

Genetics and Society

April 14, 2009

Gene Therapy & RNAi

Outline

- Viruses- dual use dilemma
- Viruses
- Immune system
 - B cells, T cells
 - Antibodies
 - Monoclonal antibodies
- Treatments
 - Antibiotics
 - Vaccination

Vaccine Dilemmas

Herd Immunity

Not everyone in a population has to be immunized for a vaccine to be effective.

Herd immunity: If most people are immunized, the ones who aren't are unlikely to come in contact with an infected individual.

If there is a cost (financial, real or perceived risk) from vaccination, this causes a dilemma.

Cultural issues

Polio vaccination programs in Nigeria and India were halted by rumors that the vaccines were a western attempt to harm them.

Only by getting local civic and religious leaders to publicly endorse the program could vaccination resume.

Suspicion of vaccination occurs even in Western countries.

HPV

Worldwide




GARDASIL.
[Quadrivalent Human Papillomavirus
(Types 6, 11, 16, 18) Recombinant Vaccine]

Key Points About GARDASIL

Who should
receive GARDASIL?

How is GARDASIL given?

Questions to ask
your doctor

Continued screening
is important

HPV and Its Consequences

Cervical cancer and HPV

GARDASIL—the only cervical cancer vaccine
For girls and young women ages 9 to 26 years



**YOU COULD BECOME
1 LESS LIFE AFFECTED
BY CERVICAL CANCER.**

Question

What are the potential costs of vaccination?

Should we have mandatory vaccination for everyone?

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Outline

- Gene Therapy
 - The Basic Idea
 - Diseases
 - Vectors
- RNAi

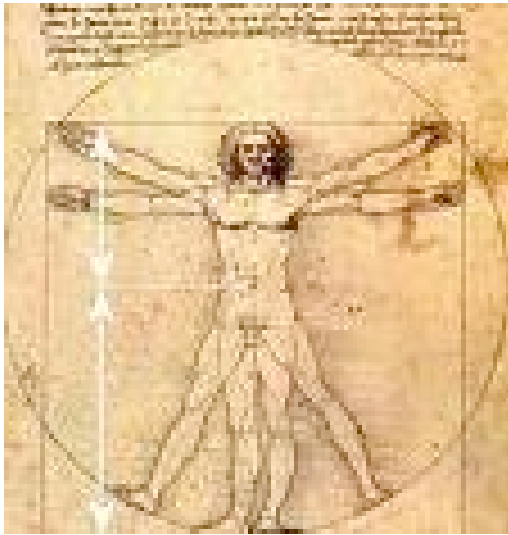
The Basic Idea

Gene therapy: Add a functioning gene to a person that lacks a functioning gene.

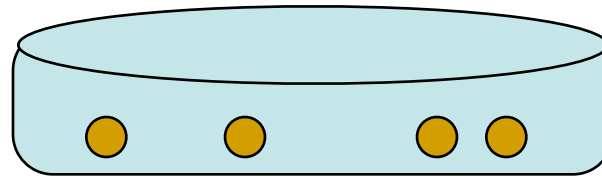
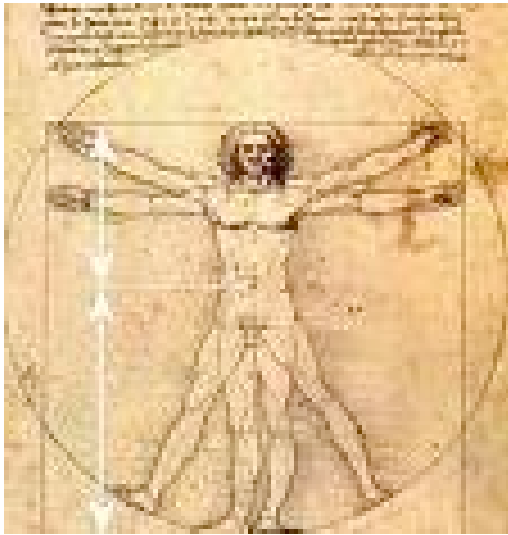
Somatic means affecting the body.
The germline (future generations) are NOT affected.

Over 1,000 clinical trials have been performed.

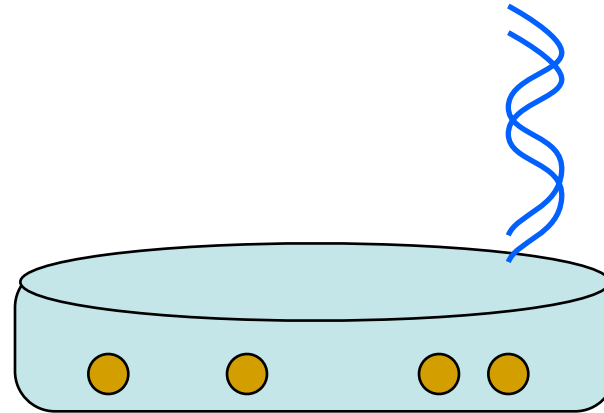
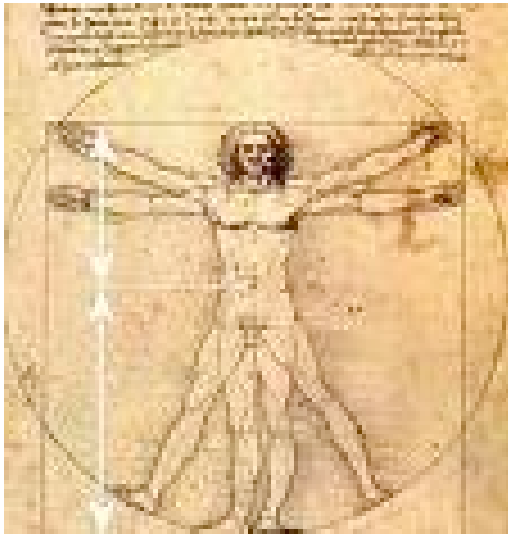
Cells can be removed, genes added,
and the cells returned



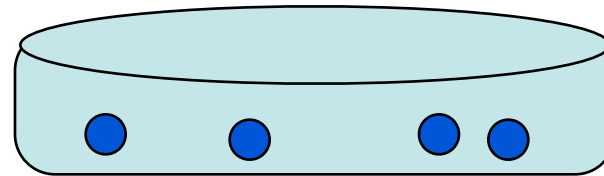
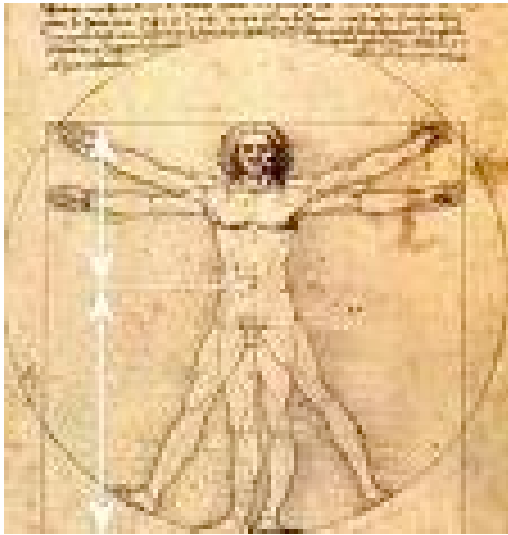
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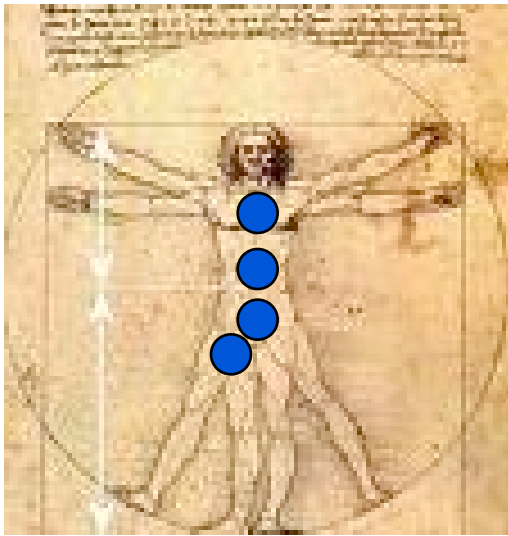
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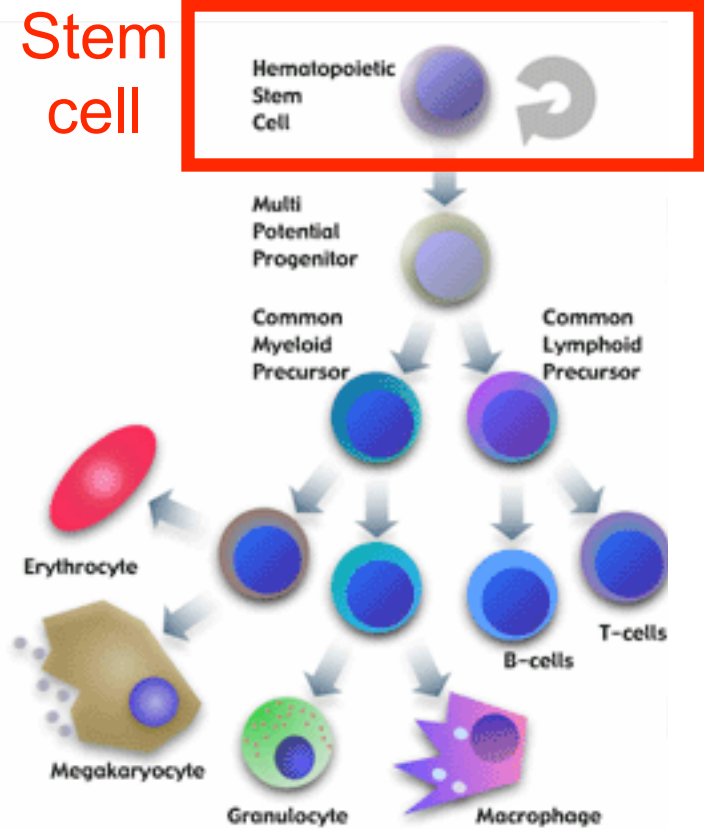


Cells can be removed, genes added,
and the cells returned



The blood is a good candidate.

- Blood cells are made constantly.
- Blood cells are made from single **stem cells**, present in the bone marrow.
- Techniques to take out and to replace bone marrow are well established.

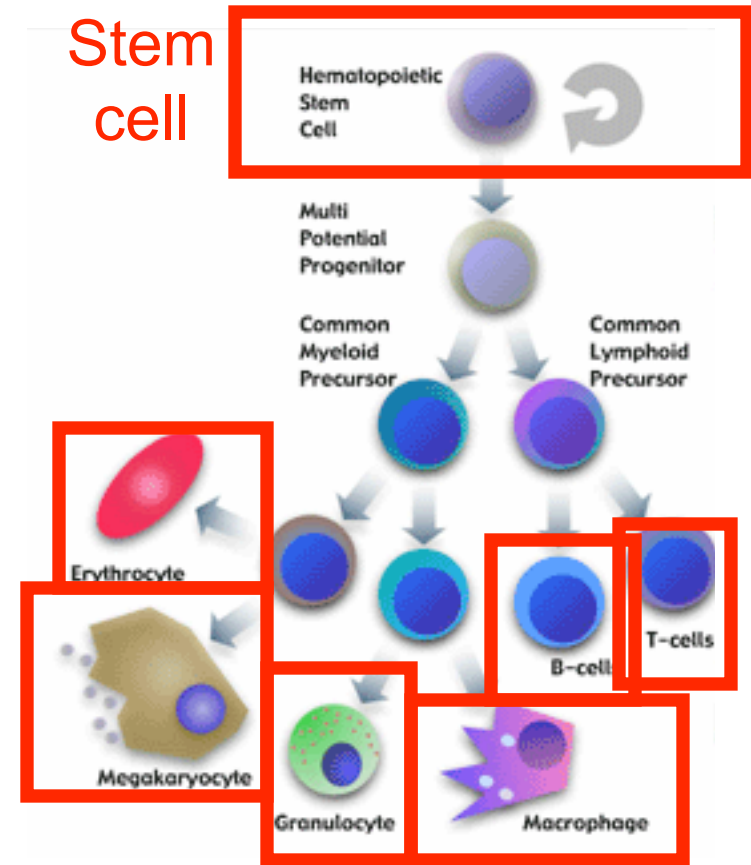


What is a stem cell?

Stem cells divide to give a **differentiated** cell and more stem cells.

Differentiated cells perform a function in the tissue but don't divide.

If you can affect the stem cell, you can affect all of the cells of the tissue.



Differentiated cells

Other stem cells

In addition to blood, liver and skin are among the tissues that regenerate rapidly from stem cells.

Muscle repair themselves from stem cells called satellite cells.

Good candidates for gene therapy

- Blood
- Muscle
- Skin
- Liver

Outline

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SCID (severe combined immunodeficiency)

Bubble boy disease



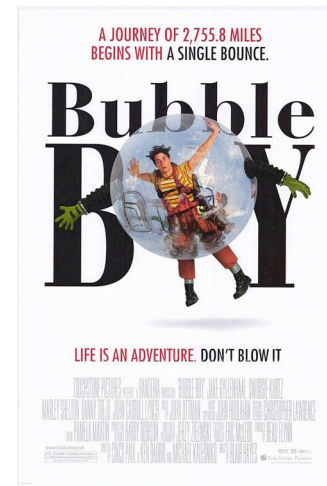
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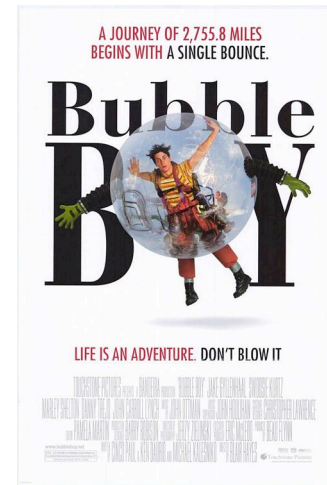
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Two types have been treated with gene therapy

Adenosine deaminase

SCID-X1

Other blood diseases that are targets for gene therapy

Hemophilia

Thalassemia

Sickle cell anemia

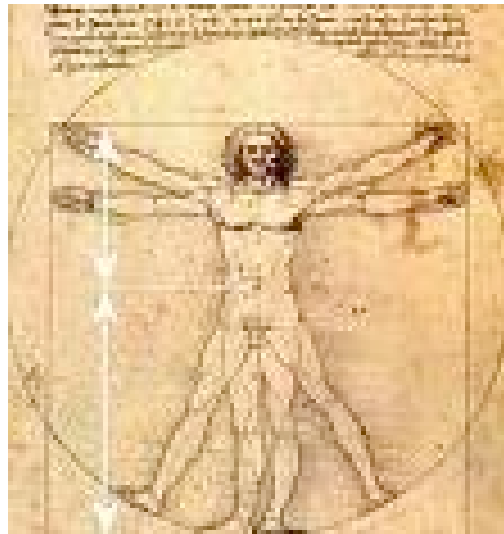
Gene therapy without stem cells

Getting access to stem cells is often difficult.

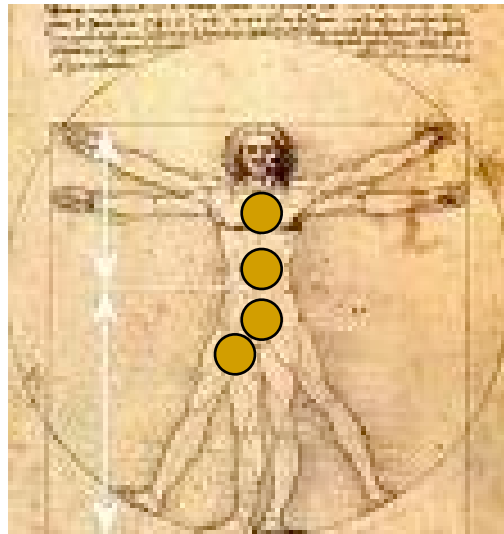
The brain and CNS don't generally have stem cells.

In these cases, gene therapy is done in the person without trying to modify the cells in the laboratory.

