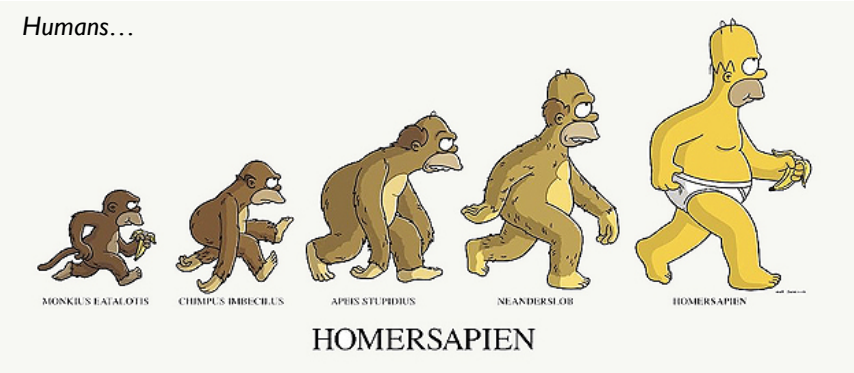
VIRUSES, VIRAL VECTORS, AND GENE TRANSFER

What are viruses? How do they cause infection and disease? How do we harness them in biology?

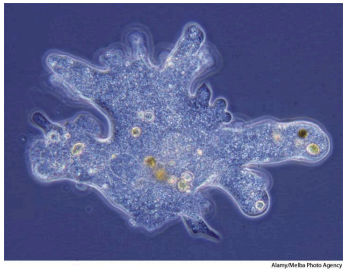
What is considered life?

- Living organisms:
  - One or more cells
  - Capable of growth
  - Capable of reproduction
  - Pretty much self-sufficient - e.g. makes energy

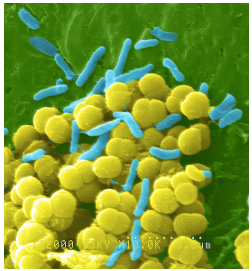
Humans…



Planaria, a flatworm



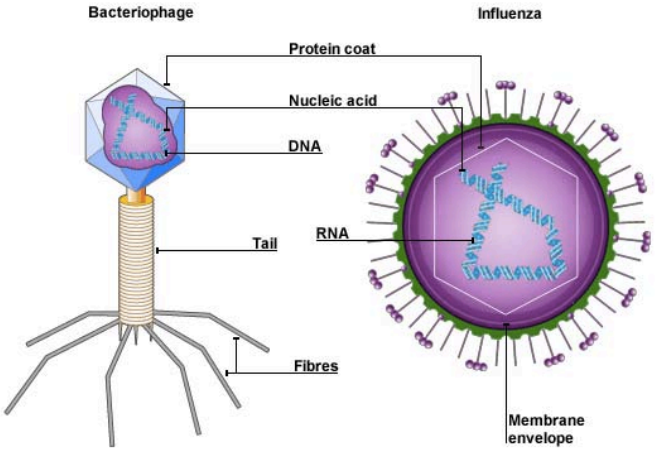
Amoeba, single-celled living organism



Some bacteria

Viruses are not considered living organisms

- Viruses are simple genetic material packaged inside a protein capsule
  - Cannot grow on its own
  - Cannot reproduce on its own
  - Does not make energy
- It can only survive by using the machinery of its host! So we decided to consider it **NON-LIVING**

