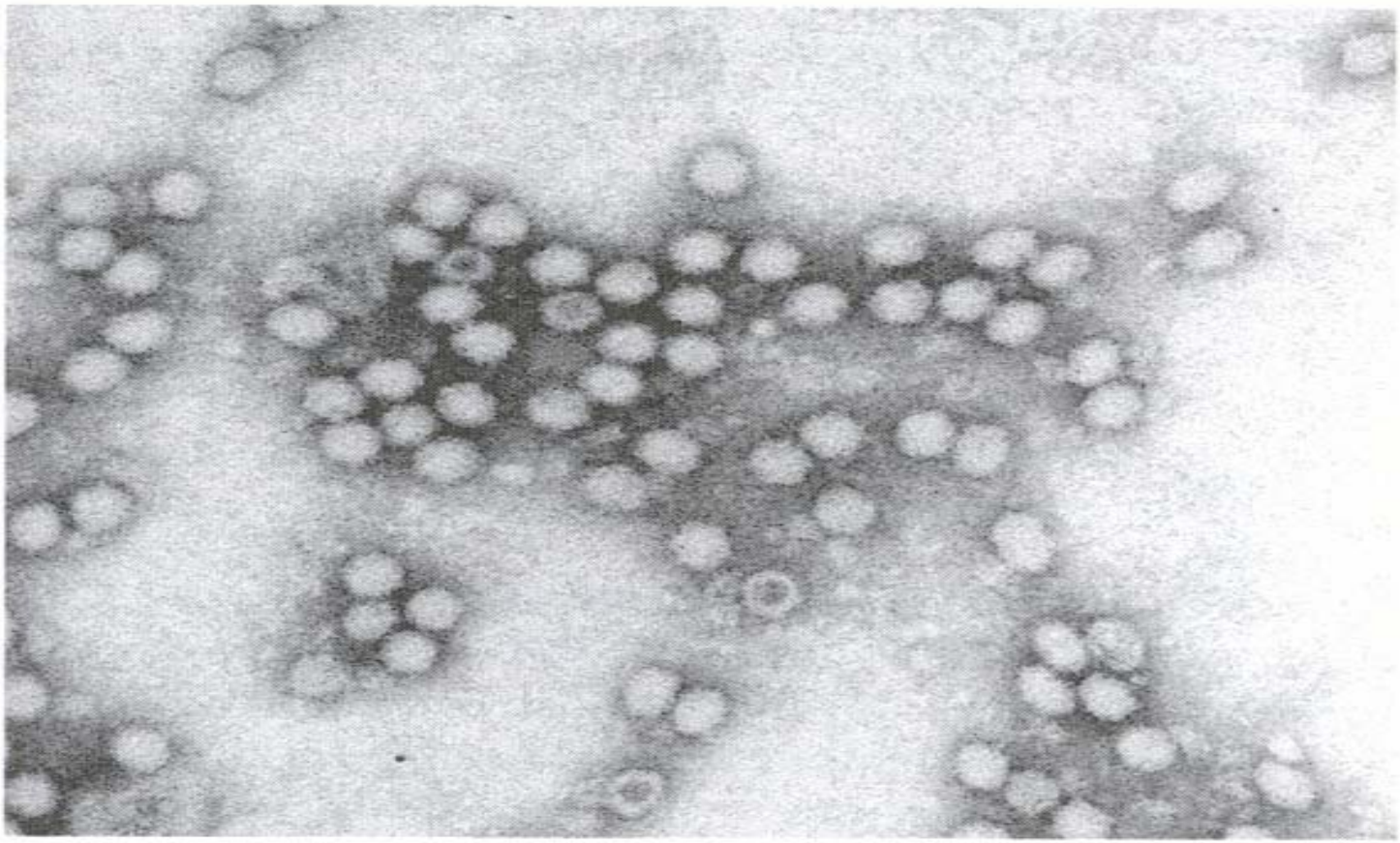
- Ease of manufacture and application allows the rapid screening of candidate anti-arthritic genes, testing of hypotheses, and evaluation of feasibility.