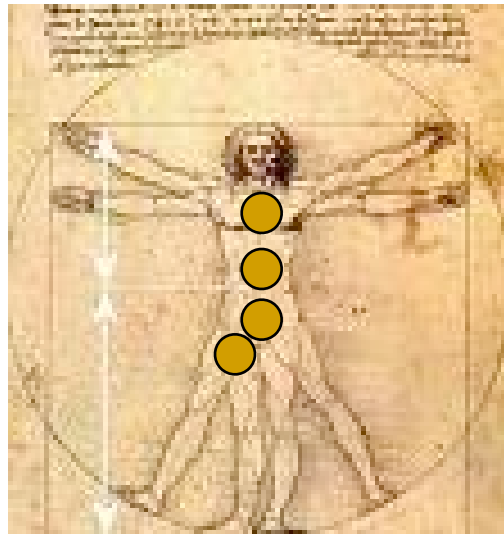
The new gene can be injected into
the person



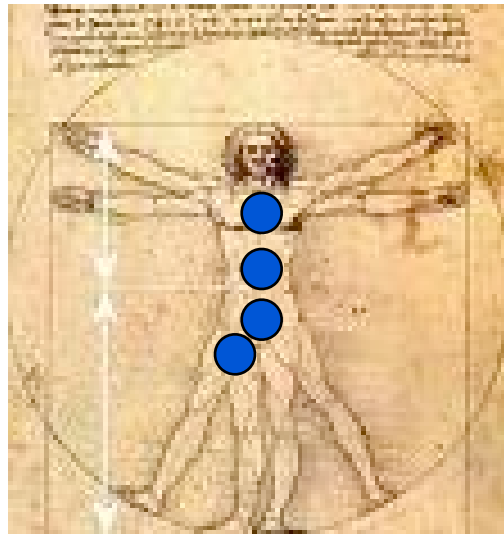
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Diseases

Heart attacks are being treated with gene therapy

Genes that promote blood vessel growth are being added to heart tissue during bypass surgeries.

This is not replacing a defective gene.

Clinical trials have shown no benefit to date.

Outline

- Gene Therapy
 - The Basic Idea
 - Diseases
 - Vectors
- RNAi

Vectors

Vectors

Question: what is a vector?

Vectors

Most gene therapy in clinical trials use a virus as the basis for a vector

The parts of the virus required for it to multiply are removed.

The therapeutic genes are added.

Gene therapy viruses

Two types:

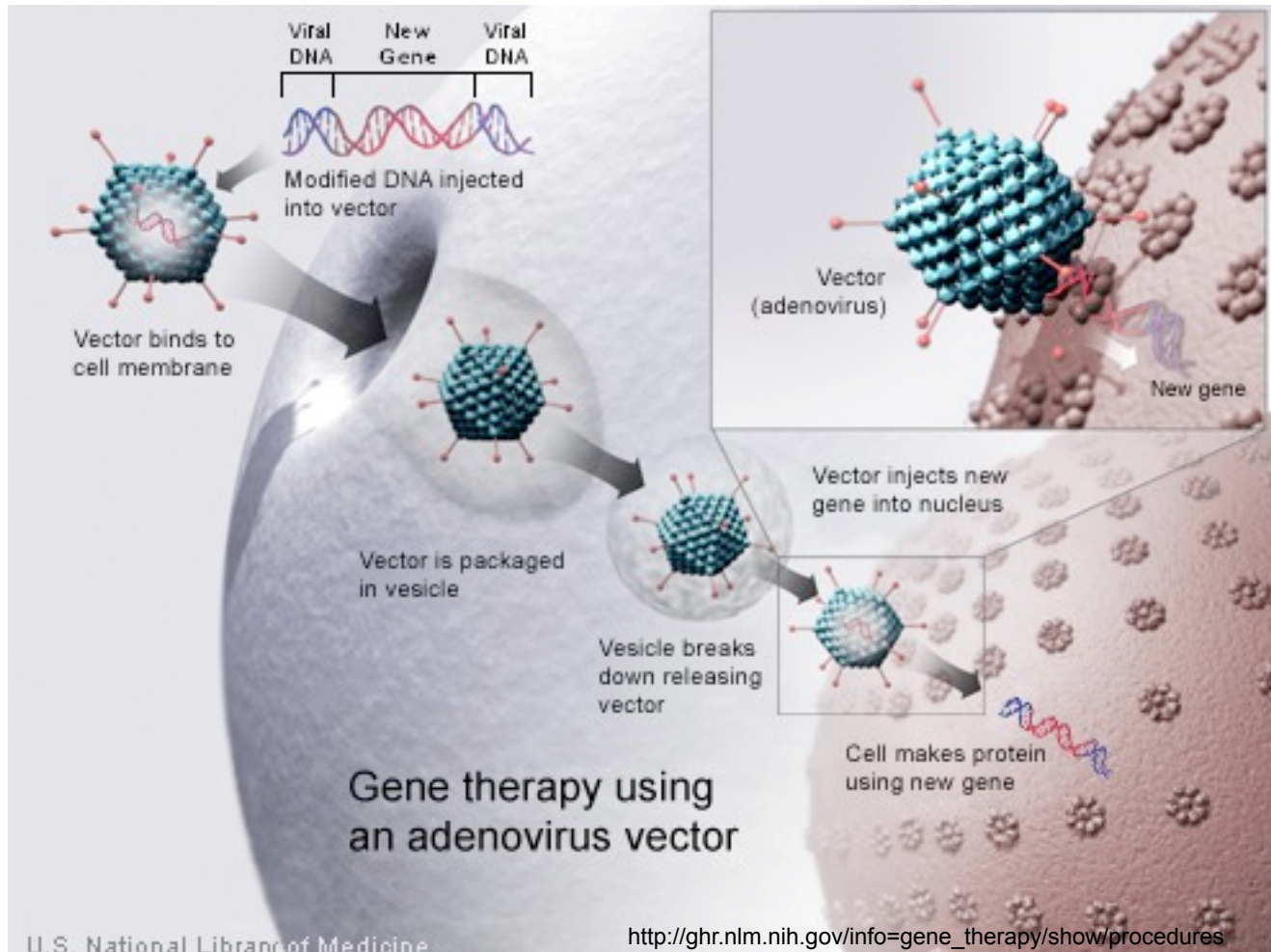
Non-integrating

Integrating

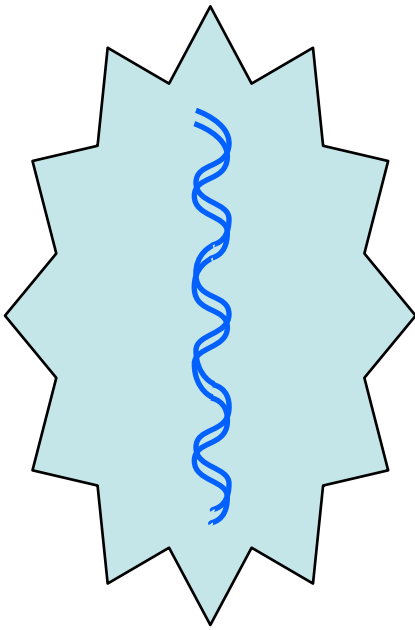
Non-integrating viruses

Do NOT insert into the host DNA

Example: adenovirus



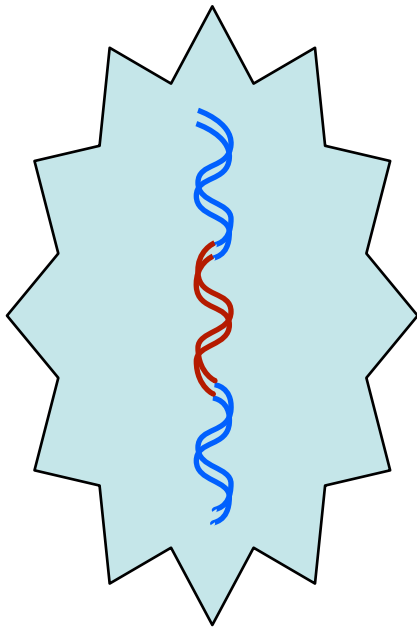
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Virus

Non-integrating viruses

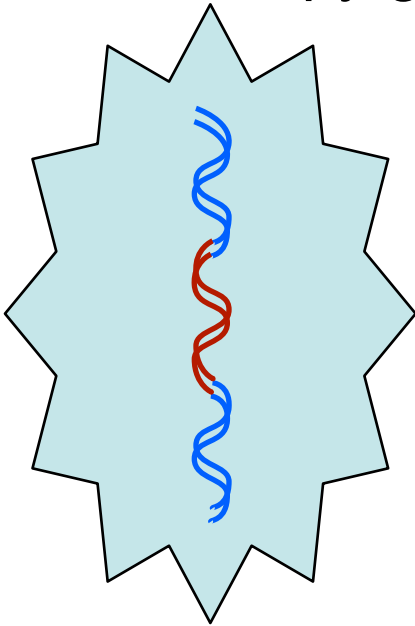
Replace virus genes
with therapy gene



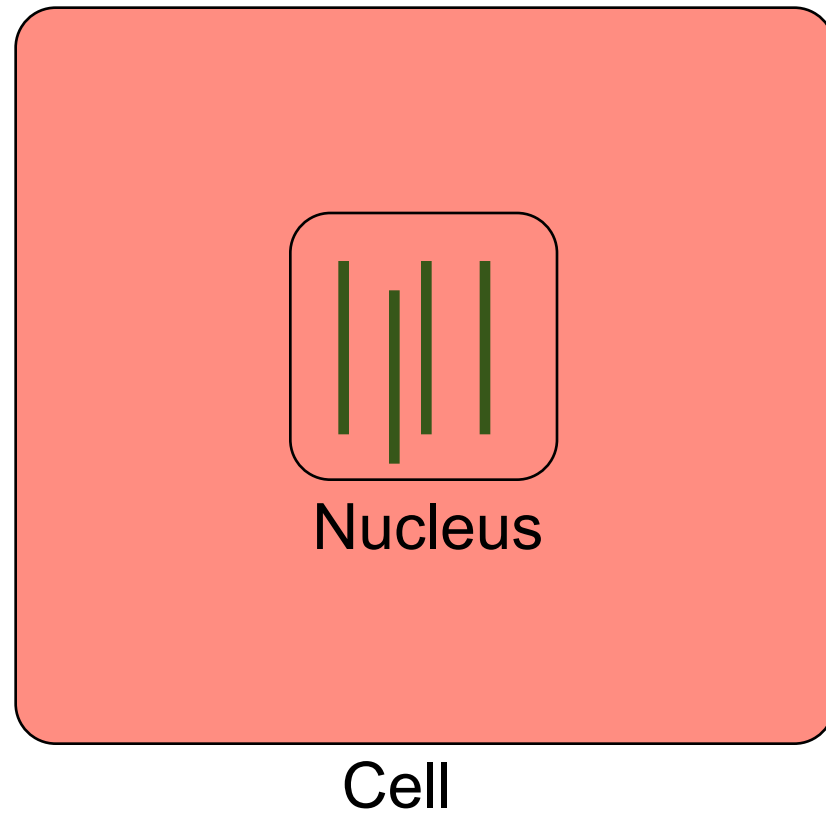
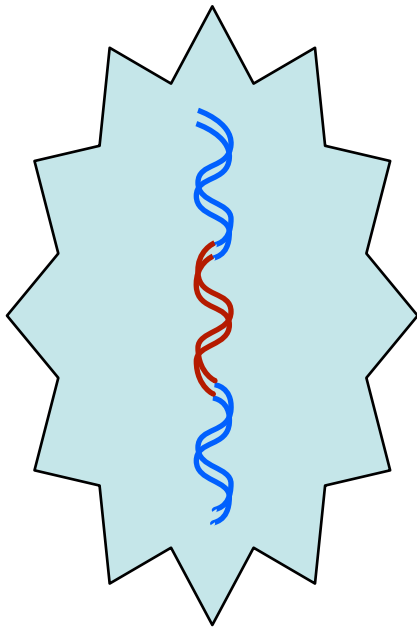
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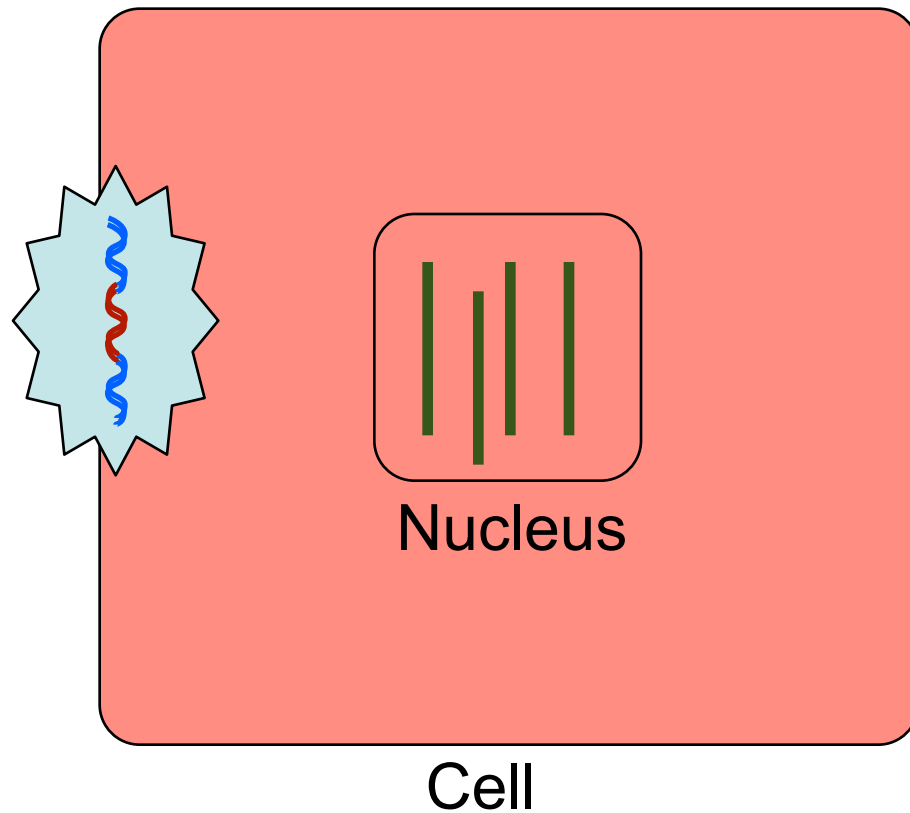
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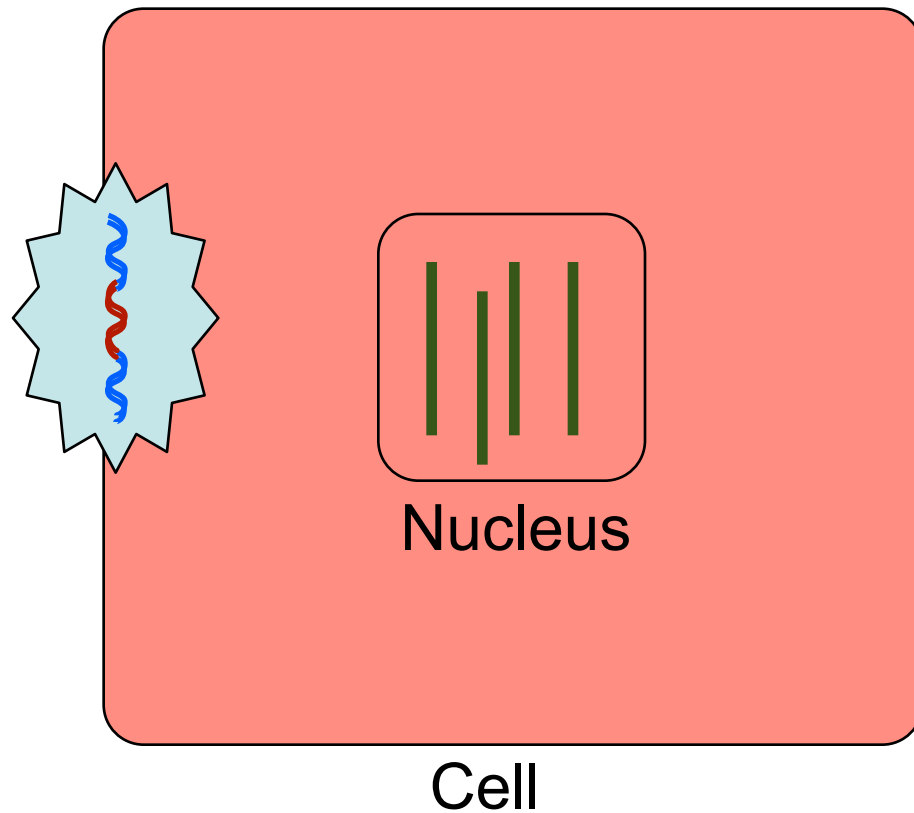
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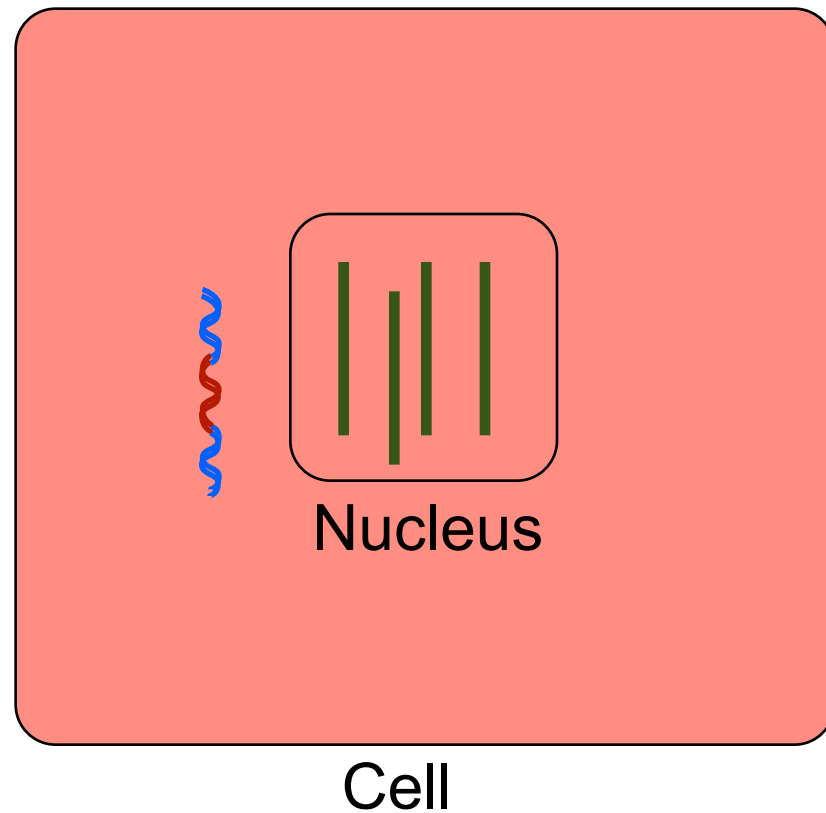
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Non-integrating viruses