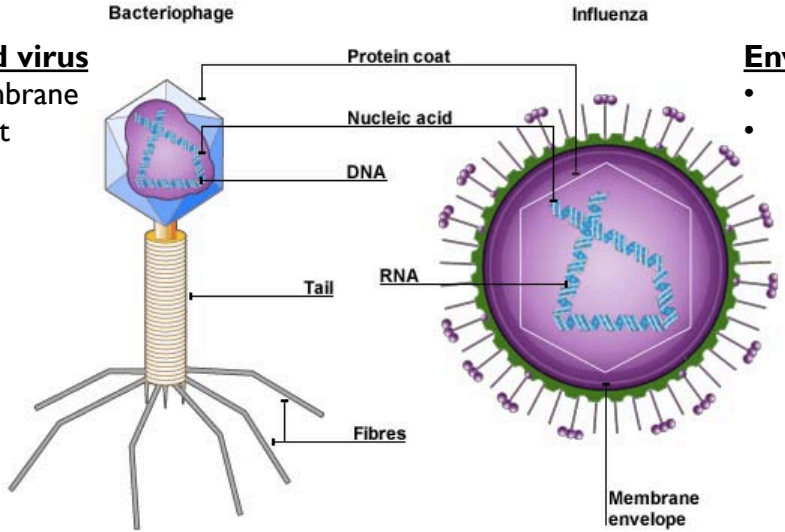


Viruses are not considered living organisms

- It's kind of like Pluto… just based on the definition.

Structure of a virus

Non-enveloped virus
- No lipid membrane
- Heat-resistant



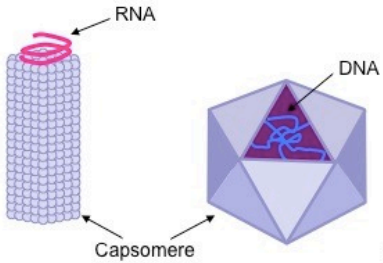
Enveloped virus
- Has lipid bilayer membrane
- Sensitive to heat

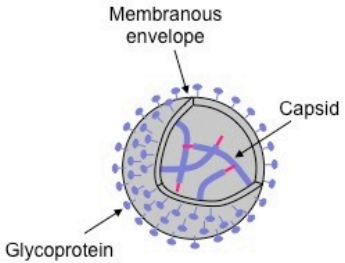
Functions of capsid or envelope of viruses:
1. Protect the nucleic acid genome
2. Interact with cell, helping with cellular entry

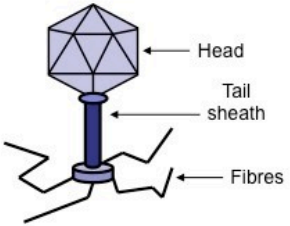
Virus classification

- What/who can they infect? **HOST RANGE**
  - Bacteria (bacteriophage)
  - Animal viruses
  - Human viruses
  - Others (e.g. amoeba, insects, plants)
- What is their genetic material like? **GENOME STRUCTURE**
  - DNA – ssDNA, dsDNA
  - RNA – ssRNA, dsRNA
  - Is the genome linear, or circular?
  - How big is it? (range can be from 2 kbp to 200 kbp!)
- Virus particle size? Typically nanometer (10^{-9}m) diameter range
- Virus particle morphology? Helical, icosahedral
- Replication strategy/life cycle?

