Gene Therapy

Current Methods and Research for Cystic Fibrosis

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June 4, 2001

What is Cystic Fibrosis?

- Cause: Mutation in cystic fibrosis transmembrane conductance regulator gene (CFTR) gene on Chromosome 7
- Affects c-AMP-regulated chloride channel in apical membrane of epithelial cells
- Most common lethal inherited disease in whites

Effects

- Chronic Pulmonary disease
- Progressive decline in pulmonary function
- Obstructive azoospermia in males
- Exocrine pancreatic insufficiency
- Patients usually die from lung damage or infection

Genotype

- Mutation in CFTR gene
- Usually Delta F508 mutation (70% of cases)
- Over 600 mutations documented
- Different mutations have slightly different problems
- Variation of expressivity

Delta F 508 Mutation

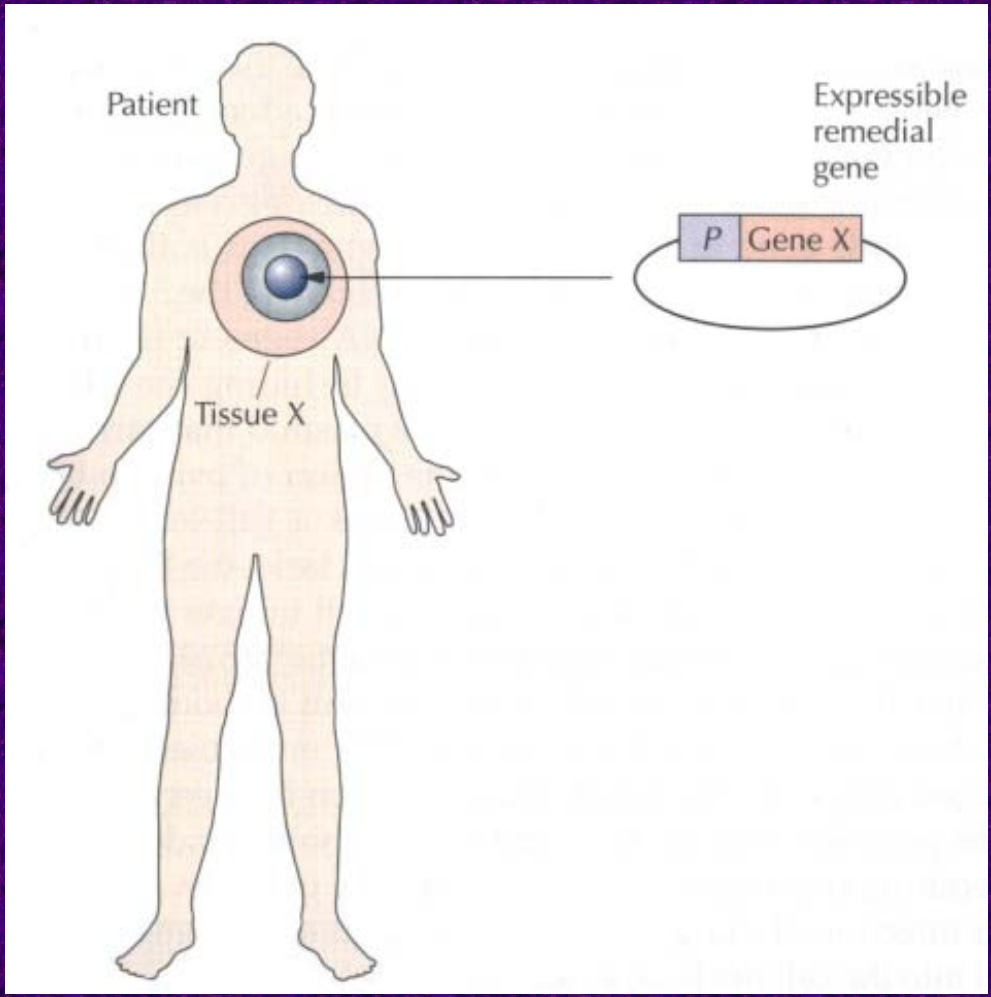
- Mutation in 70% of cystic fibrosis patients
- Class II biosynthetic trafficking defect
- Mutant protein degraded before it reaches cell membrane
- “Subtle defects in pulmonary function”

Gene Therapy for CF

- General Principles and Methodology
- Vectors
- Delivery
- Current Status of Research
- Challenges

General Principles

- The goal of gene therapy is to cure disease by altering the genome to include or exclude a desired set of genes.