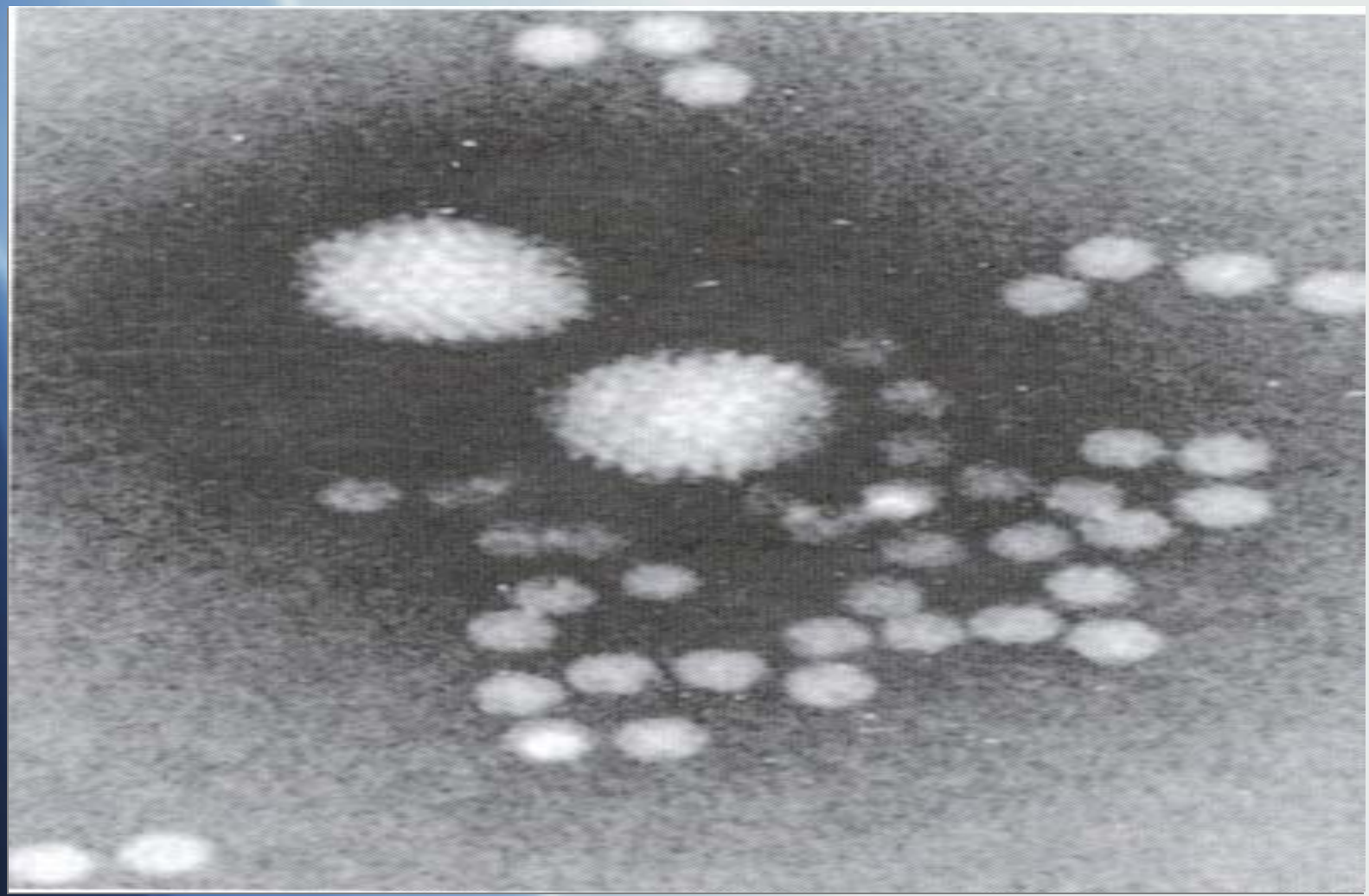
Limitation in clinical studies…

- Limited duration of gene expression.
- Difficulties in repeat dosing.
- Inflammatory responses.
- Questionable safety.
- The use of present vectors seems restricted to local, acute therapies.

AAV

- Parvoviridae/the simplest DNA virus
- The genus Dependovirus contains members that are defective and depend on a helper virus (usually an adenovirus) for replication. Human adenoassociated viruses belong to this genus.





- Advantages:

  Integration at a specific site in the genome of infected cells.

- Disadvantages:

  Lack of helper cell line for preparation of large amount of recombinant virus.

RETROVIRUS

- RNA tumor virus
- (+) ssRNA, linear, diploid
- 3 subfamilies:

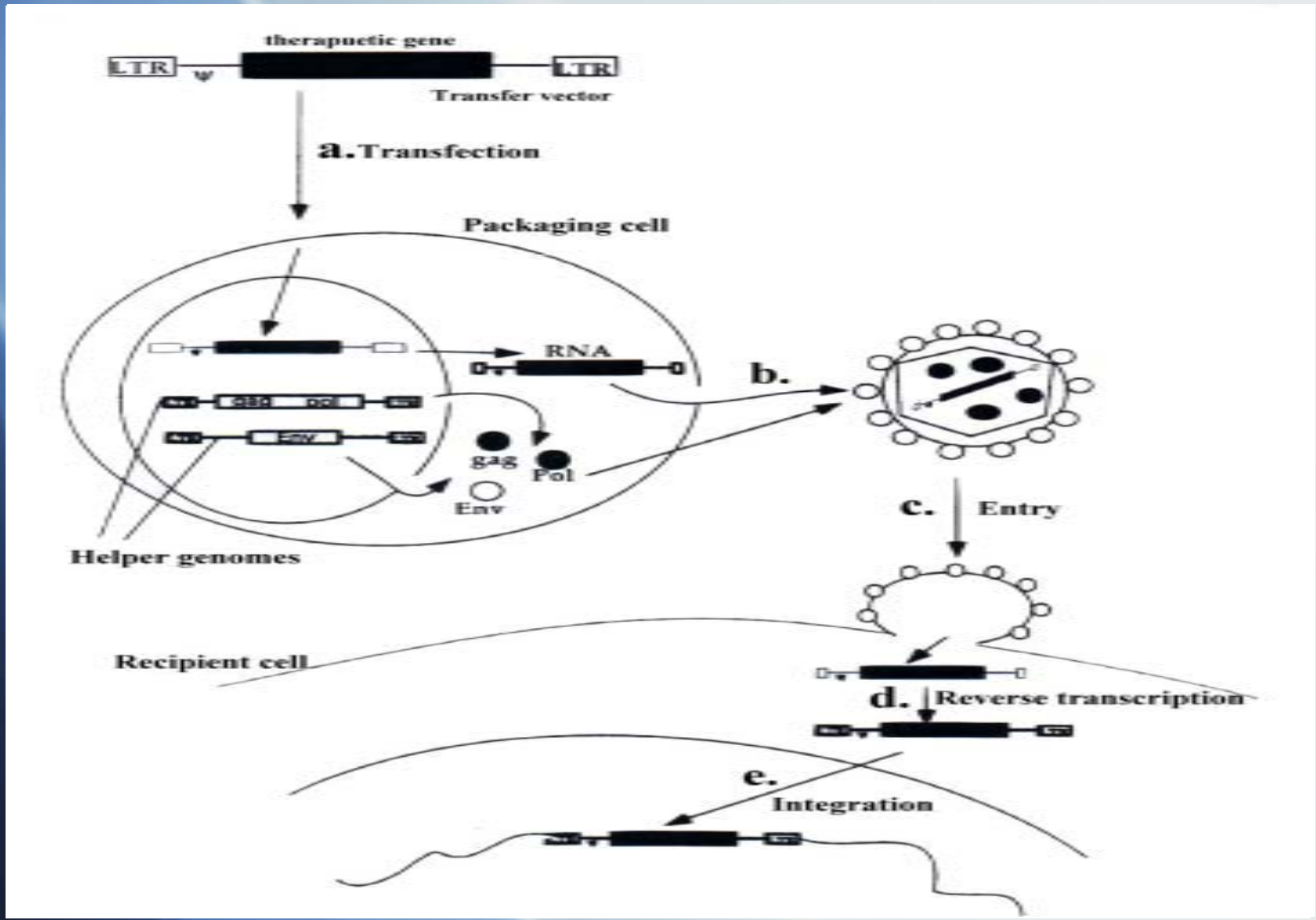
  -Oncovirinae, Spumavirinae, Lentivirinae