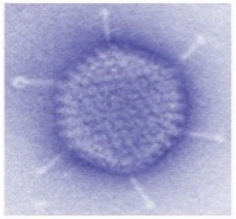
Viruse are extremely diverse



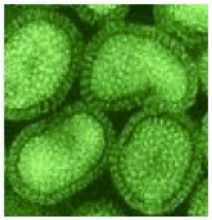




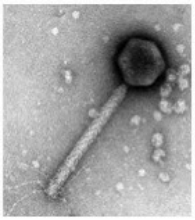
Tobacco Mosaic Virus



Adenovirus

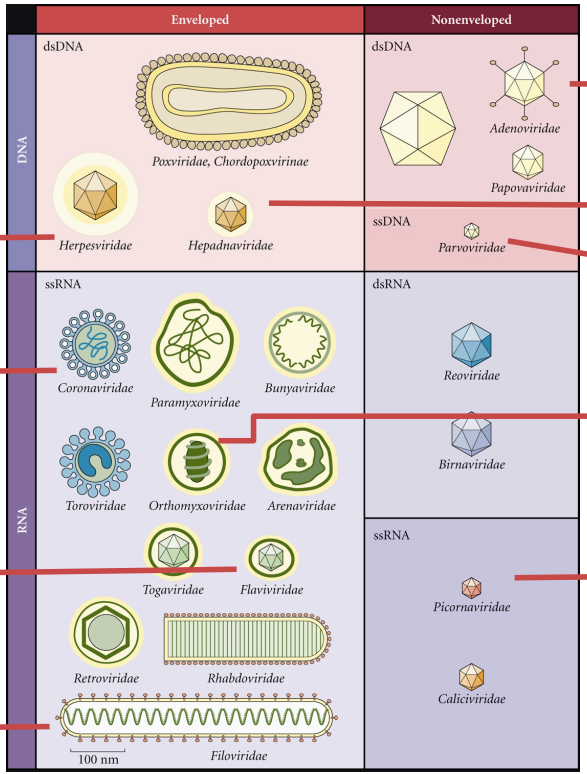


Influenza Virus



Bacteriophage

Viruses are extremely diverse



Oral/genital herpes ("cold sores"); Burkitt's Lymphoma; Mononucleosis (mono/"kissing disease")

SARS, MERS

Zika virus

Ebola (ebolavirus)

Cold-like symptoms; pneumonia; bronchitis

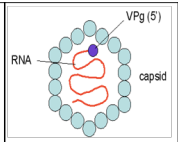
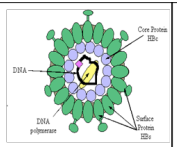
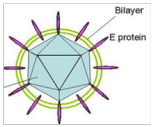
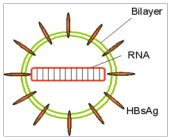
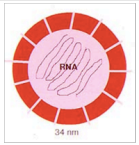
Hepatitis B

Very deadly for dogs

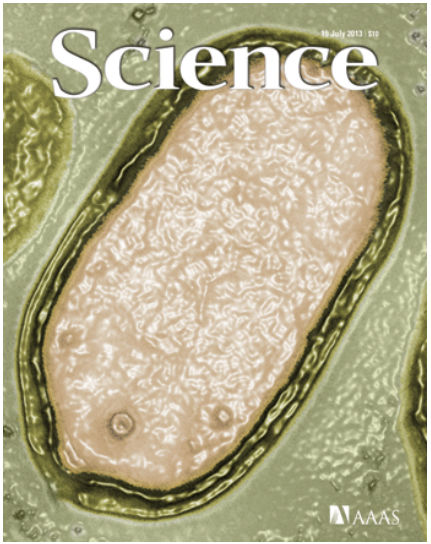
Influenza (the flu), including swine flu, chicken flu

Common cold (Rhinovirus)

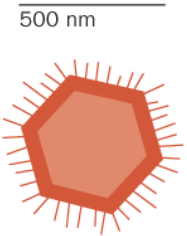
Viruses are extremely diverse

					
Name of Virus	Hepatitis A Virus (HAV)	Hepatitis B Virus (HBV)	Hepatitis C Virus (HCV)	Hepatitis D Virus (HDV)	Hepatitis E Virus (HEV)
Classification	Picornavirus	Hepadnavirus	Flavivirus	Deltavirus	Hepevirus
Viral genome	ssRNA	dsDNA	ssRNA	-ssRNA (-ve)	ssRNA
Transmission	Enteric	Parental	Parental	Parental	Enteric
Incubation period	15-45 days	45-160 days	15-150 days	30-60 days	15-60 days
Chronic Hepatitis	No.	Yes. 10% chance	Yes. >50% chance	Yes. <5% of coinfectious >80% of superinfectious	No.
Cure?	No cure. Treatments usually tackle the symptoms.	No cure. Treatments usually tackle the symptoms.	No cure. Treatments usually tackle the symptoms.	No cure. Treatment: Alpha interferon for 12 months.	No cure. Treatments usually tackle the symptoms