Non-integrating viruses



Gene therapy viruses

Two types:

Non-integrating

- Limited capacity
- Limited infectivity
(not all cells affected)
- Infection of non-target cells.
- Stability

Integrating

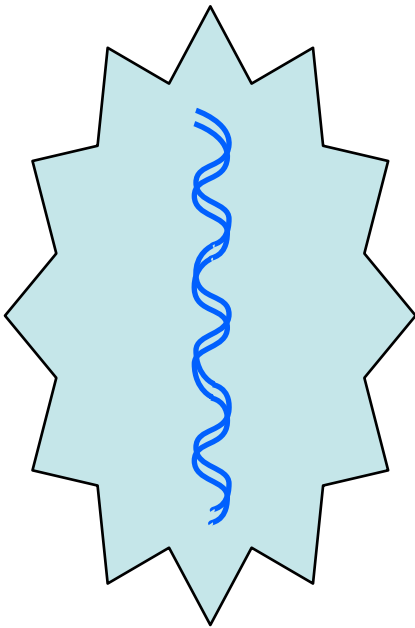
Integrating viruses

Insert themselves into the host DNA

Retroviruses are the most common.

HIV is one of the retroviruses commonly used to create gene therapy vectors

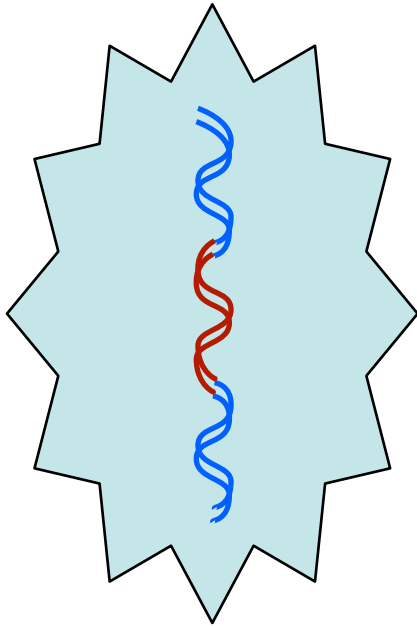
Non-integrating viruses



Virus

Non-integrating viruses

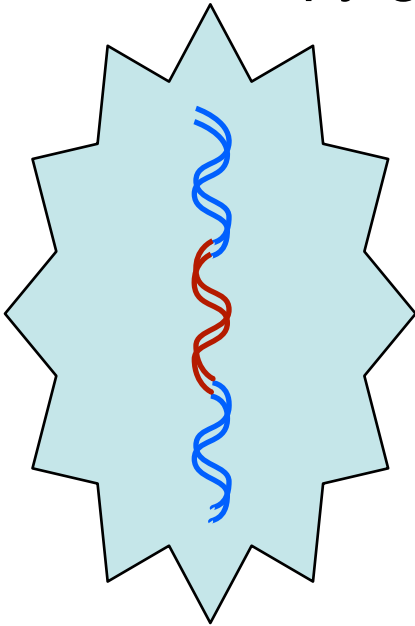
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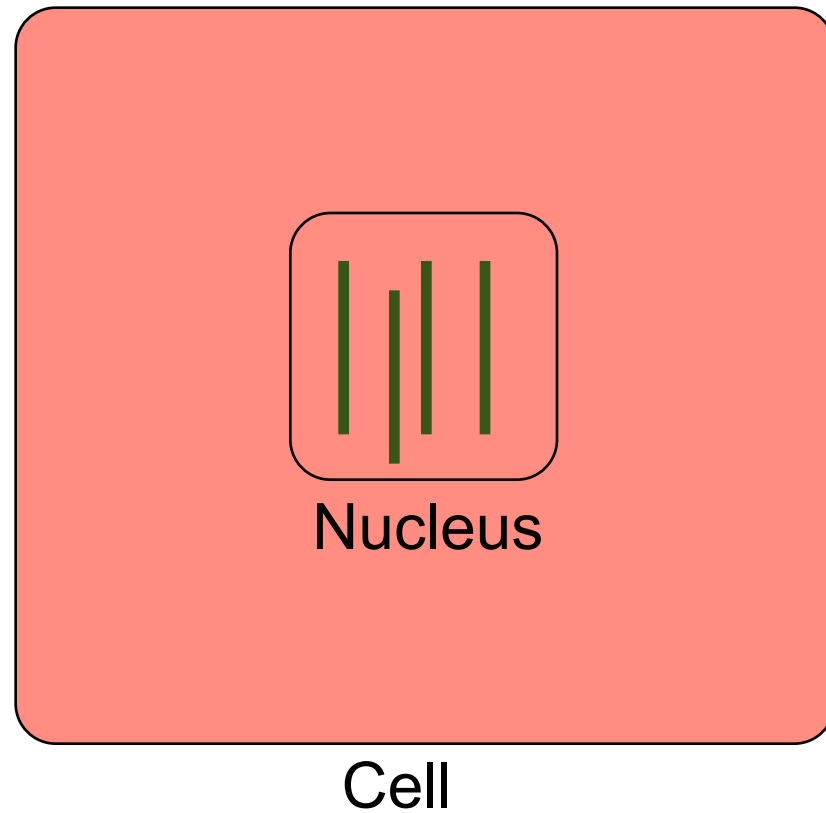
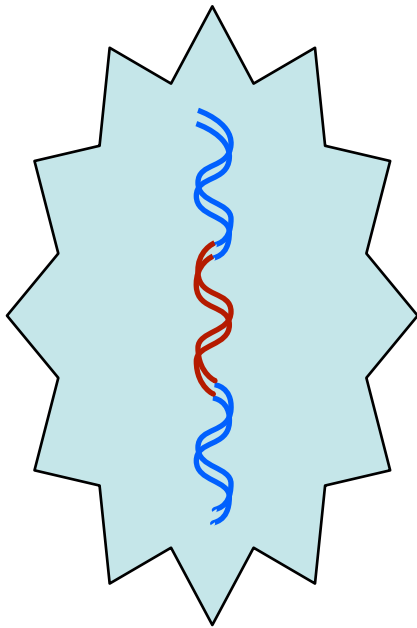
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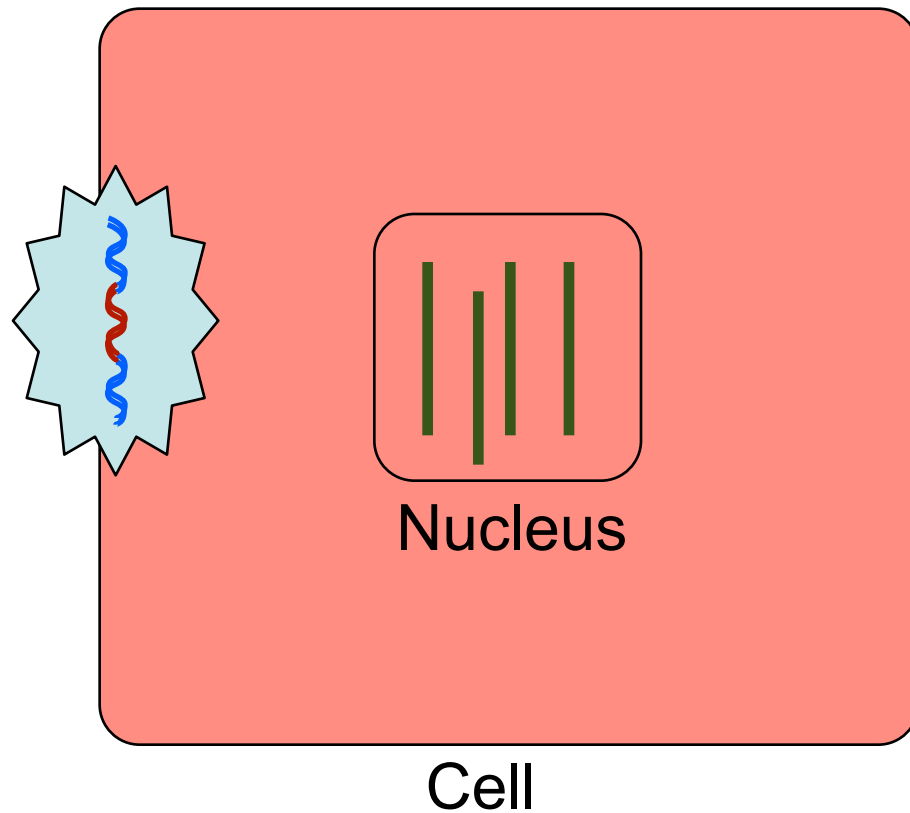
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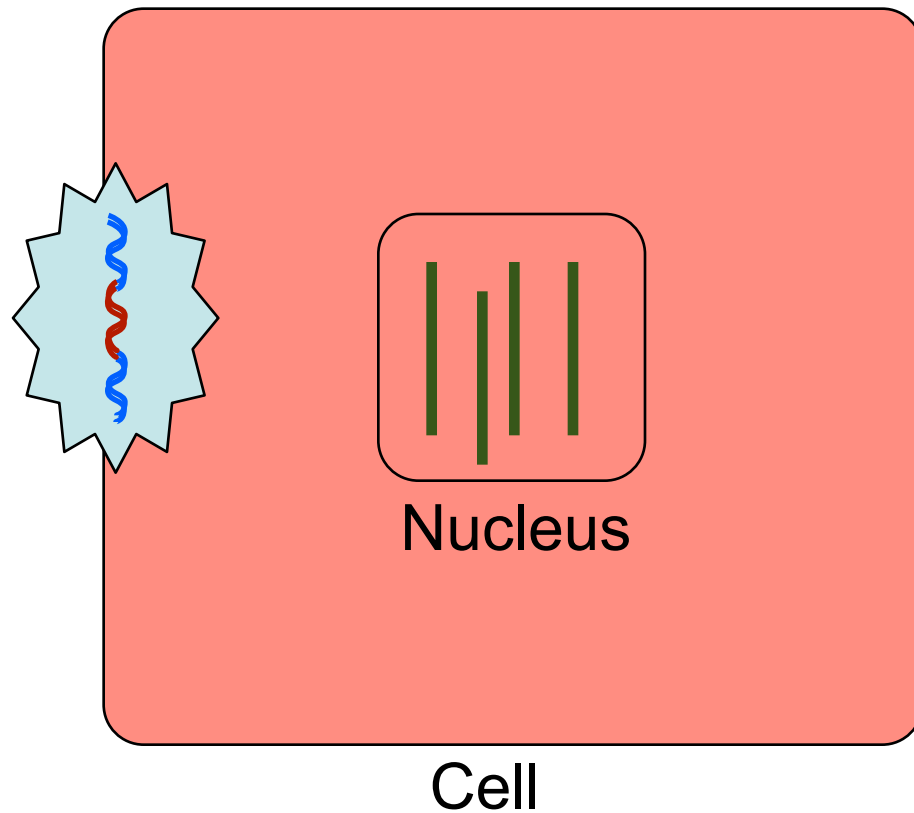
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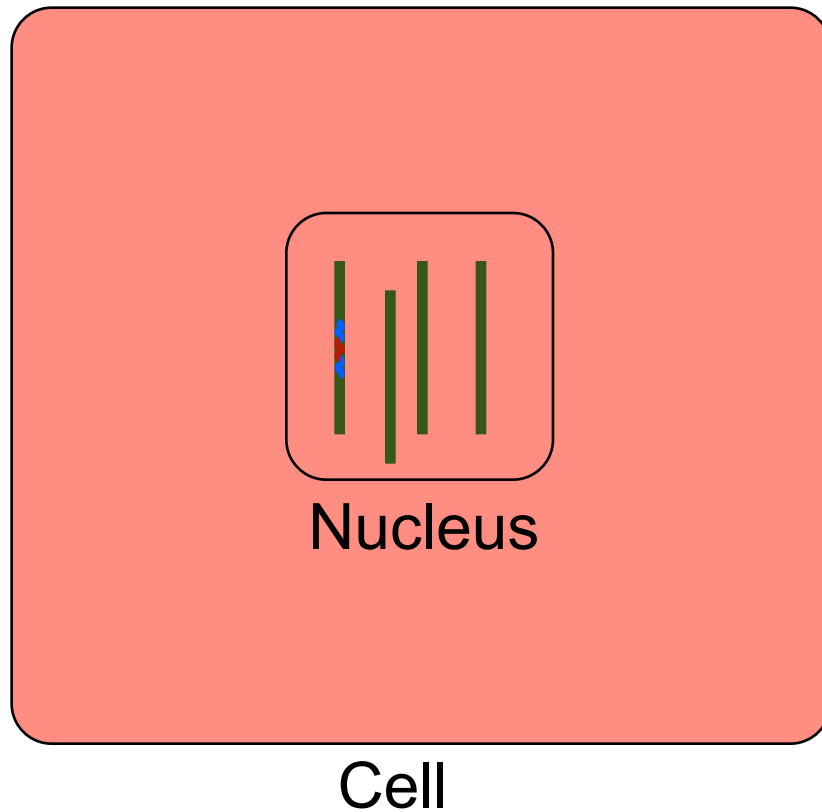
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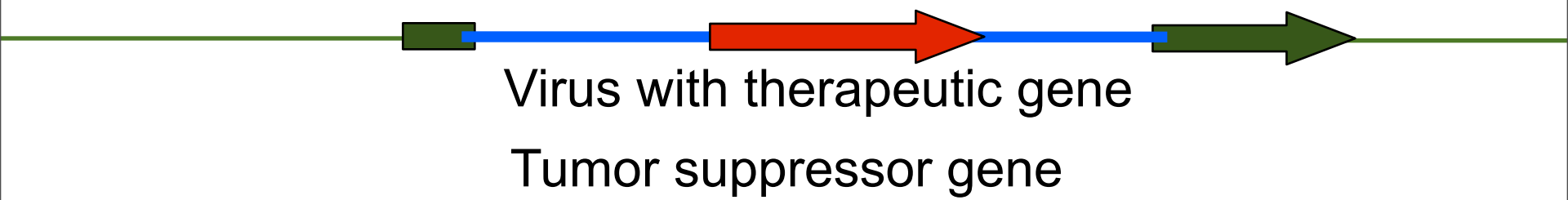
Non-integrating viruses



Safety concerns of integrating vectors



Safety concerns of integrating vectors



Safety concerns of integrating vectors

Three patients in the SCID-X1 trial developed a form of leukemia.

None of the Adenosine deaminase patients have had this problem yet.