Methodology

- Insert DNA coding for correct protein into selected vector
- Deliver vector to target site
- Vector integrates DNA into the host cell
- Desired protein product is produced by the host cell

Terminology

- Recombinant: two or more genes linked due to a crossover event
- Transfection: the transfer of DNA to a eukaryotic cell
- Transduction: the transfer of nonviral DNA by a virus to a cell
- Vector

What is a vector?

- Cannot simply deliver raw DNA to cells -- would be degraded, or not make it into cells
- Vector is “a DNA molecule that can carry inserted DNA and be perpetuated in a host cell”

Desired traits in CF Vector

- Low/no immune system response
- Able to integrate into non-dividing cells
- Able to integrate into epithelial cells
- Remains in body for long periods of time
- Deliver large DNA sequences
- Easy/cheap to produce in large quantities

Vectors for CF Gene Therapy

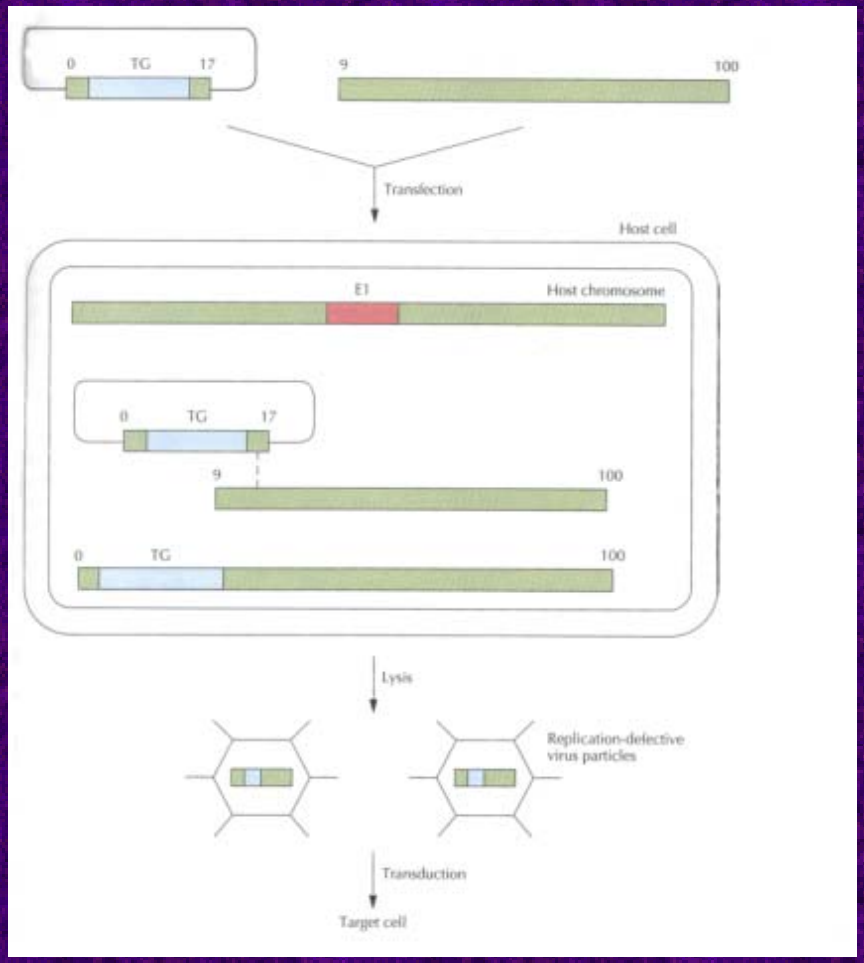
- Adenoviruses
- Adeno-associated viruses
- Liposomes
- HIV and Ebola