- Advantages:

  Efficient entry into Dividing cells and integration to create stable expression.

- Disadvantages:

  Cannot infect and integrate Non-dividing cells that happened in most of the target sites.


therapuetic gene
LTR
Ψ
LTR
Transfer vector
a. Transfection
Packaging cell
RNA
b.
gag
Pol
Env
Helper genomes
c. Entry
Recipient cell
d. Reverse transcription
e.
Integration

Protein Carrier

- Liposome
- Oligofectamine
- Lithium acetate(LiAc)
- The new method is très chic but not well understood

  ---Protein-transduction domain(PTD).

Liposome

- Advantages:

  Easy to prepare and no size constrain of introduced gene.
- Disadvantages:

  Low efficiency of gene transfer and mechanism of maintenance of introduced DNA in lacking.

Supersize Cargoes Pack

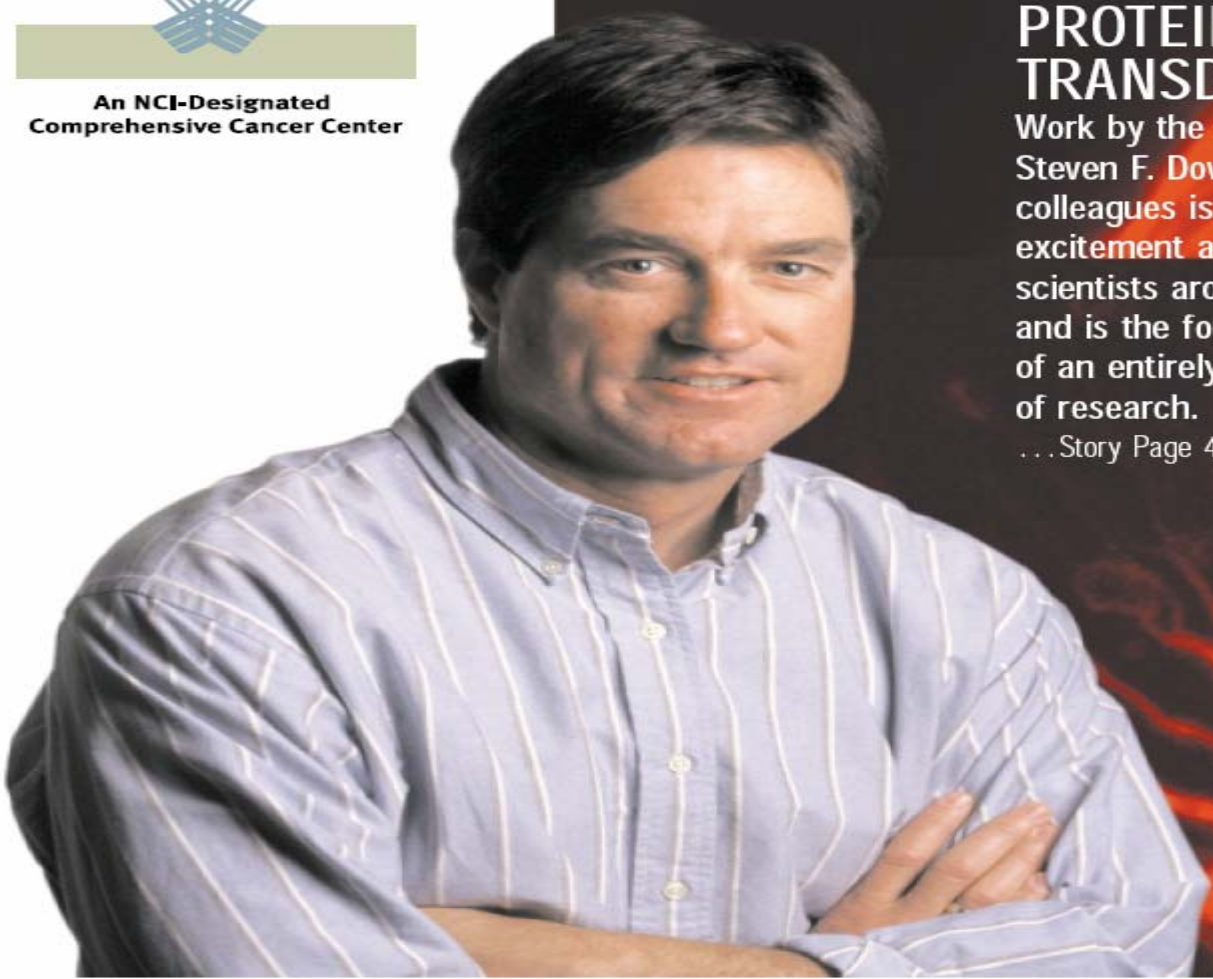
-Big Molecular Punch

"Protein transduction is like putting a computer through a mail slot. We can get vastly more information inside the cell than ever before."