Virus with therapeutic gene

Tumor suppressor gene

Gene therapy viruses

Two types:

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(not all cells affected)
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- Stability

Integrating

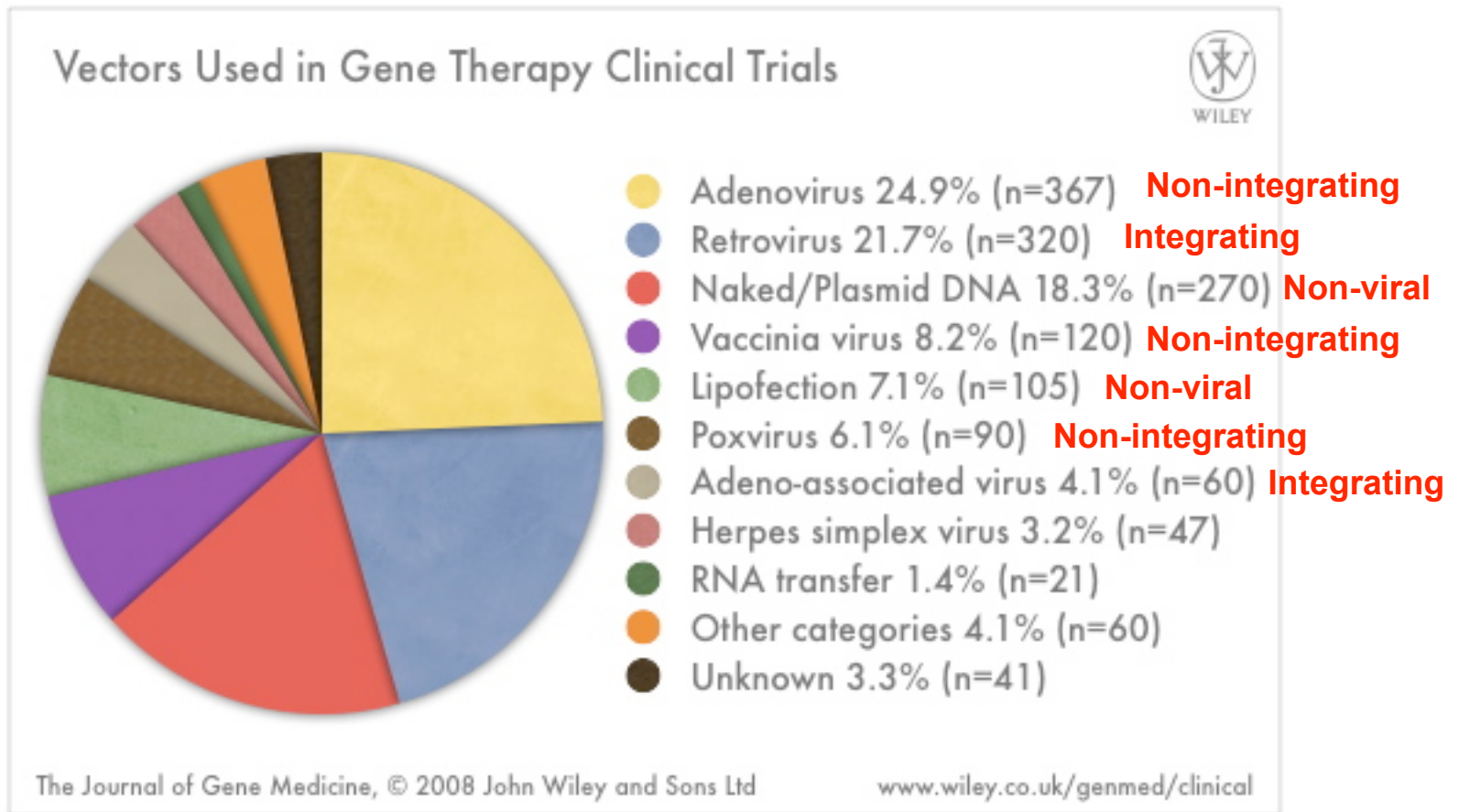
- Limited capacity
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- Mutations

Non-viral methods

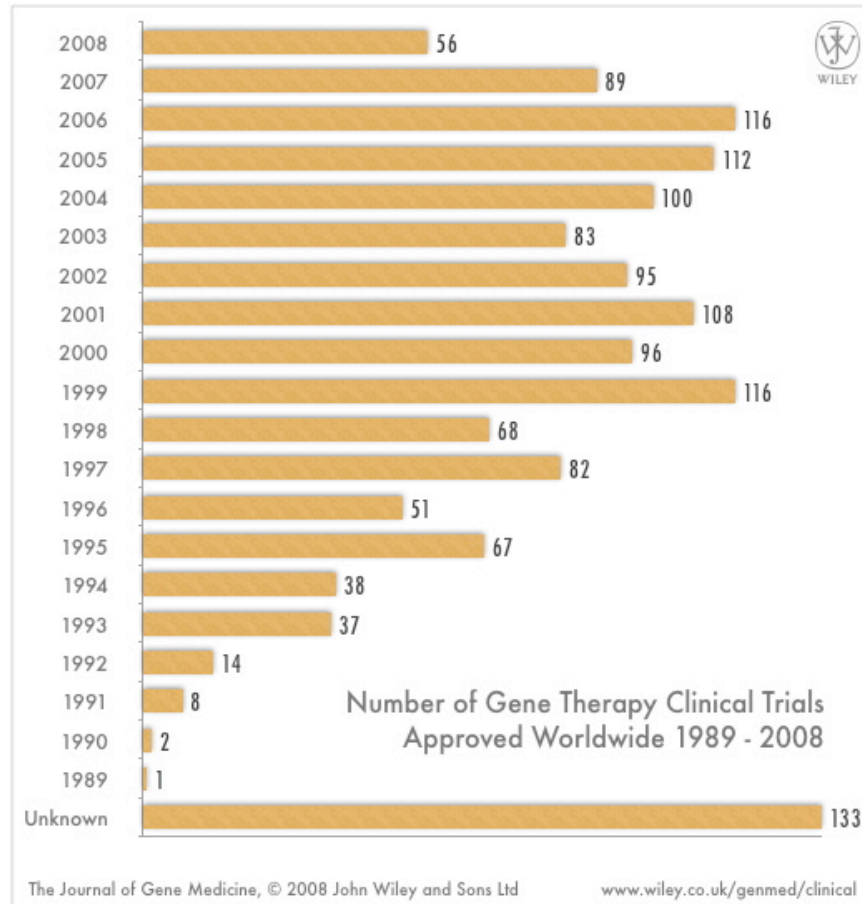
Some trials inject plain DNA or DNA coated with lipids.

This method affects few cells, and the DNA is usually not integrated into the chromosome

Vectors used in trials

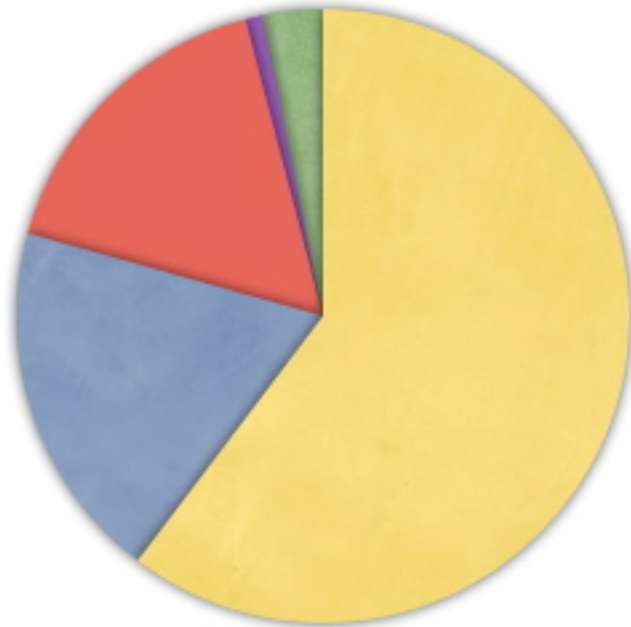


Clinical trials



Gene therapy trials

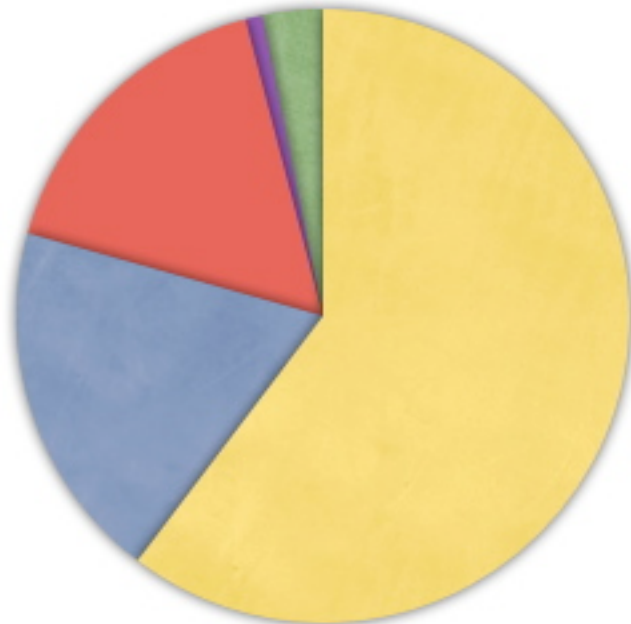
Phases of Gene Therapy Clinical Trials



- Phase I 60.3% (n=888)
- Phase I/II 19% (n=280)
- Phase II 16.6% (n=244)
- Phase II/III 0.9% (n=13)
- Phase III 3.2% (n=47)

Gene therapy trials

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Safety

Safety/efficacy

Efficacy

Vector problems

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- Limited infectivity (not all cells affected)
- Infection of non-target cells.
- Stability (if non-integrating)
- Mutations (if integrating)

Vector problems

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- Limited infectivity (not all cells affected)
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- Stability (if non-integrating)
- Mutations (if integrating)
- Immune response

Immune response

Immune response to the vector

Immune response to the therapeutic protein

Adenovirus vectors

“Jesse Gelsinger, an 18-year-old from Arizona, died on Sept. 17 [1999], four days after being injected with a high dose of genetically engineered adenovirus intended to correct his condition.”

NICHOLAS WADE
NYT September 30, 1999

The problem was an out of control immune response to the virus.

Summary

- Gene therapy
- Somatic
- Vectors- two types
- Problems with gene therapy vectors

Outline

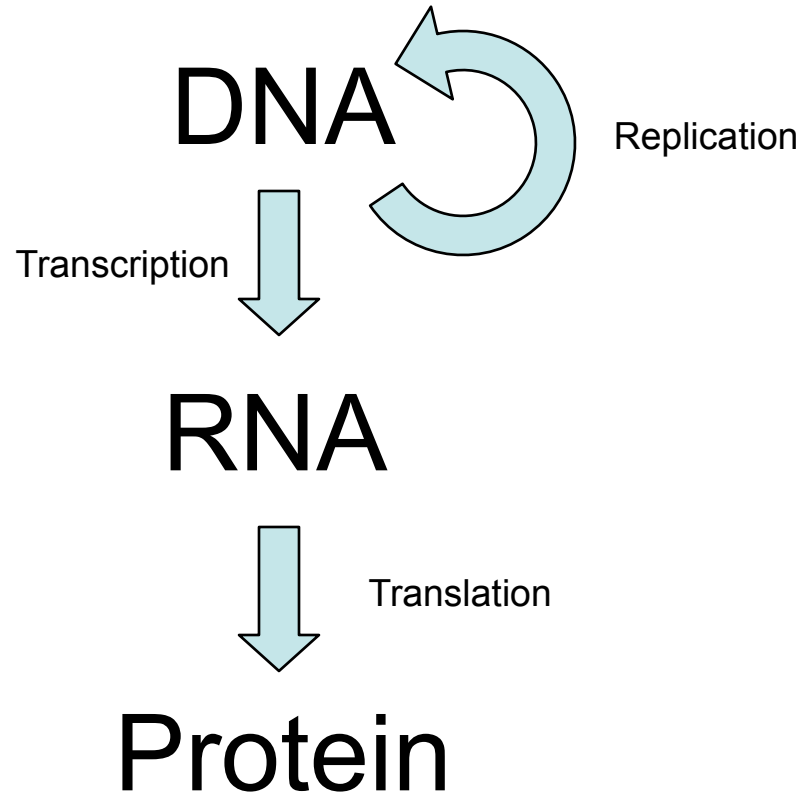
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RNAi

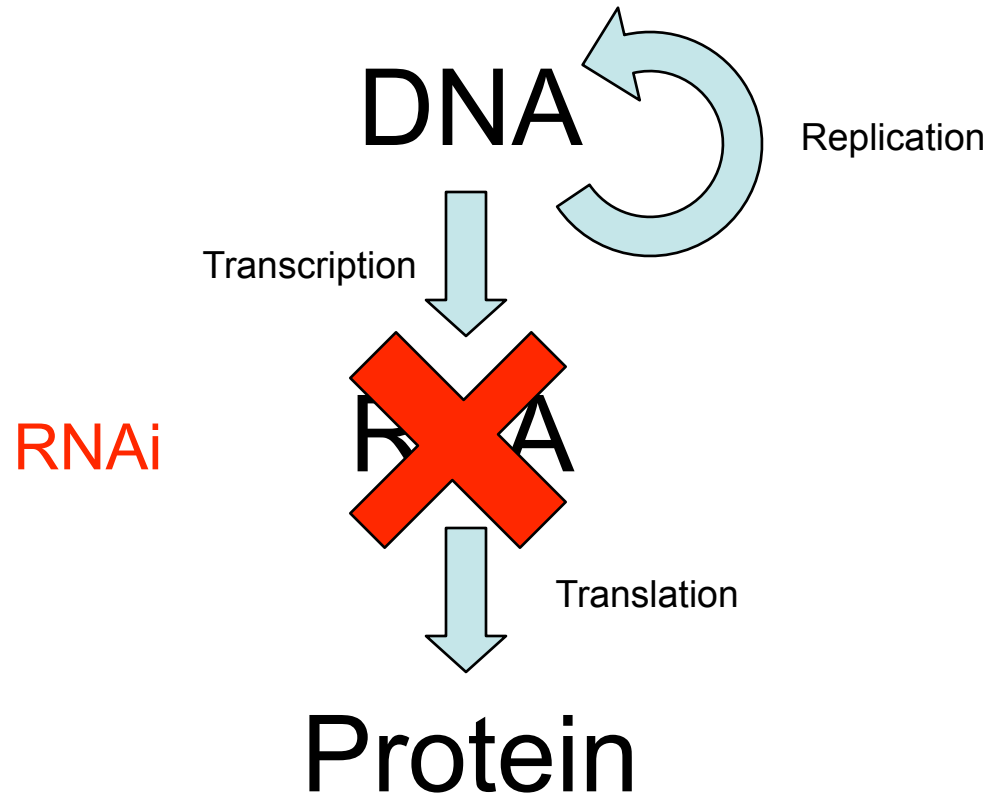


- Effect was first characterized in worms in 1998
- 2006 Nobel prize in Physiology and medicine.