Adenovirus Vector

- Infect non-dividing human cells
- E1 region of cell needed for replication
- No integration into chromosome
- Can accommodate 36kb of double-stranded DNA
- In gene therapy, will replace viral replication genes with CFTR gene

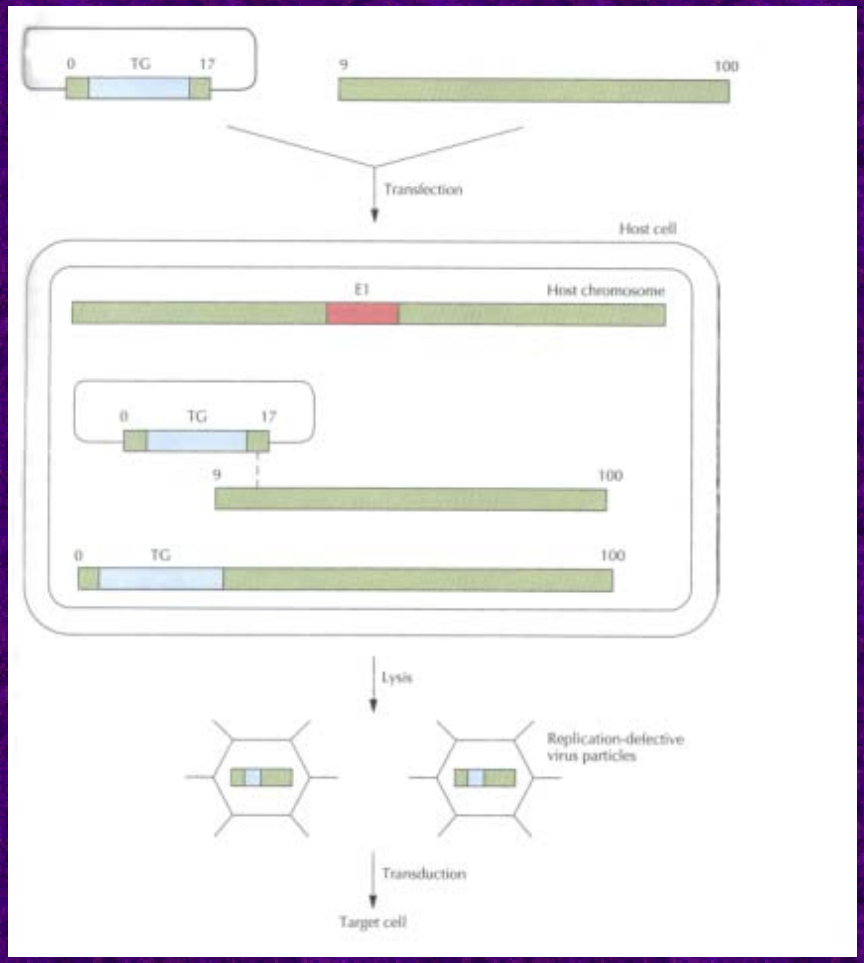
Making an Adenovirus Vector

- Insert TG plasmid into adenovirus genome map units 0-17
- Introduce above and linear adenovirus DNA (map units 9-100) into host cell containing adenovirus E1 gene



Making an Adenovirus Vector

- Recombination
- Recombinant gene released during cell lysis
- No E1 for virus to replicate
- Adenovirus infects target cell
- CFTR gene expressed