~Steven Dowdy, Ph.D.

Rebecca and John Moores
UCSD Cancer Center NEWS
An NCI-Designated Comprehensive Cancer Center

University of California, San Diego



PROTEIN TRANSDUCTION:

Work by the Cancer Center's Steven F. Dowdy and colleagues is generating excitement among scientists around the globe, and is the foundation of an entirely new field of research.

...Story Page 4

SPRING 2003

Protein transduction domain(PTD)

- It’s positive charge; the surface of the cell is negative charge.
  - Powerful fusion → cross the cell membrane (mechanism is not yet understood!!)

- Every chemical structure can utilize this approach-nucleic acid, proteins, peptides, synthetic molecules, carbohydrates and more.

Nucleic Acids

- Surprisingly, muscle cells (cardiac and skeletal) can take up DNA without any special coating or delivery vehicles.

Gene therapy

What is gene therapy?

Gene therapy :

1、 A technique for correcting defective genes responsible for disease development

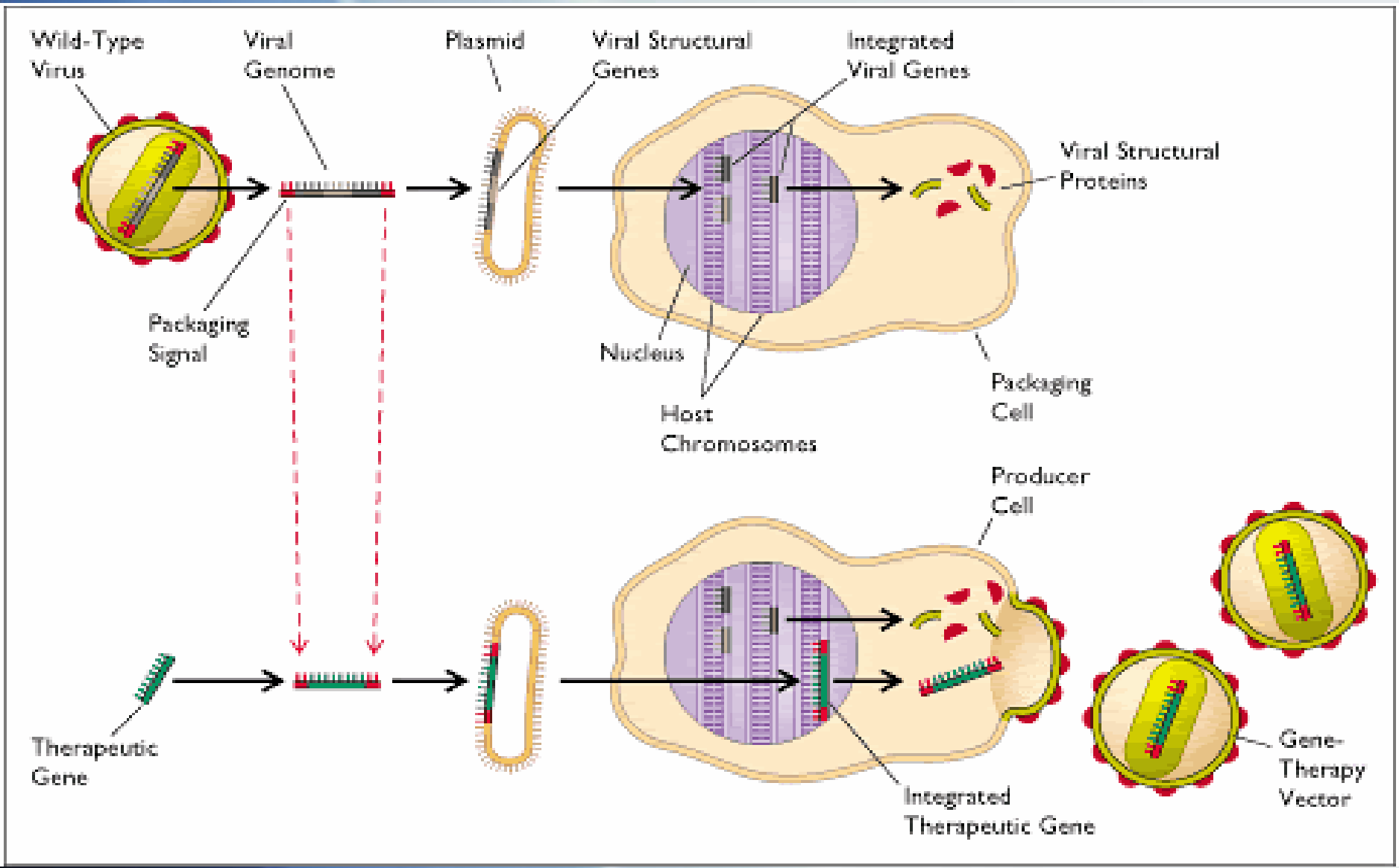
2、 A normal gene is inserted into the genome to replace an abnormal, disease-causing gene

How does gene therapy work?