Biggest known virus: Pandoravirus



Pandoravirus salinus
Base pairs: 2.5 million
Length: 1,000 nm
Diameter: 500 nm



Megavirus chilensis
Base pairs: 1.26 million
Diameter: 500 nm

Influenza type A
Base pairs: 13,500
Diameter: 100 nm

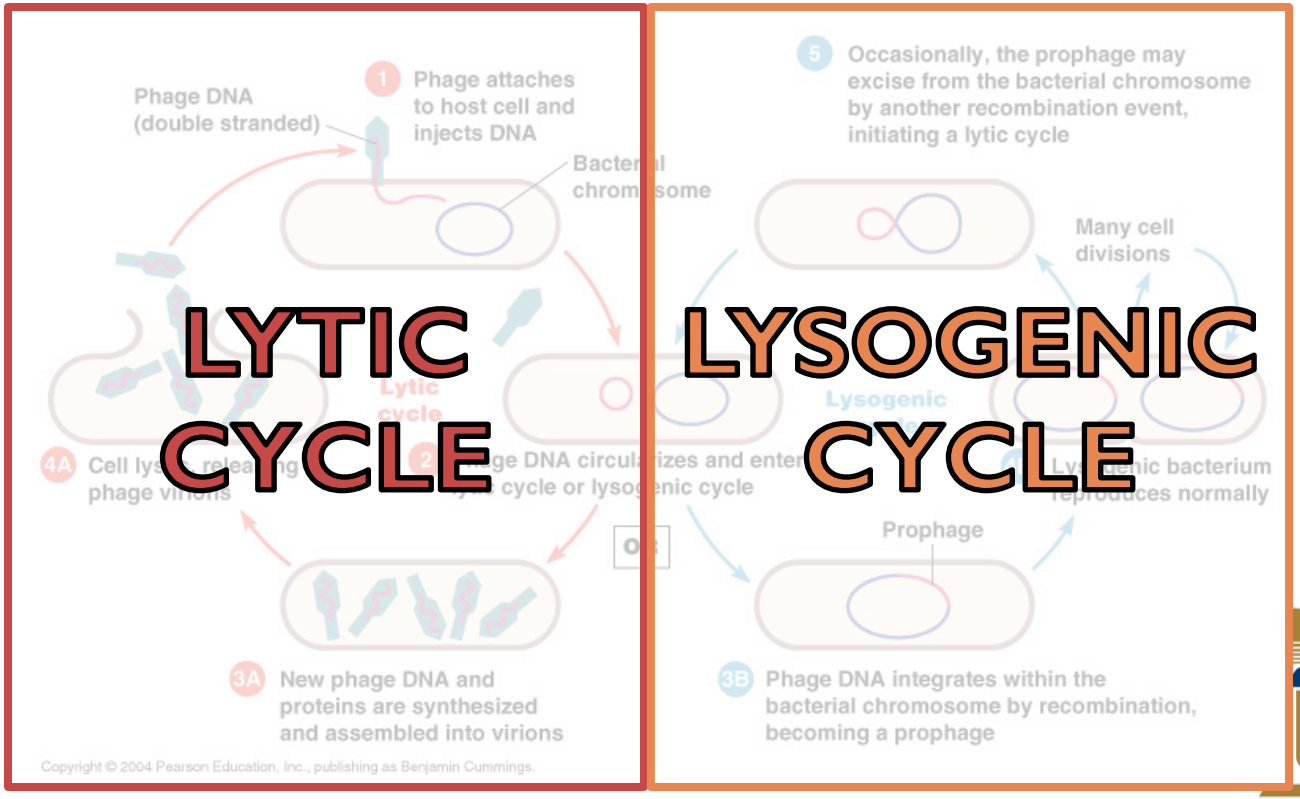
It is half the size of *E. coli (~2um)*

https://bio113.weebly.com/pandoravirus-salinus.html

DNA virus, discovered in 2013
It infects amoeba, not humans
Massive 2.5 Mbp genome size