Adenovirus for Cystic Fibrosis

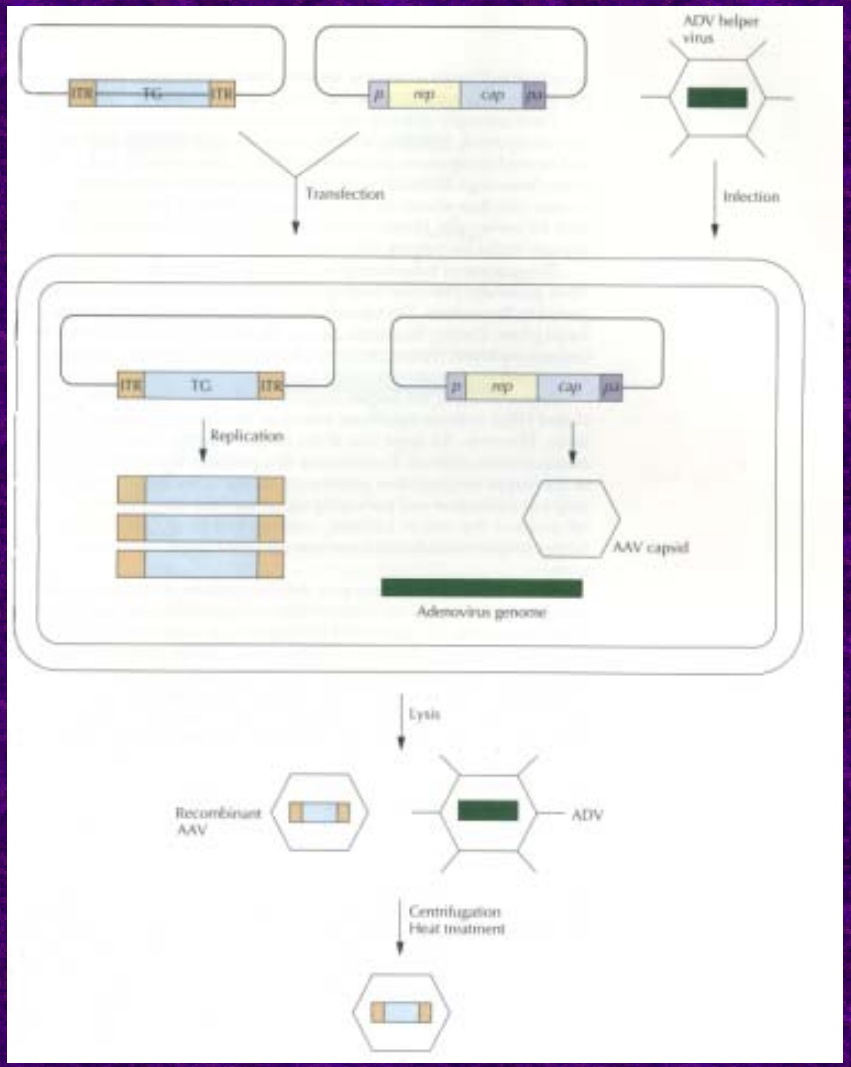
- Vector stable in aerosol delivery
- Few cells transduced with CFTR
- Does not remain in body for long period of time
- Immune response both to vector and to transformed cells

Adeno-Associated Virus (AAV)

- Not pathogenic
- Single-stranded human DNA virus
- Integrates onto chromosome 19
- Needs proteins from helper virus (such as adenovirus)
- Host cell polymerases convert adeno-associated virus genome into double-stranded DNA which is then transcribed