1、Target cells infected with the vector

2、The vector then unloads its genetic material containing the therapeutic gene into the target cell

3、The generation of a functional protein product from the therapeutic gene restores the target cell to a normal state



http://www.fda.gov/fdac/features/2000/gene.html

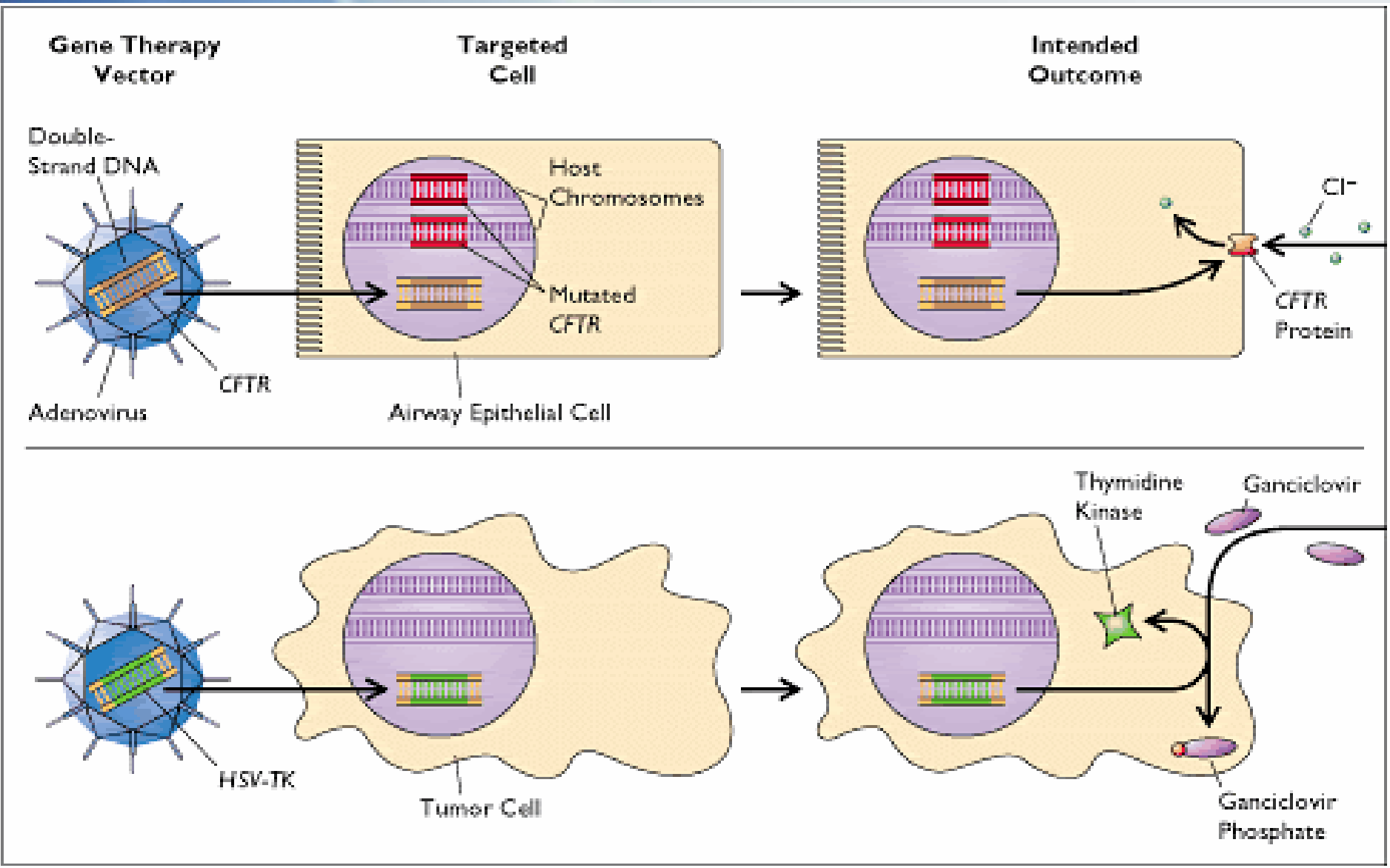


Illustration: Seward Hung

http://www.fda.gov/fdac/features/2000/gene.html

Vectors

1、 Viral vectors

2、 Non-Viral Vectors

Viral vectors

Advantages of viral vectors

1、They're very good at targeting and entering cells

2、They can be modified so that they can't replicate and destroy the cell

Disadvantages of virus vectors

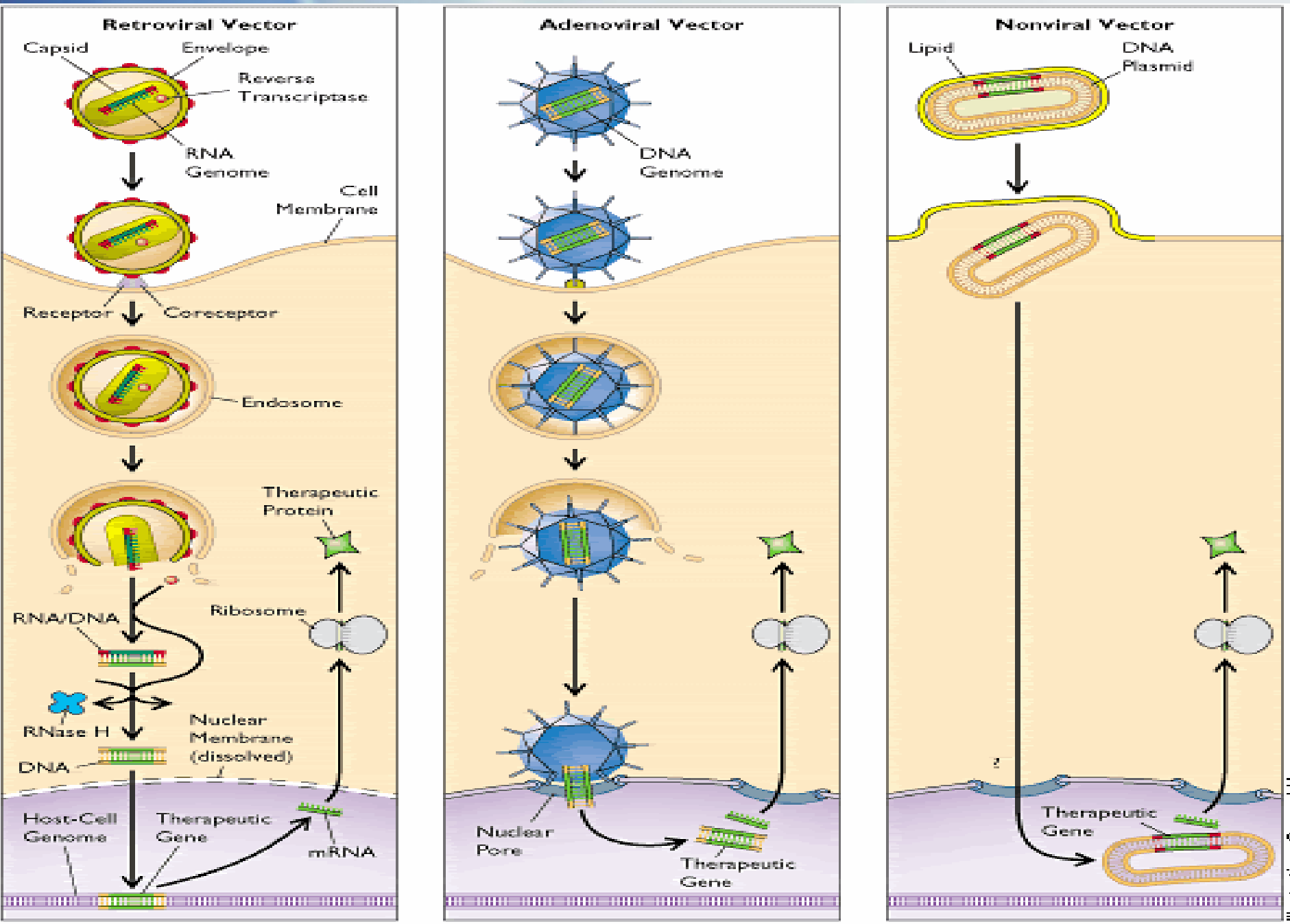
1、Some genes may be too big to fit into a certain type of virus

2、Viruses can cause immune responses in patients

Non-Viral Vectors

1、Non-viral vectors are typically circular DNA molecules, also known as plasmid

2、Liposome



http://www.fda.gov/fdac/features/2000/gene.htm

Process

1. Learn about the disease
2. Design a gene therapy
3. Get money and approval for clinical trials
4. Phase One clinical trial
5. Phase Two clinical trial
6. Phase Three clinical trial
7. Get FDA approval for general clinical use
8. Phase Four clinical trial

Disadvantages

Short-lived nature of gene therapy

Problems with integrating therapeutic DNA into the genome and the rapidly dividing nature of many cells prevent gene therapy from achieving any long- term benefits.

Immune response

The immune system's enhanced response to invaders it has seen before makes it difficult for gene therapy to be repeated in patients.

Disadvantages

Problems with viral vectors
- Toxicity
- Immune and inflammatory responses

Multigene disorders