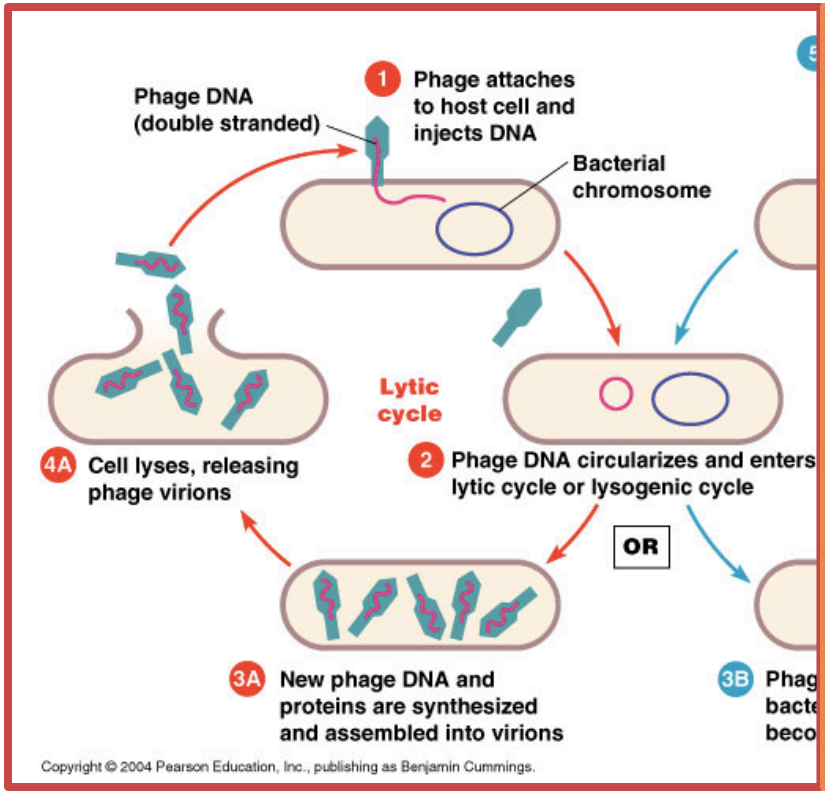
Philippe et al., Science, 2013. https://doi.org/10.1126/science.1239181

The life cycle of viruses



NOTE: this uses bacteria and bacteriophage (virus that infects bacteria) as example

The life cycle of viruses



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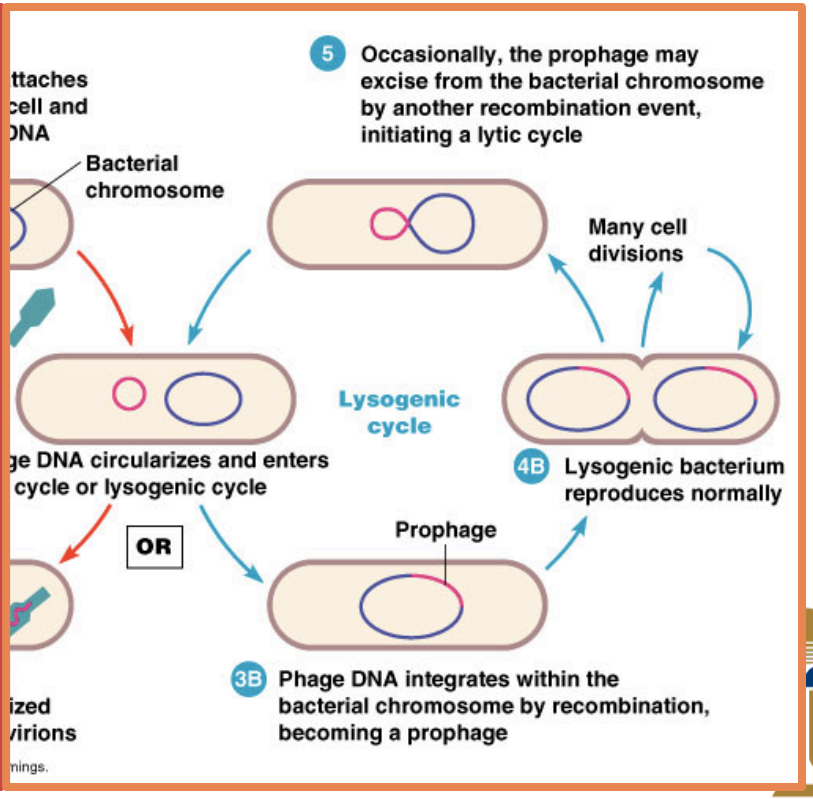
The life cycle of viruses