Central Dogma



Central Dogma



RNAi vs. Gene Therapy

RNAi vs. Gene Therapy

RNAi

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- Eliminates function

RNAi vs. Gene Therapy

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RNAi vs. Gene Therapy

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- Adds function

RNAi vs. Gene Therapy

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Gene Therapy

- Adds function
- Adds DNA

RNAi vs. Gene Therapy

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Gene Therapy

- Adds function
- Adds DNA
- Often Permanent

RNAi vs. Gene Therapy

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RNAi vs. Gene Therapy

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Gene Therapy

- Adds function
- Adds DNA
- Often Permanent
- Doesn't affect offspring
- Usually done in a hospital

RNAi is an anti-viral defense

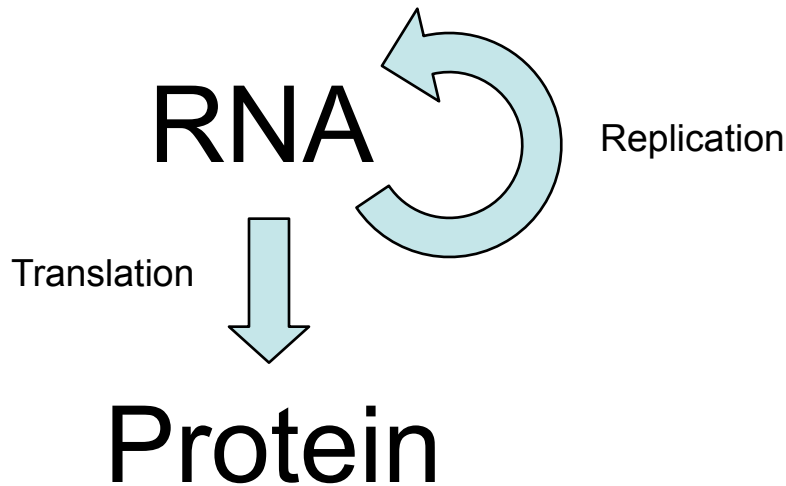
Many viruses contain RNA
instead of DNA

—
C C U A G A G G A G A C A U A C U U G C G C C

RNA

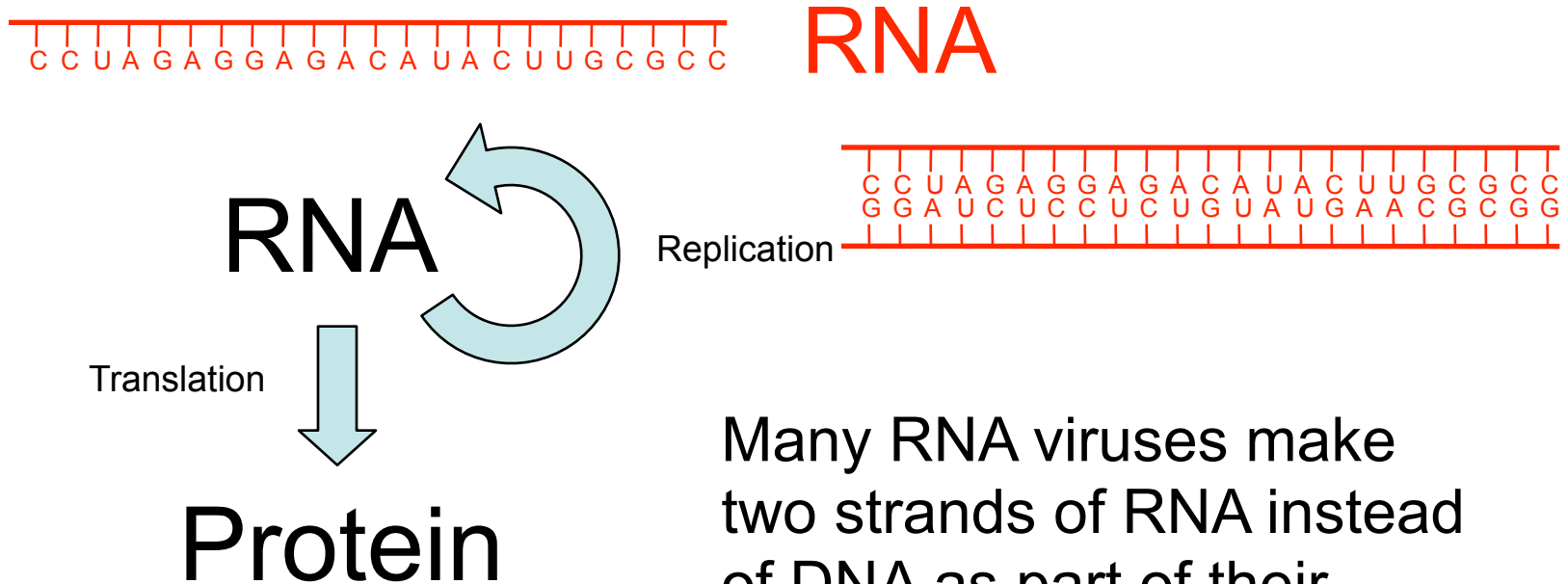
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Many viruses contain RNA instead of DNA



Many RNA viruses make two strands of RNA instead of DNA as part of their copying mechanism.

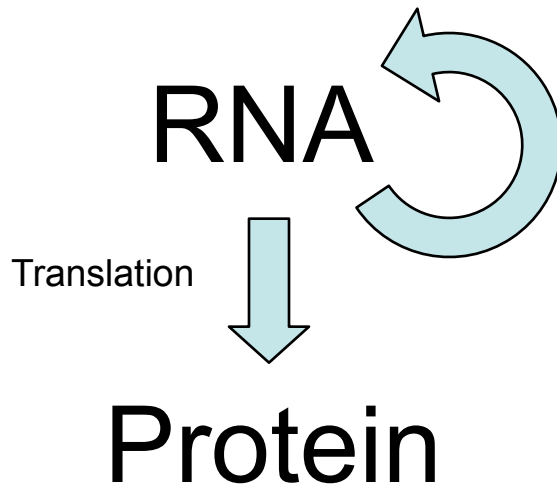
RNAi is an anti-viral defense

Many viruses contain RNA instead of DNA

The cell should NEVER have double-stranded RNA

C C U A G A G G A G A C A U A C U U G C G C C

RNA



Replication

C C U A G A G G A G A C A U A C U U G C G C C
G G A U C U C C U C U G U A U G A A C G C G G

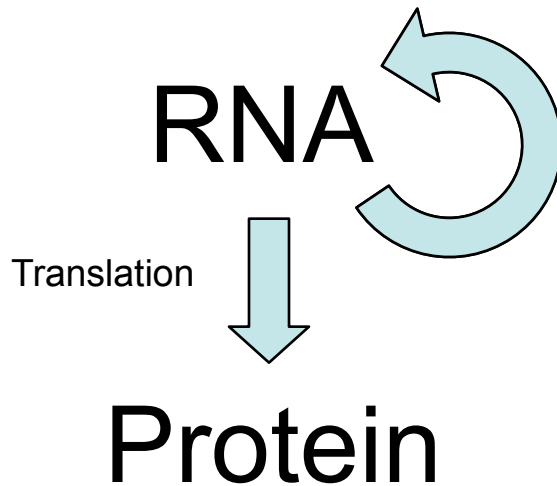
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RNA



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G G A U C U C C U C U G U A U G A A C G C G G

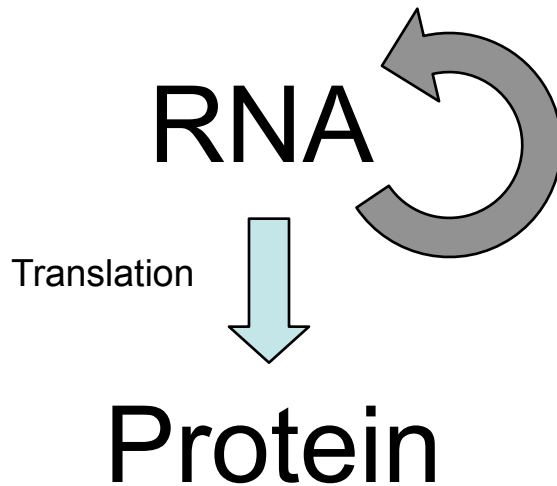
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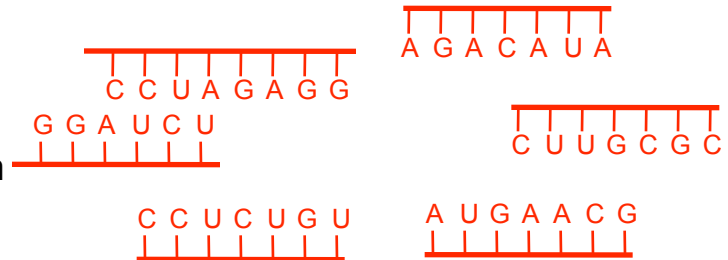
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CCUAGAGGAGACAUAUCUUGCGCC

RNA



Replication



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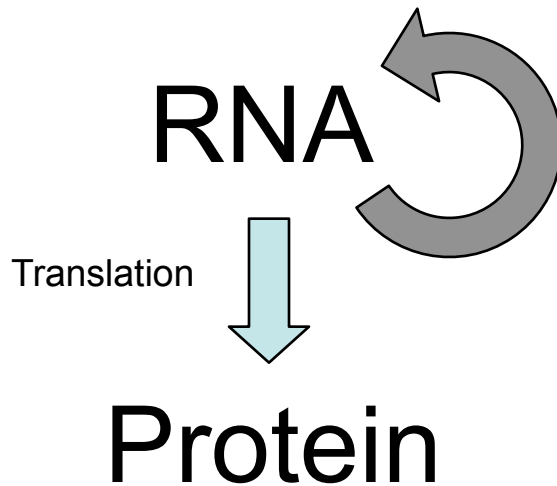
RNAi is an anti-viral defense

How do you get rid of the single stranded?

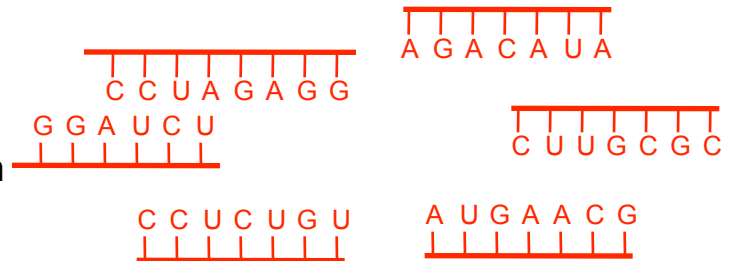
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CCUAGAGGAGACAUAUCUUGC GCC

RNA



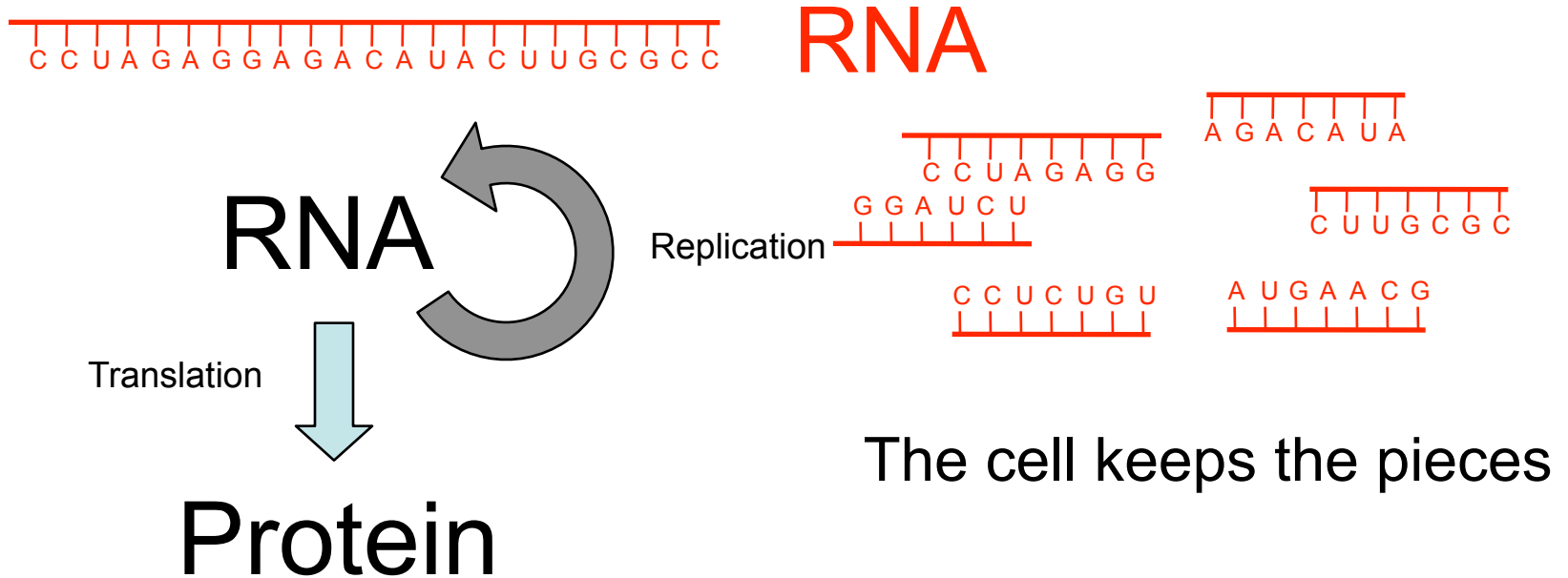
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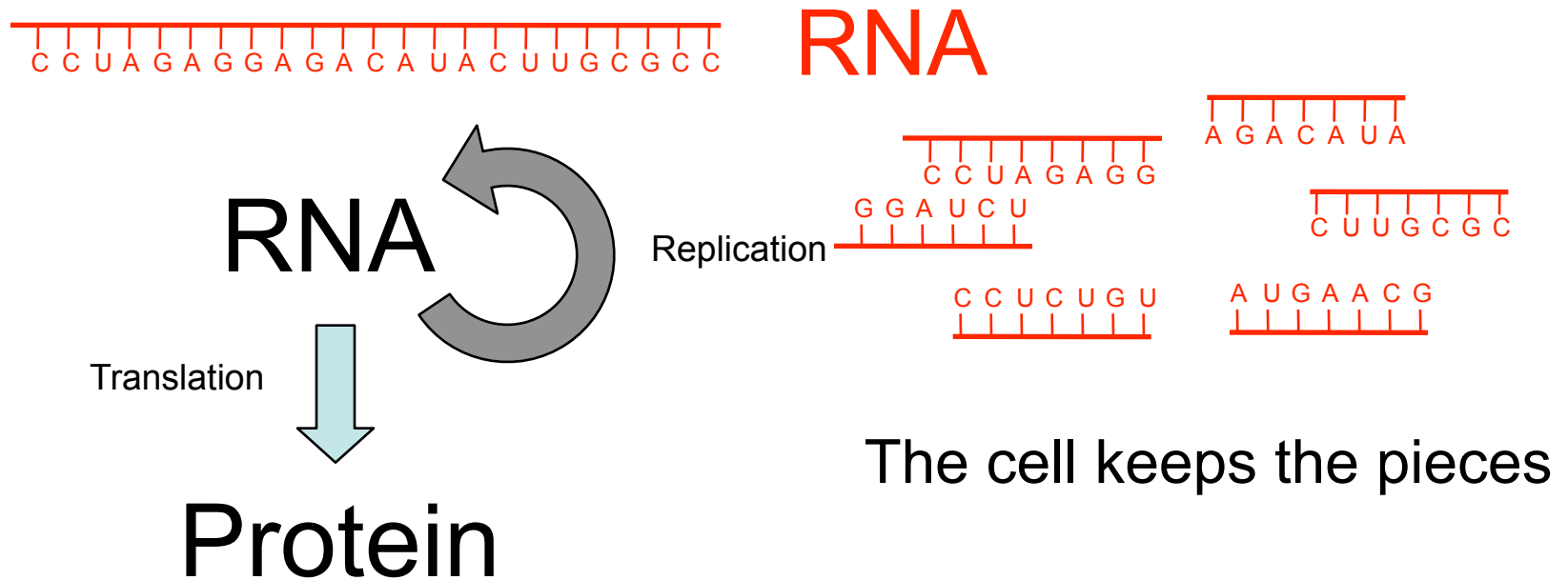
The cell eliminates all double-stranded RNA

RNAi is an anti-viral defense

How do you get rid of the single stranded?