- Multigene or multifactorial disorders would be difficult to treat effectively using gene therapy

The most difficult
- To the cells which need it

Pharmacogenetics

What's Pharmacogenetics?

1. Pharmacogenetics vs. Pharmacogenomics
2. Genetic polymorphisms vs. Drugs
   a、 Ingestion, absorption, metabolism, clearance, and excretion
   b、 Receptors
3. Human genome project

1. Genes encode the drug target
2. Genes encode drug metabolizing enzymes
3. Genes related to drug clearance mechanisms
4. Genes causally linked to the disease
5. Genes causally linked to mechanisms underlying adverse events

History of Pharmacogenetics

Ethnic variation:

1. Phenylthiourea (PTU)

Race	Incidence
Eastern Eskimos	40%
American Whites	30%
American Blacks	23%
Asian Chinese	6%
African Blacks	6%

2. Glucose-6-phosphate dehydrogenase

3. Alcohol dehydrogenase

History of Pharmacogenetics

Allelic frequency:

1. N-acetyl transferase
2. Glutathione-S-transferase

Molecular level:

Cytochrome P450 family

Ethnic classification → Molecular viewpoint

Pharmacogenetics vs. Technology

*DNA sequencer and Protein sequencer

1. Phenotyping
2. Genotyping
    a. PCR and RFLP
    b. Oligonucleotide ligation assay (OLA)
    c. DNA chips
    d. Single-stranded conformation polymorphism (SSCP)

Current and future applications

1. Cardiology
   a. Long QT syndrome
   b. CYP2D6
2. Neurology
   a. Alzheimer's disease: ApoE → ApoE-4
3. Oncology, asthma, depression, AIDS

Future

1. Disease → Molecular level
2. Highly specific diagnosis
3. Data bank
4. Drug development → Molecular therapy
    a. High efficacy
    b. Low adverse effect

Drug design

New drugs

- Discovery
  - Mechanism of disease
  - Target of drugs
- Development
  - The way of drug screen
  - Drug modification