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The life cycle of viruses

LYTIC CYCLE

LYSOGENIC CYCLE

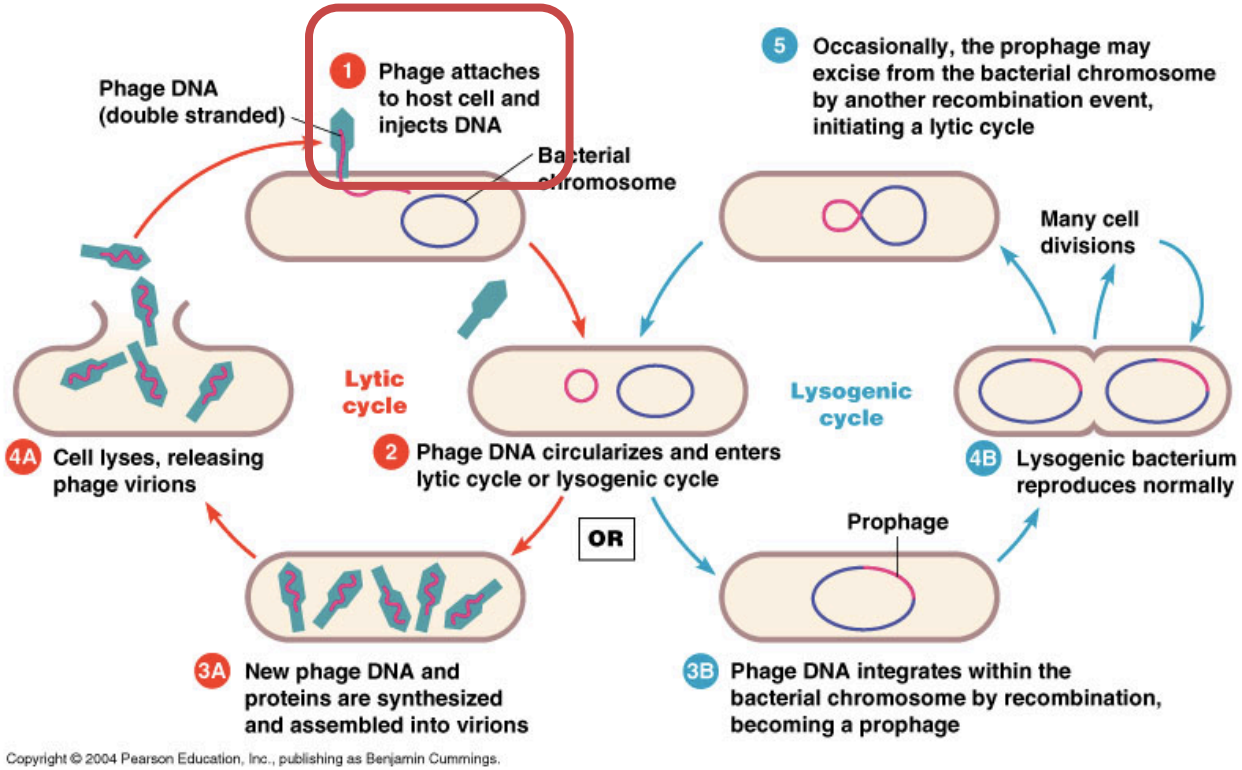
A word about viral latency

- **Latency** = Ability of pathogenic virus to lay dormant in a cell (i.e. during the lysogenic stage)
- **Reservoir** = A cell type or anatomical site where the virus lies dormant/remains latent (e.g. in Hep B it is hepatocytes)
- Viruses can be latent in many forms:
  - **Episomal latency** – the viral DNA is separate from the nuclear DNA/host genome, like a plasmid, except it's called an **EPISOME**, it just floats around the cytoplasm, but it could also enter nucleus. (e.g. HSV)
  - **Proviral latency** – the provirus is a viral genome that was integrated into the host genome. (e.g. HIV)

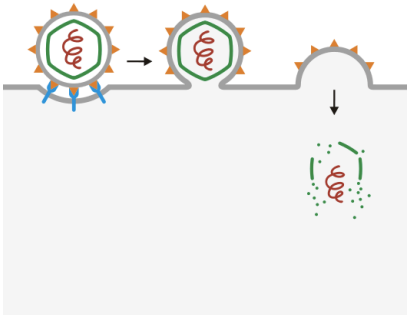