RNAi is an anti-viral defense



RNAi is an anti-viral defense

Any single strands that match are eliminated too



RNA



RNA

Replication

Translation



Protein

The cell keeps the pieces

RNAi is an anti-viral defense

Any single strands that match are eliminated too



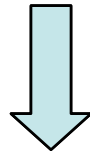
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RNA

Replication

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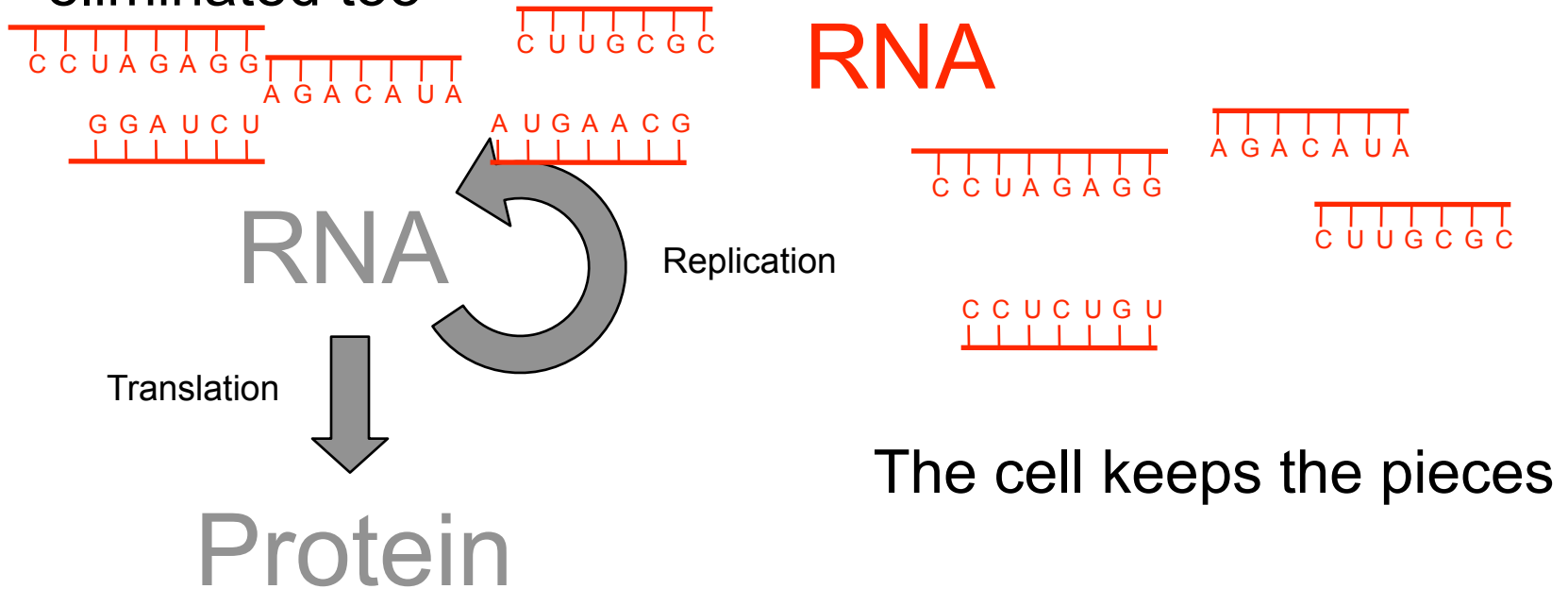


Protein

The cell keeps the pieces

RNAi is an anti-viral defense

Any single strands that match are eliminated too

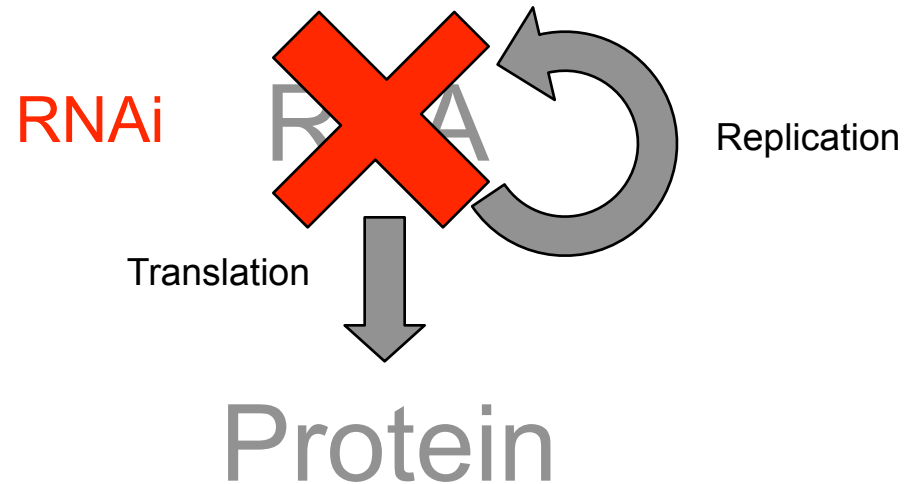


How do we use this?

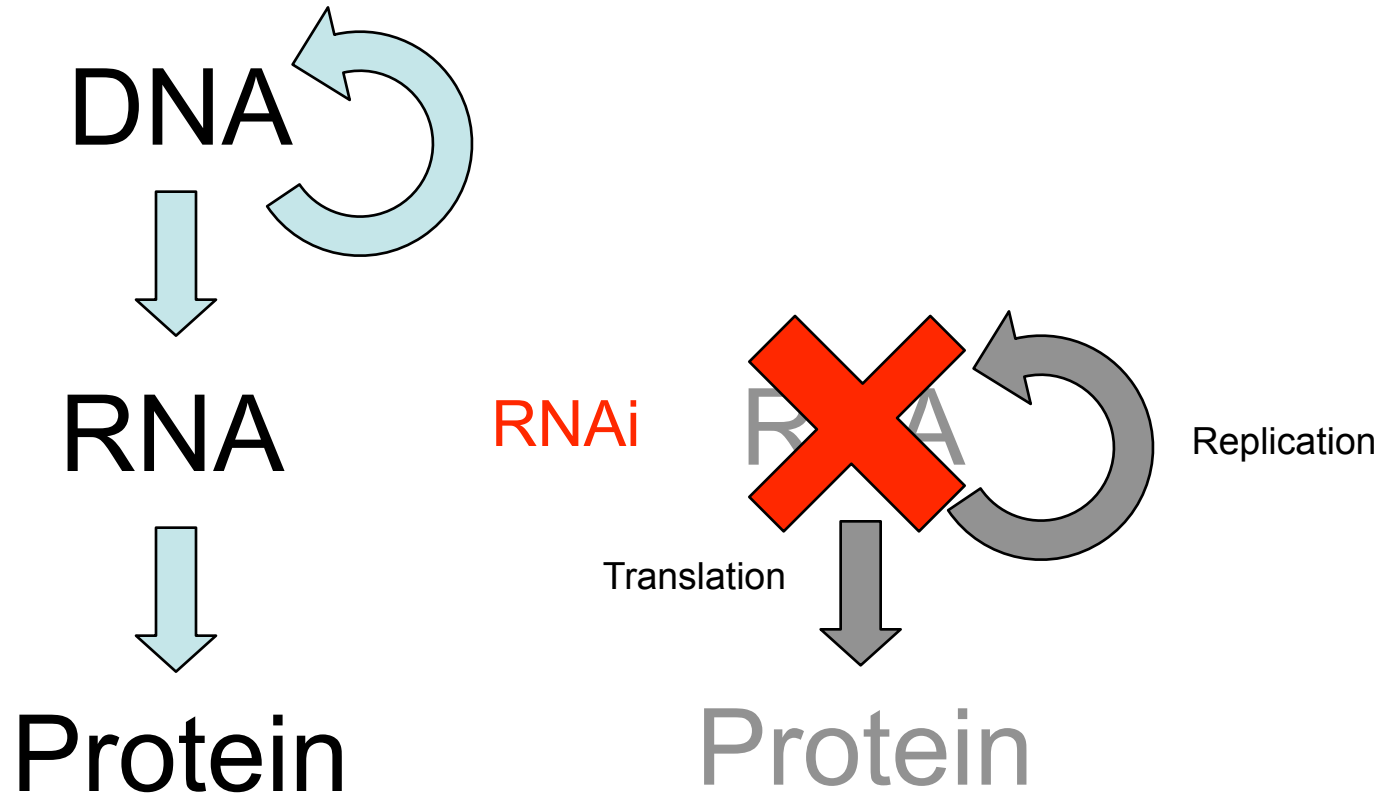
How do we use this?

ANY single-stranded RNA that
matches a double-stranded
RNA gets eliminated

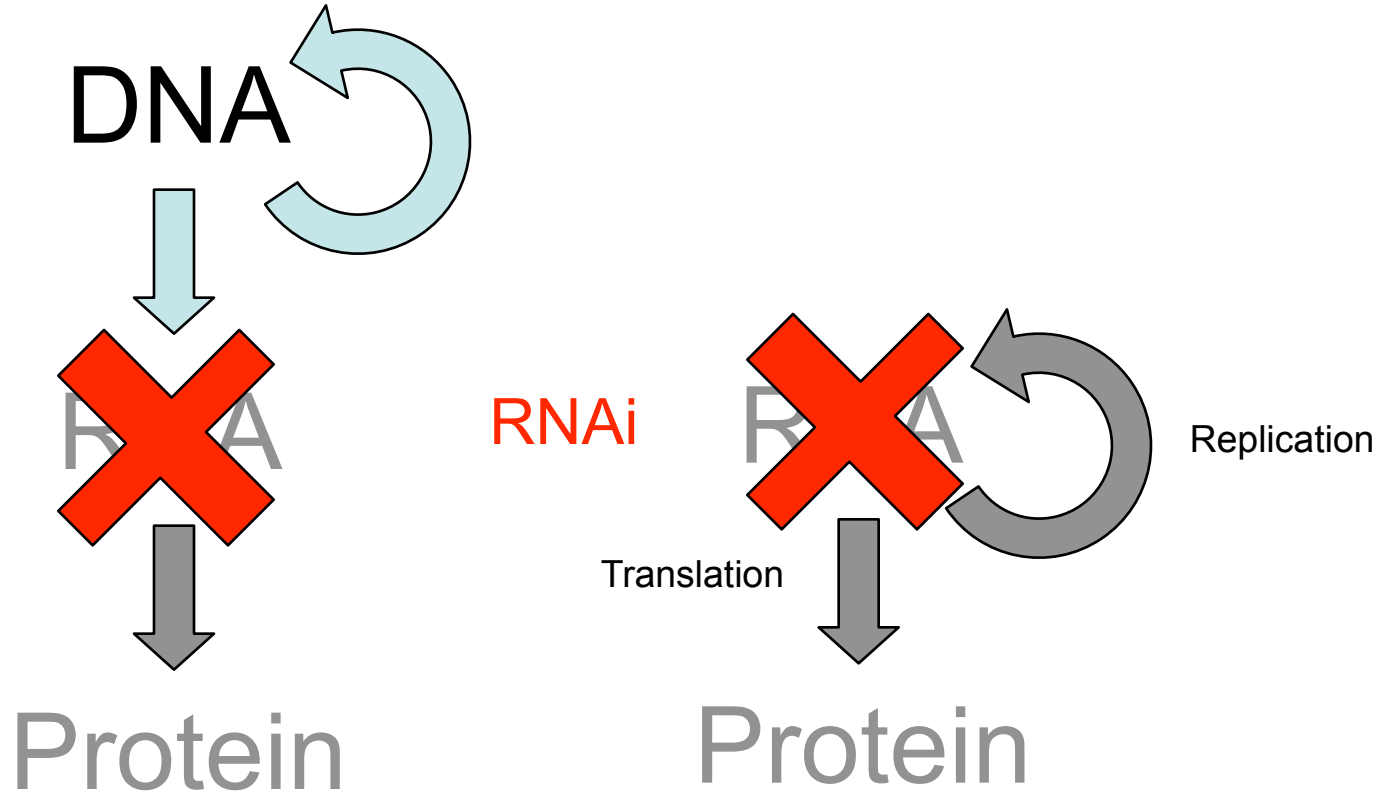
How do we use this?



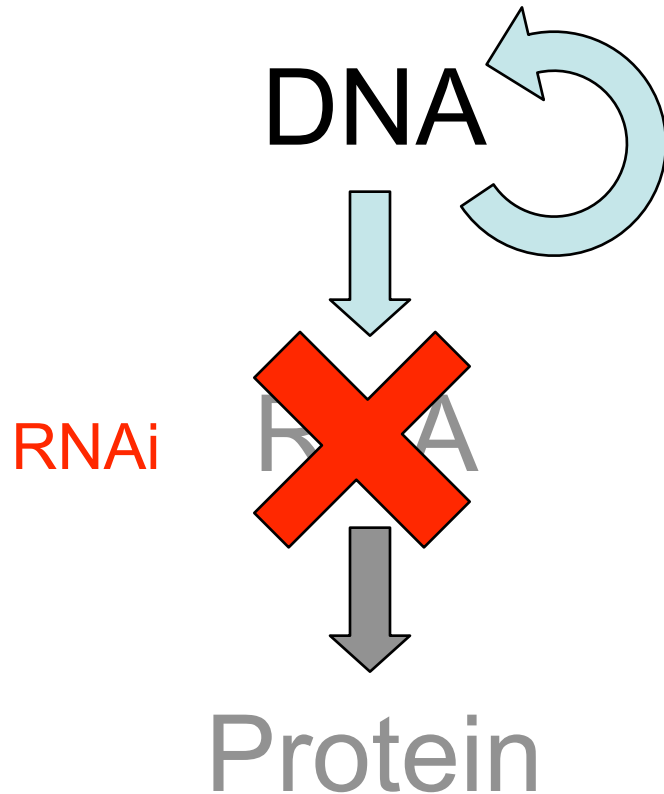
How do we use this?



How do we use this?



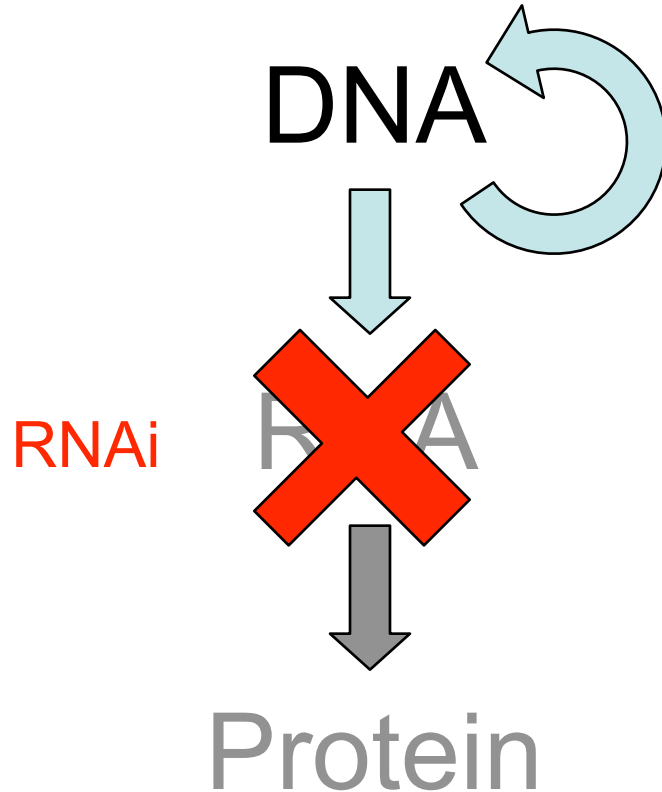
How do we use this?



If we can eliminate mRNA,
we can eliminate the function
of a gene.

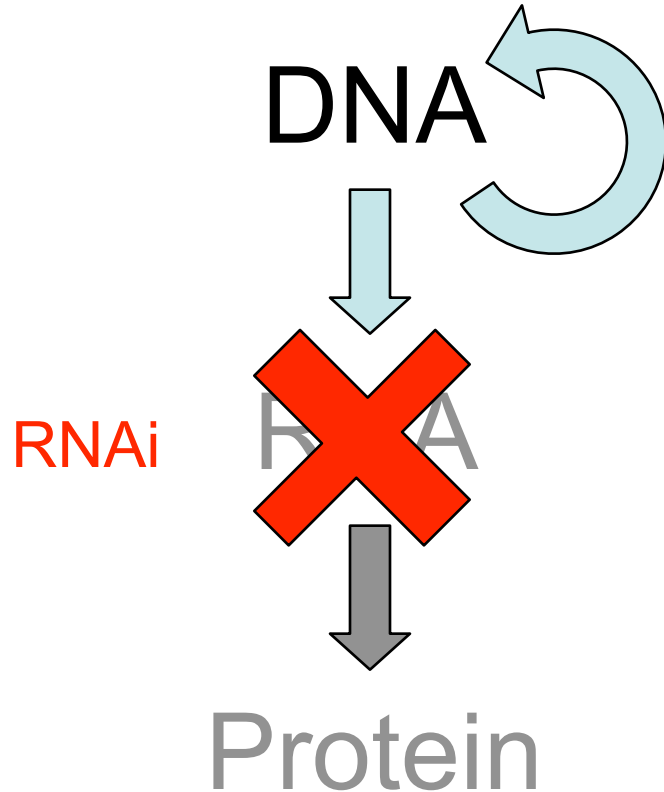
DNA without RNA is pointless

How do we use this?



If we give the cell double-stranded RNA that matches only the mRNA for a gene, the cell will eliminate the mRNA for that gene.

How do we use this?



Double-stranded RNA can be given as a drug.