The tendency of new drug research

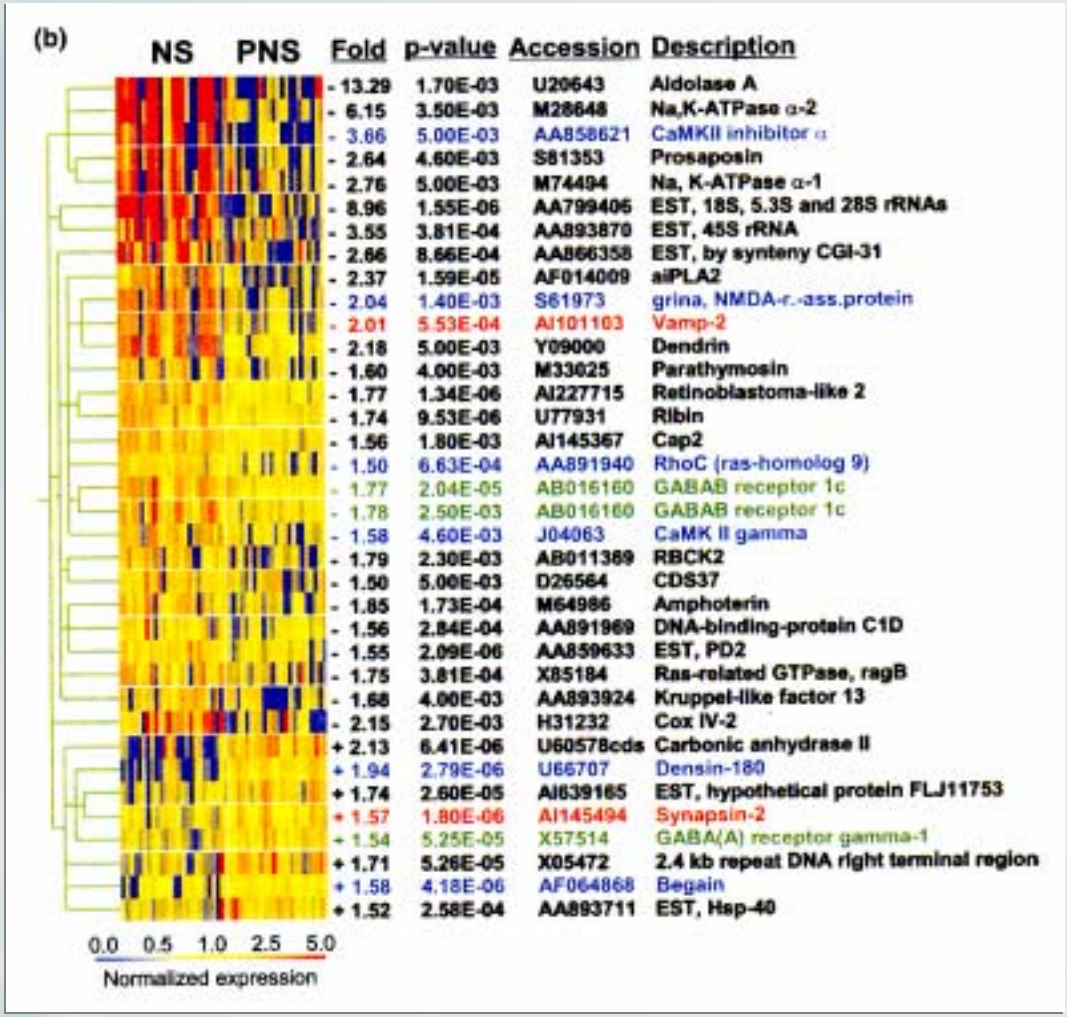
1. The new targets of drugs
2. Drug screen
3. Structural biology
4. Bioinformation
5. Computer-Aided Drug Design (CADD)
6. Combinatorial Chemistry

The new targets of drugs

1. Human genome project
2. Proteomics: 2D electrophoresis
3. DNA microarray

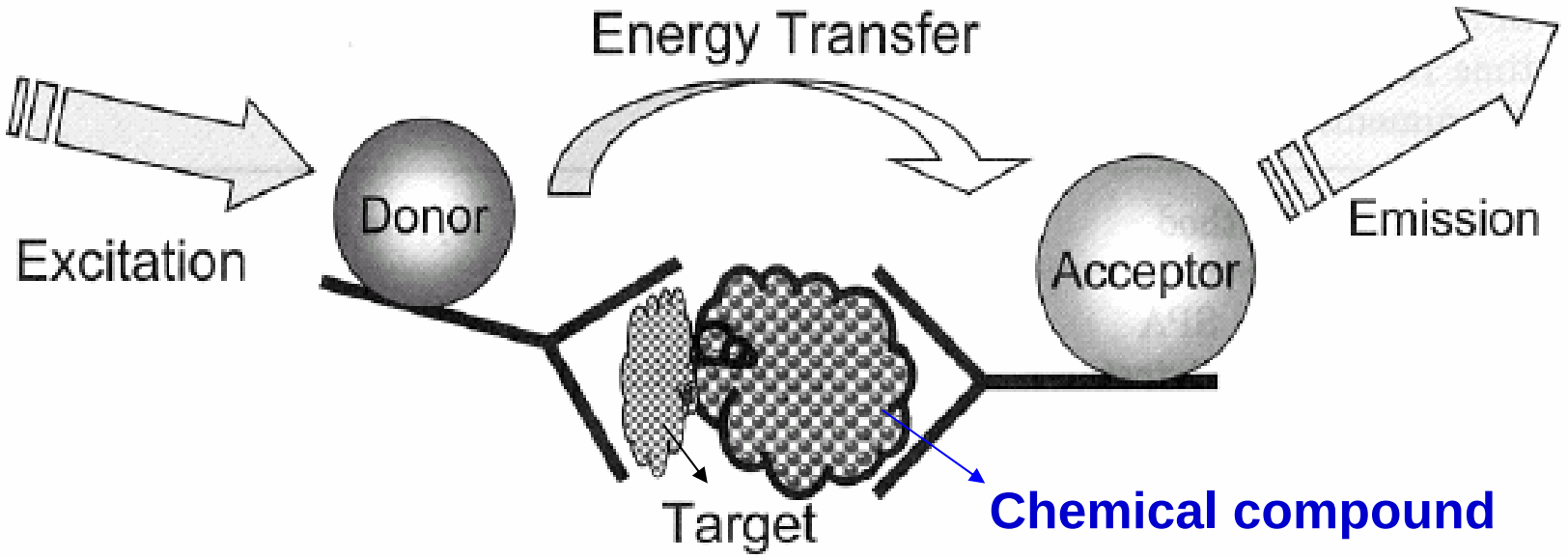
The related structure and function could improve the target of drug