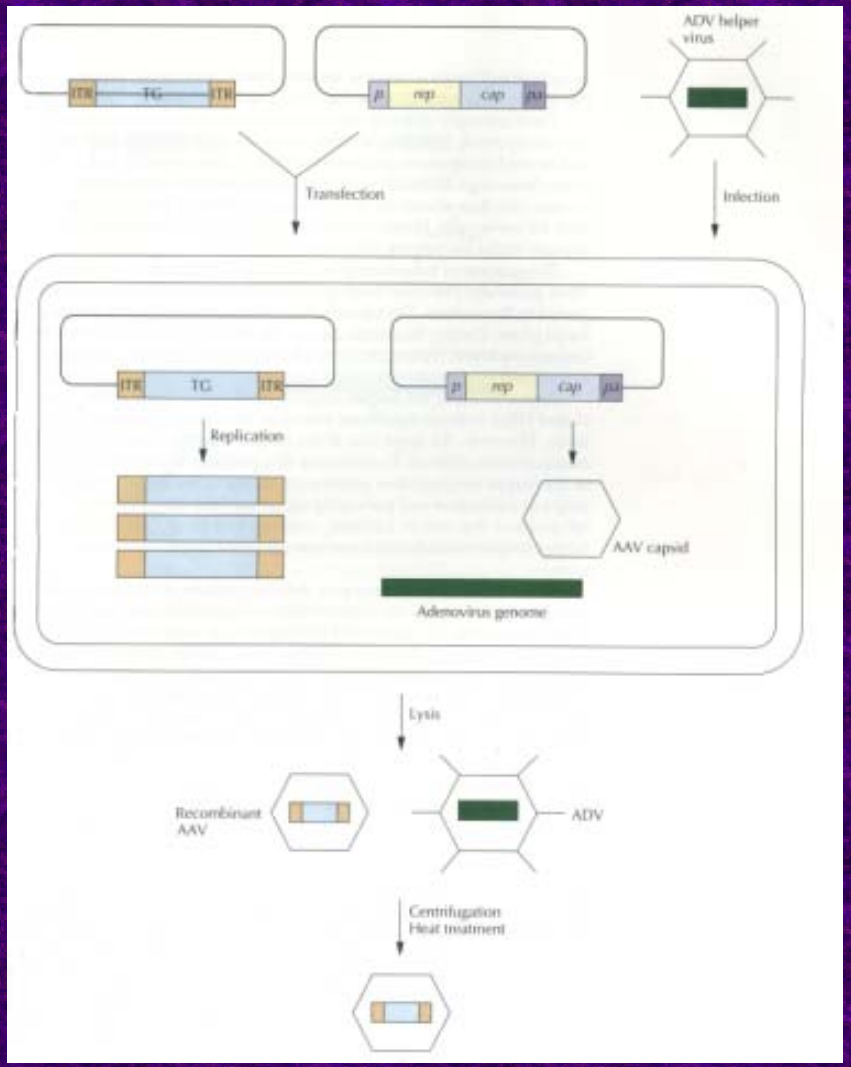
Making an AAV

- Infect a cell with helper adenovirus
- Cotransfect the cell with two plasmids, one containing CFTR and one containing genes for replication (rep) and for capsid formation (cap)



Making an AAV

- Cell lysis releases recombinant AAV and ADV particles
- Separate AAV from ADV by centrifugation
- Deactivate any remaining ADV with heat
- Deliver recombinant AAV to patient



Adeno-Associated Virus

- Lower efficiency than adenovirus
- Limited capacity for large DNA sequences
- Phase I clinical trials:
  - No inflammatory reponse
  - Vector persisted 70 days

Liposomes

- Cationic-lipid-mediated gene transfer
- Can deliver large DNA sequences
- Easy to produce in large quantities

Liposomes

- No toxic effects yet observed
- Some flu-like symptoms, resolved within four days
- Significant increase in chloride channel function, but no sodium or pulmonary difference yet observed
- May reduce bacterial adherence to respiratory epithelial cells
- Transient effect

HIV/Ebola Vectors

- Most recently tried vector
- HIV persists in the body
- Ebola attaches well to lung cells
- Vector does not contain parts of the HIV and Ebola genomes needed to cause disease
- HIV genes inside of Ebola viral envelope

HIV/Ebola Vectors

- Controversy!
  - **"I wouldn't want that thing put into me."**
    - Robert Gallo, Director of Institute for Human Virology and co-discoverer of HIV
  - **"It's not even HIV anymore; it's just pieces. And Ebola sounds horrible, but this has nothing to do with the Ebola virus that knocks out all your defense mechanisms and kills you. Those genes are gone."**
    - W. French Anderson, USC Researcher

Vector Delivery

- It is important that the vector actually have a chance to be in physical contact with the host cell.
- The best way to deliver a drug to the lungs is through inhalation.
- One of the reasons why cystic fibrosis has been popular to study is that drug delivery to the lungs is so simple!

Current Research

Includes:

- Work on HIV/Ebola vector
- Improvements to Liposome vector by adding integrin-binding motif
- Binding of adeno-associated virus to epithelial cells

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