RNAi can be used as a drug

Cellular mRNA

CCUAGAGGAGACAUA CUUGCGCC

RNA

RNAi can be used as a drug

Cellular mRNA

CCUAGAGGAGACAUAUCUUGCGCC

RNA

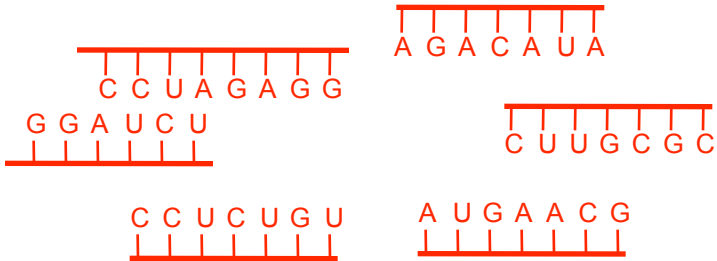
CCUAGAGGAGACAUAUCUUGCGCC
GGAUCUCCUCUGUAUGAACGCGG

Double
stranded RNA
drug

RNAi can be used as a drug

CCUAGAGGAGACAUA CUUGCGCC

RNA

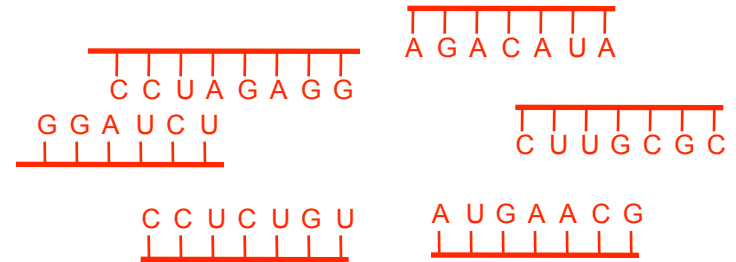


The cell eliminates all double-stranded RNA

RNAi can be used as a drug

CCUAGAGGAGACAUA CUUGCGCC

RNA



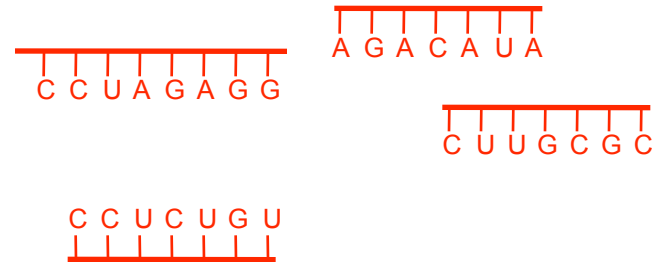
The cell keeps the pieces

RNAi can be used as a drug

Any single strands
that match are
eliminated too



RNA



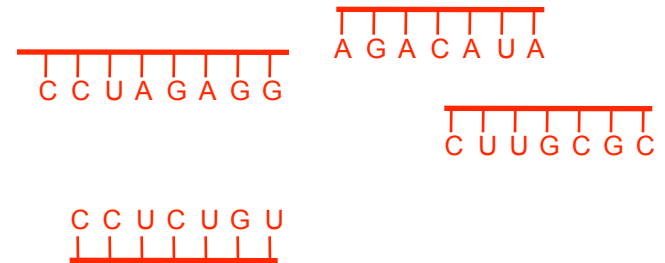
The cell keeps the pieces

RNAi can be used as a drug

Any single strands
that match are
eliminated too

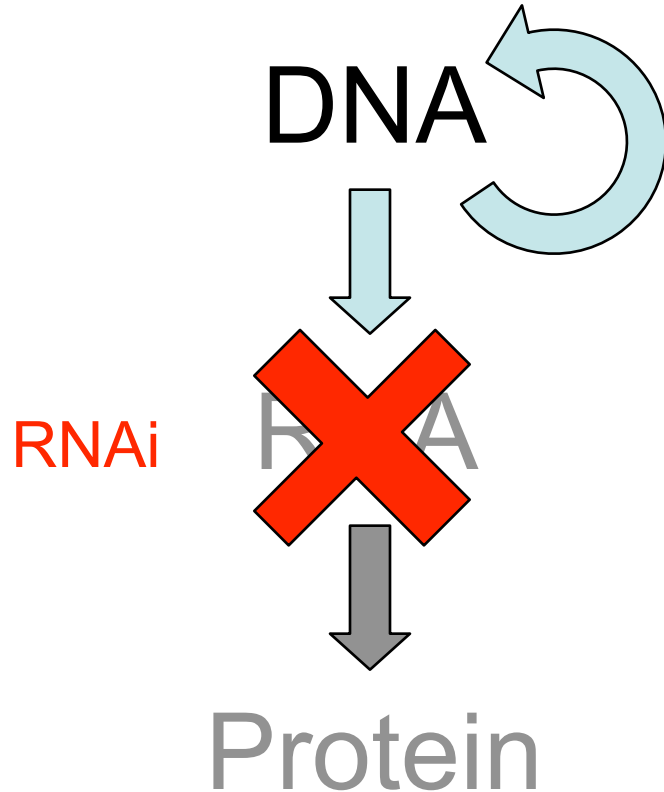


RNA



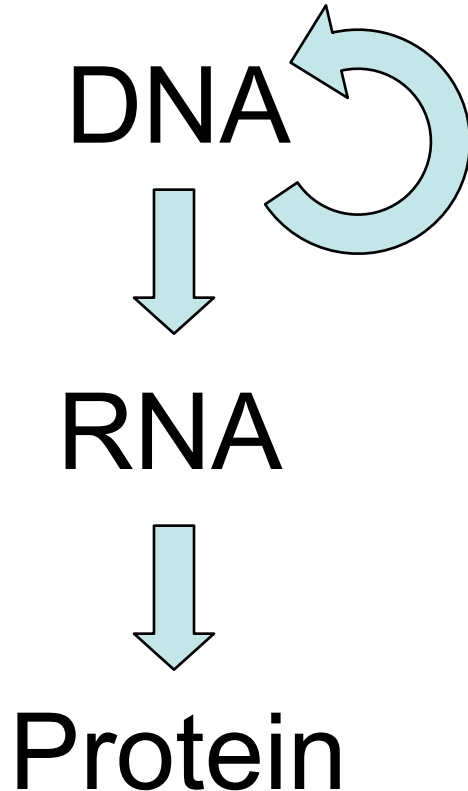
The cell keeps the pieces

How do we use this?



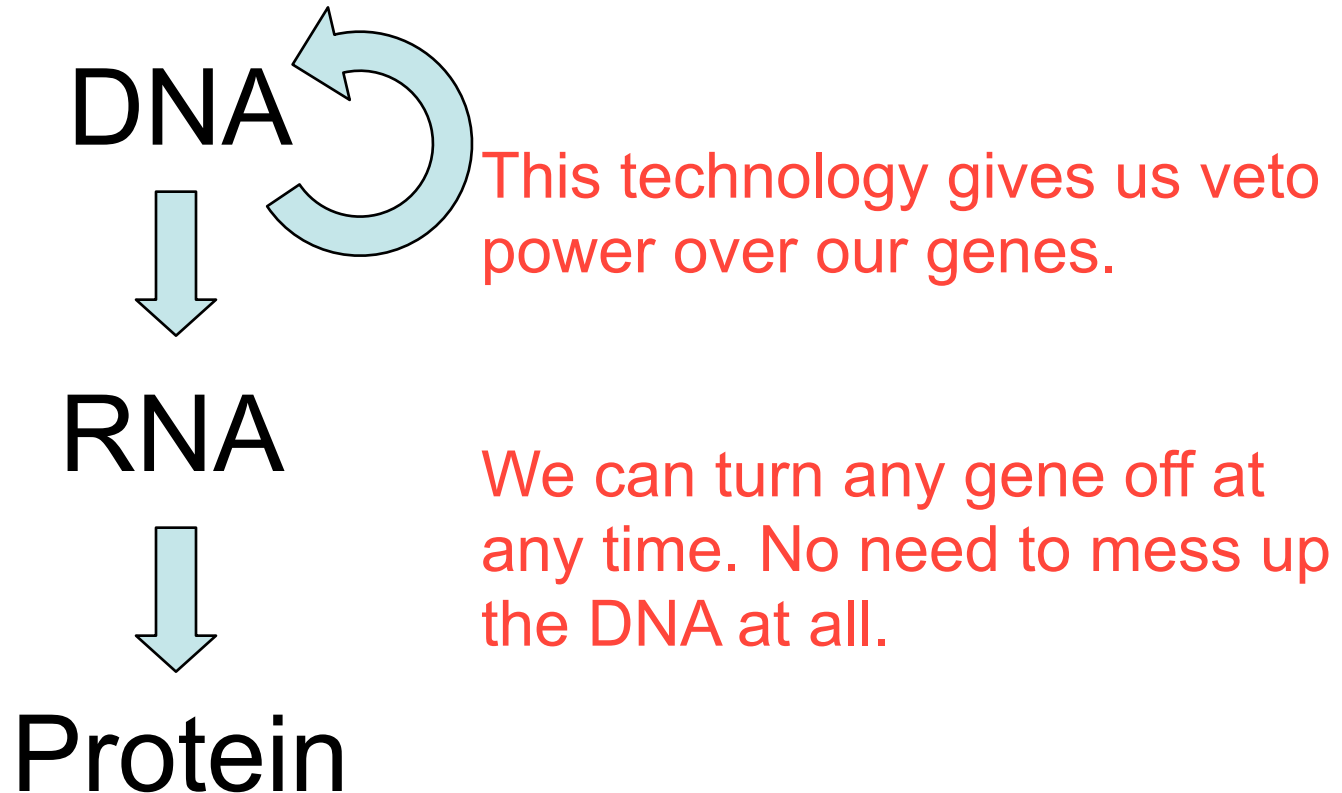
Double-stranded RNA can be given as a drug.

How do we use this?



If we stop giving the RNA drug, the cell makes the mRNA again.

How do we use this?



Companies developing RNAi drugs

* **Acuity Pharmaceuticals** 0.00 - Acuity Pharmaceuticals is a product-focused ophthalmic pharmaceutical company leveraging its strong technology portfolio in RNA interference. Acuity is focusing on developing treatments for age-related macular degeneration (AMD) and diabetic retinopathy (DR), two of the leading causes of vision loss in the developed world.

* **Alnylam Pharmaceuticals** 0.00 - Alnylam Pharmaceuticals (ALNY, NASDAQ), a leader in RNAi therapeutics, is a biopharmaceutical company developing novel therapeutics based on a breakthrough in biology known as RNA interference, or RNAi; a discovery that enables the creation of a broad new class of human therapeutics. Using RNAi, Alnylam has built a product engine to develop a deep pipeline of drug products to treat a wide array of important diseases.

* **Atugen AG** 0.00 - Atugen AG, the RNAi Therapeutics Company, is a biopharmaceutical company based in Berlin, Germany currently employing about 40 individuals. Atugen's therapeutic siRNA programs focus on in vivo applications in oncology and liver diseases, exploiting its proprietary oligonucleotide delivery technologies and chemically stabilized siRNA structures (atuRNAi).

* **Benitec** 0.00 - Benitec, a leading international biotechnology company listed on the Australian Stock Exchange, develops RNA interference (RNAi)-based therapeutics to treat serious diseases such as AIDS and hepatitis C.

* **Calando Pharmaceuticals** 0.00 - Calando Pharmaceuticals is focused on designing, developing and commercializing new RNAi therapeutics to treat and cure diseases and other medical conditions by combining effective RNAi therapeutics with our patented and proprietary delivery technologies.

* **CytRx** 0.00 - CytRx Corporation (NASDAQ: CYTR), located in Los Angeles, California, is a biopharmaceutical company engaged in the development of high-value therapeutics utilizing its core technologies in small molecule drugs, RNAi drug discovery and DNA vaccines.

<http://www.rnaiweb.com>

* **Sirna Therapeutics** 0.00 - Sirna Therapeutics (RNAI, NASDAQ) initiated its first clinical trial of a chemically modified short interfering

Companies developing RNAi drugs

Massachusetts-based **Anylam Pharmaceuticals Inc** <ALNY.O> has used RNAi to block a gene involved in cholesterol metabolism, for example, and this week won a grant from the National Institute of Allergy and Infectious Diseases to use the technology against H5N1 avian influenza.

San Francisco-based **Sirna** <RNAI.O> is working with **Allergan Inc.** <AGN.N> to test RNAi in people with age-related macular degeneration, a leading cause of blindness which is caused by a proliferation of blood vessels in the eye.

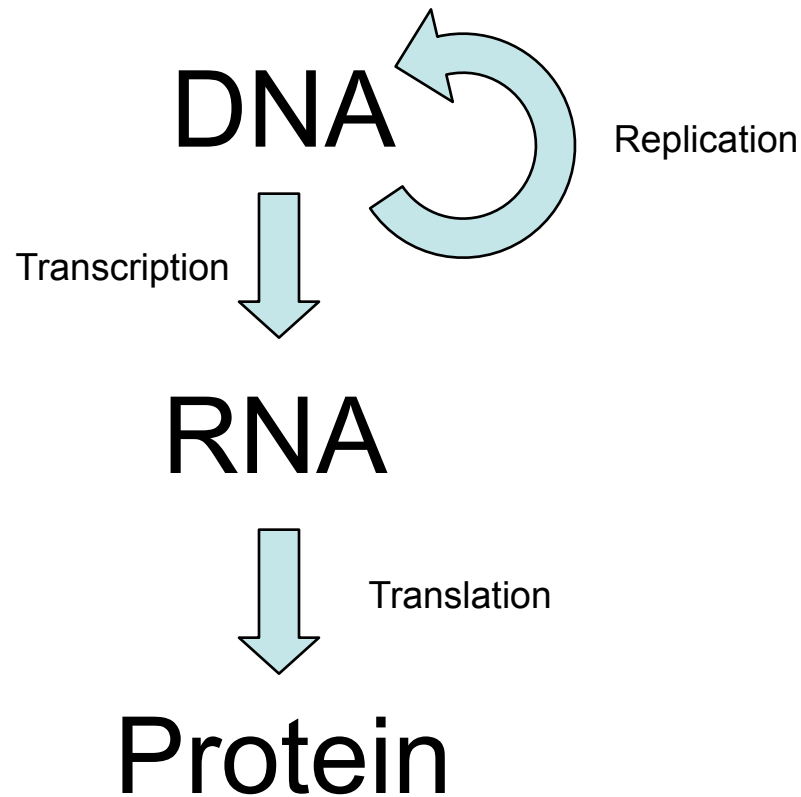
Sirna is working with **GlaxoSmithKline** <GSK.L> <GSK.N> to develop RNAi to fight respiratory diseases.

A German company called **Cenix BioScience** has teamed up with **Merck and Co.** <MRK.N> to develop RNAi treatments and Maryland-based biotech company called **Intradigm**, a small spinoff from Swiss drugs giant **Novartis** <NOVN.VX> found a way to use RNAi to treat SARS in monkeys.

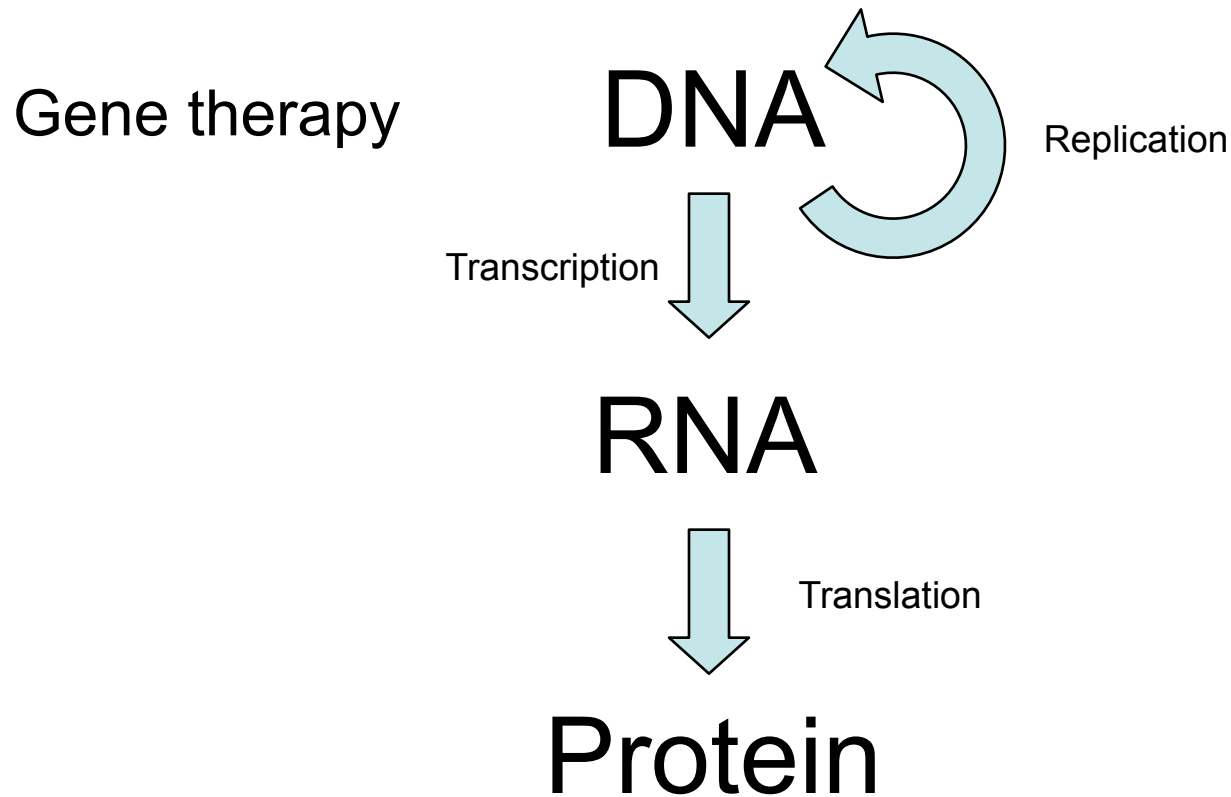
Summary

- RNAi gives us veto power over our genes
- Double stranded RNA can be introduced, and any RNA in the body with the same sequence will be eliminated