2D electrophoresis



DNA microarray

High throughput screening (HTS)



Methods	Detection	Excitation	Donor	Acceptor	Emmision
SPA	Scintillation	–	Radioisotope	SPA beads	light
HTRF/LANCE	Fluorescence	337 nm	Eu3+ Chelate	XL-665/APC	665 nm
AlphaScreen	Fluorescence	680 nm	Donor beads	Acceptor beads	520-620 nm

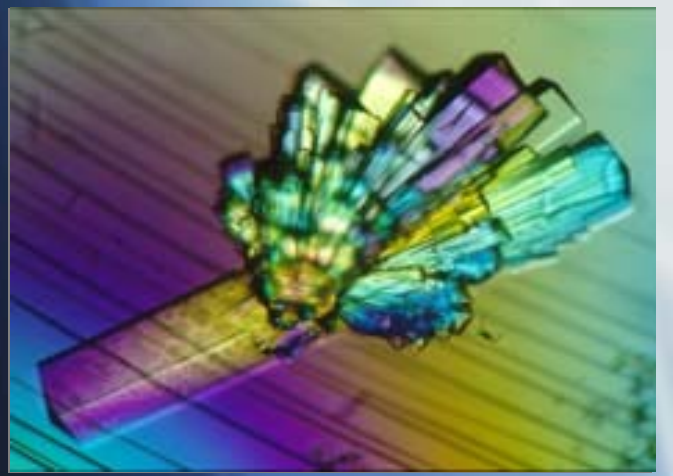
(Source: Nippon Yakurigaku Zasshi. 2001 Aug;118(2):81-8.)

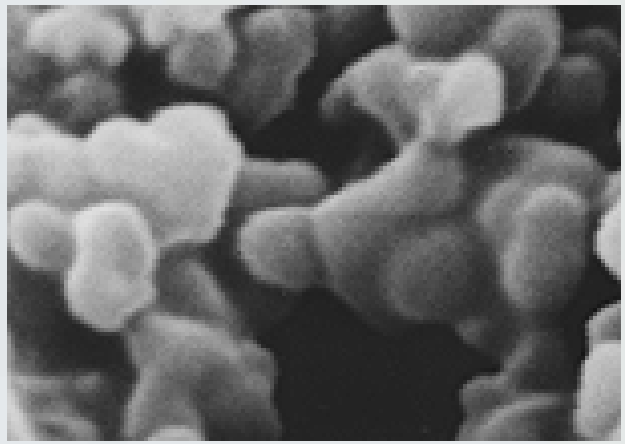
Protein structure

X-ray crystallography

NMR spectroscopy

Scanning electronmicroscope (SEM)





Computer-Aided Drug Design (CADD)

- Based on: structural biology, bioinformation
- 3 groups:
  - Ligand-based drug design
  - Receptor-based drug design
  - Mechanism-based drug design: transportation, distribution, metabolism

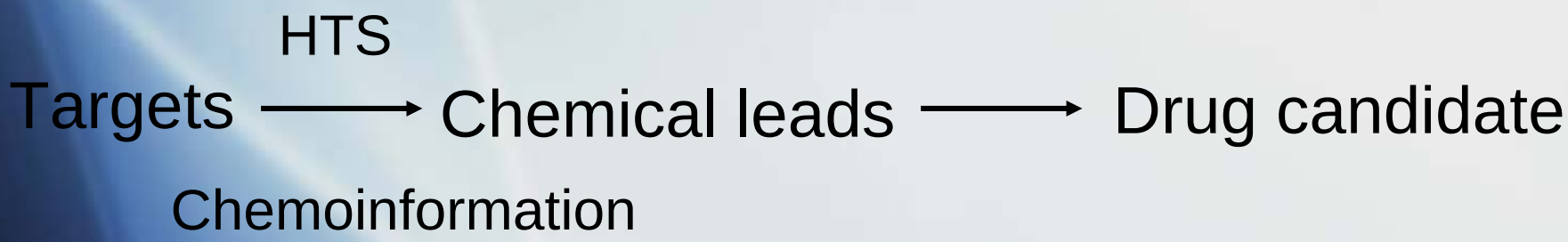
Combinatorial Chemistry

1. in 1980
2. Chemical compounds were synthesized and screened to set up a database.
3. High chemical library improve the rate of the screen.

The environment of drug design

1. Human genomics & proteomics
2. Combinatorial chemistry
3. Molecular biology
4. High throughput automated
5. Biochemistry biophysics
6. Drug metabolism/ Absorption
7. Analytical techniques

The stage of drug discovery



The characteristics of drug candidate

1. Potent
2. Specific
3. Minimum-modest metabolism
4. High absorption
5. Low toxicity
6. Wide therapeutic index
7. Not a potent Cytochrome p-450 (CYP 450) inhibitor or inducer
8. Feasible dosage from human to eat