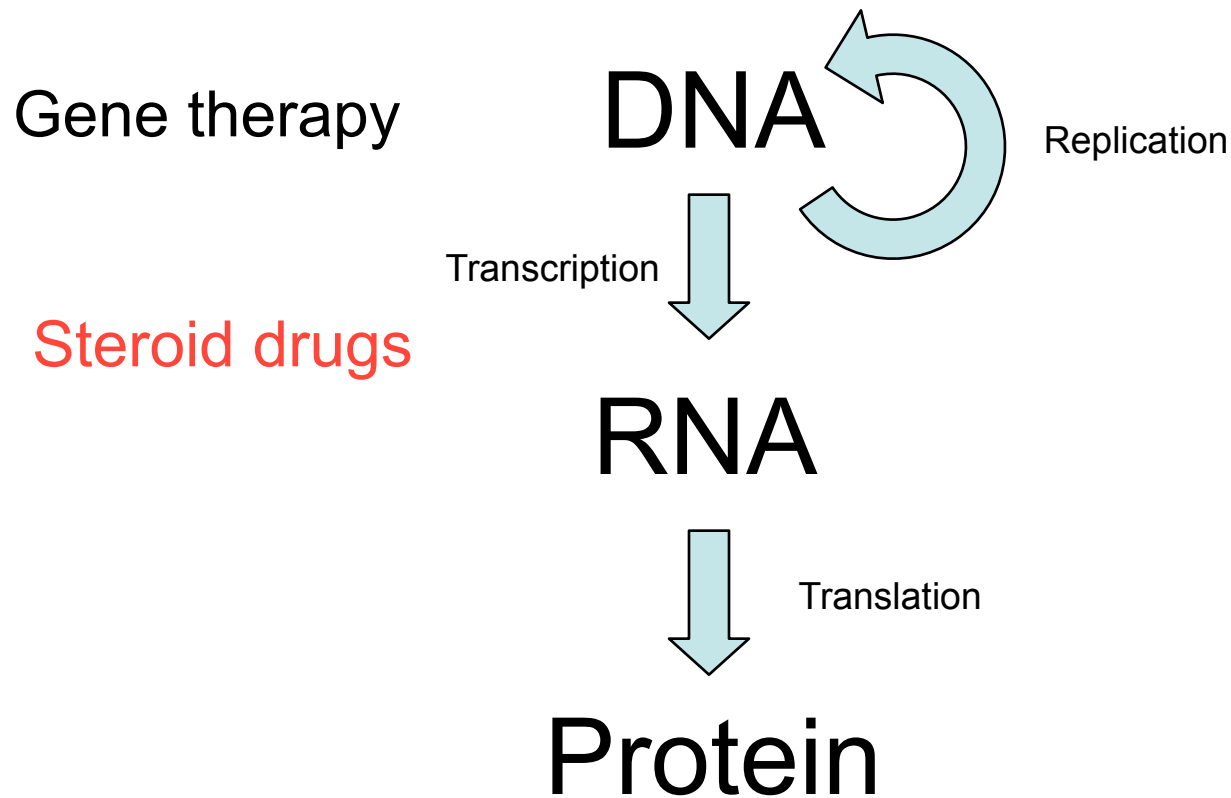
Gene therapy isn't always needed



Gene therapy isn't always needed

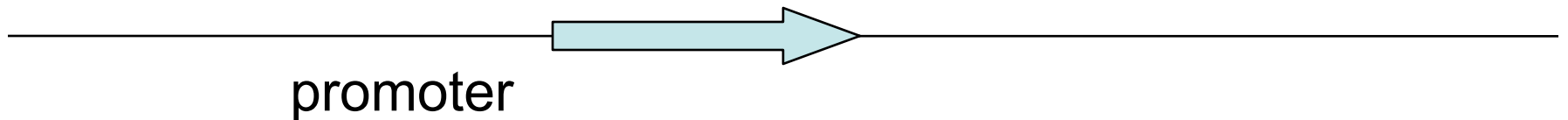


Gene therapy isn't always needed



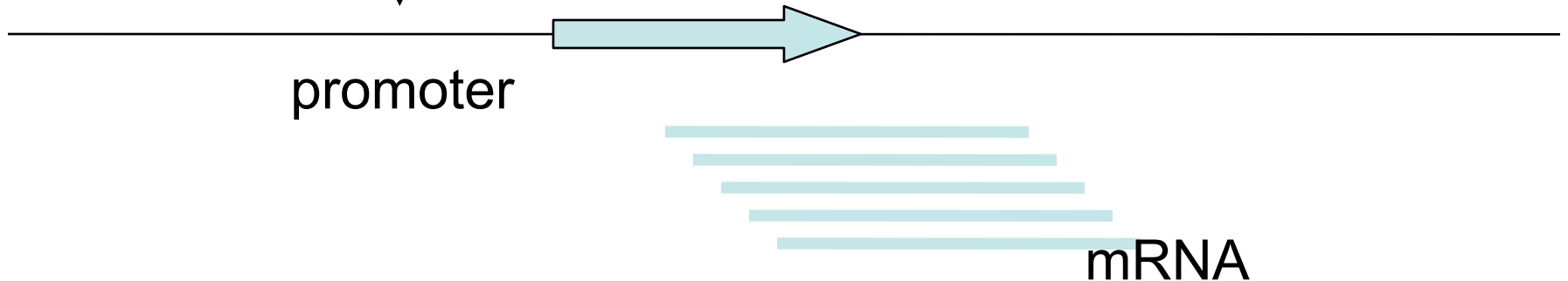
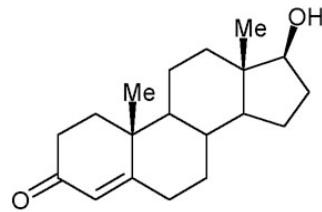
Anabolic steroids

Anabolic steroids (testosterone etc.) work by changing gene expression

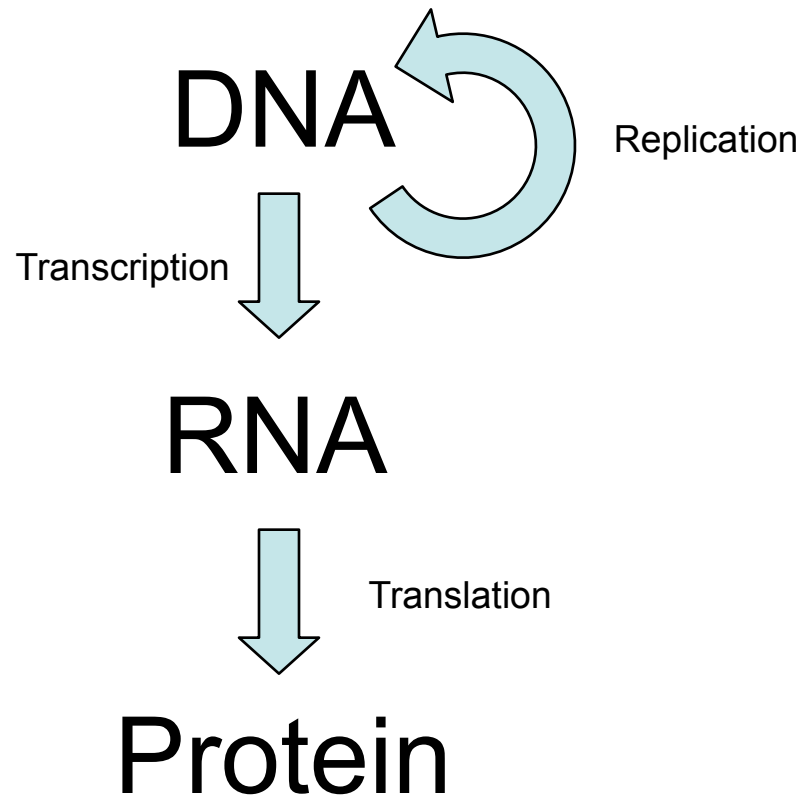


Anabolic steroids

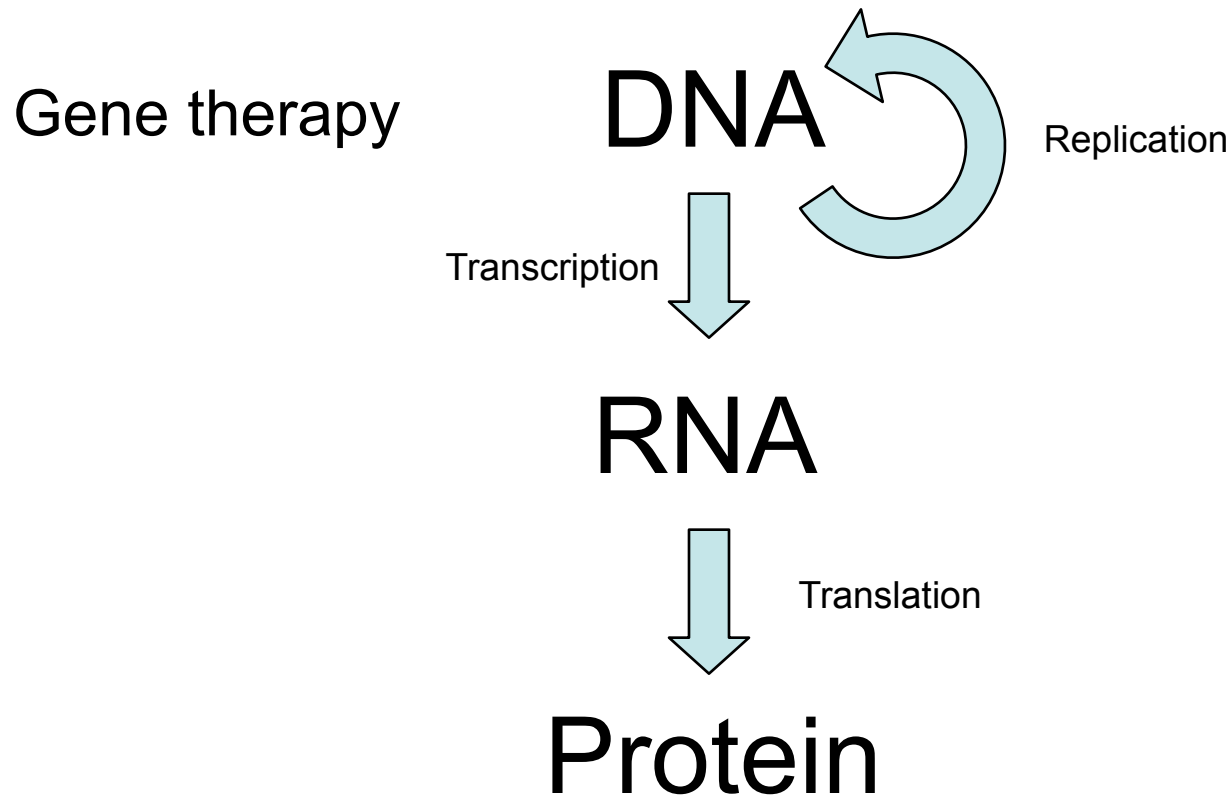
Anabolic steroids (testosterone etc.) work by changing gene expression



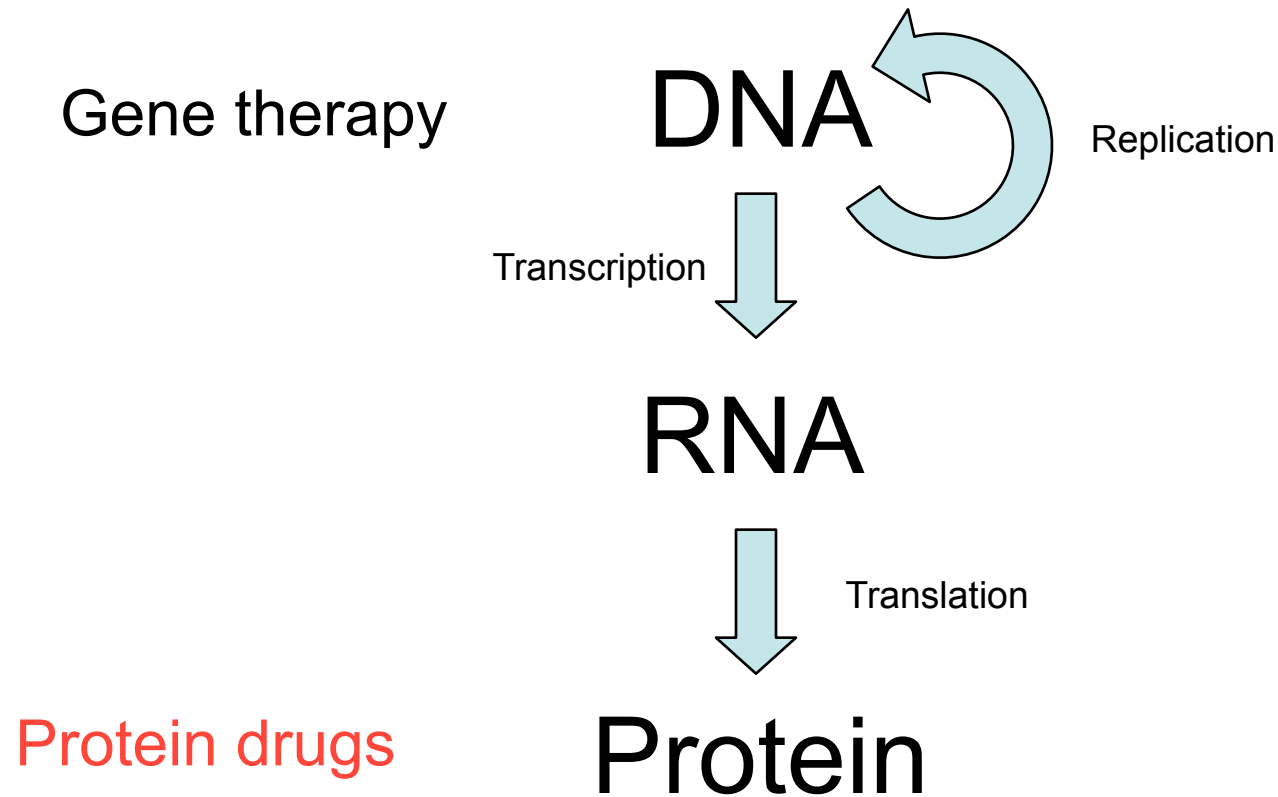
Gene therapy isn't always needed



Gene therapy isn't always needed



Gene therapy isn't always needed



Gene therapy isn't always needed

- Diabetes
- Growth hormone deficiency
- Anemia

Gene therapy isn't always needed

- Diabetes Insulin
- Growth hormone deficiency Growth hormone
- Anemia Erythropetin (EPO)

Growth hormone (humatrope)

In 2003 the FDA approved Eli Lilly's drug humatrope for use in children of short stature (shortest 1.2%).

This would correspond to heights of less than 5' 3" and 4' 11" in adult men and women, respectively.

Drug trials showed increases of 3" in final height.

Estimated costs: \$20-40K per year.

Erythropetin (EPO)

EPO is a natural hormone that regulates red blood cell production

It is given to cancer patients to treat anemia caused by chemotherapy

It is taken by endurance athletes to improve their oxygen carrying capacity.

EPO



http://www.nrk.no/sport/annan_idrett/4988390.html

Lance Armstrong 7 time champ
(took EPO for chemotherapy
induced anemia)



<http://instantpunditry.blogspot.com/archive/2005/09/index.html>

David Millar banned
for 2 years

EPO

Autosomal Dominant Erythrocytosis Caused By Increased Sensitivity to Erythropoietin

Eeva Juvonen, Eero Ikkala, Frej Fyhrquist, and Tapani Ruutu
Blood, Vol 78, No 11 (December I), 1991

Describes a Finnish family with a mutation that makes them hypersensitive to EPO...

“The erythrocytosis has not had any obvious effect on the health or life-span of the affected individuals. Many of them have reached an advanced age, and one of the affected family members has won several Olympic gold medals and world championships in endurance sports. “