*Note: **Plasmid vs Episome**, very roughly – both are extrachromosomal; can be replicated separately from the chromosomes of the cell; but EPISOMES have the ability to be integrated into the chromosome, whereas PLASMIDS do not.*

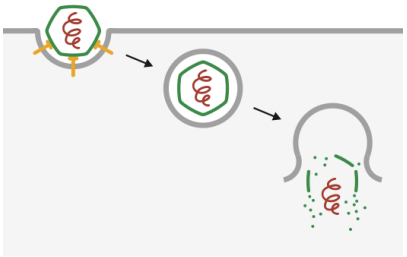
NOTE: this uses bacteria and bacteriophage (virus that infects bacteria) as example

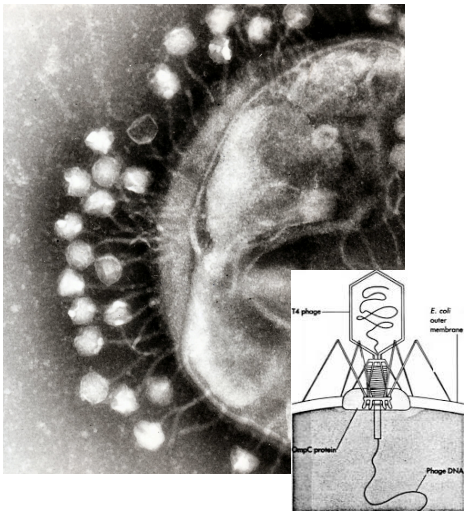
The life cycle of viruses



Attachment and entry





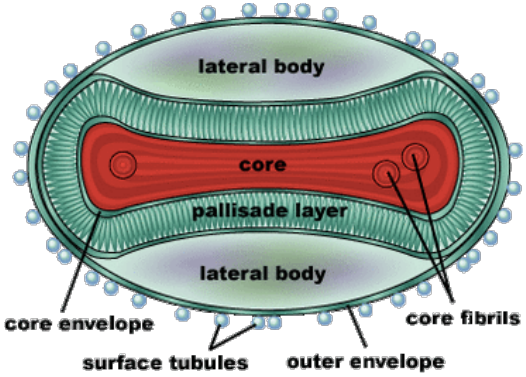


Membrane Fusion
- Viruses with envelope, infecting cells with a lipid bilayer membrane
- Bilayer membrane of virus is same as cell
- Needs receptors

Endocytosis
- Must have the right surface receptors
- Virus carried in by vesicle/endosome to the nucleus

Genetic Injection
- Bacteriophage infecting a bacteria
- Genetic material gets pooped into the bacteria
- Very high speed of injection!

Smallpox 💀💀💀



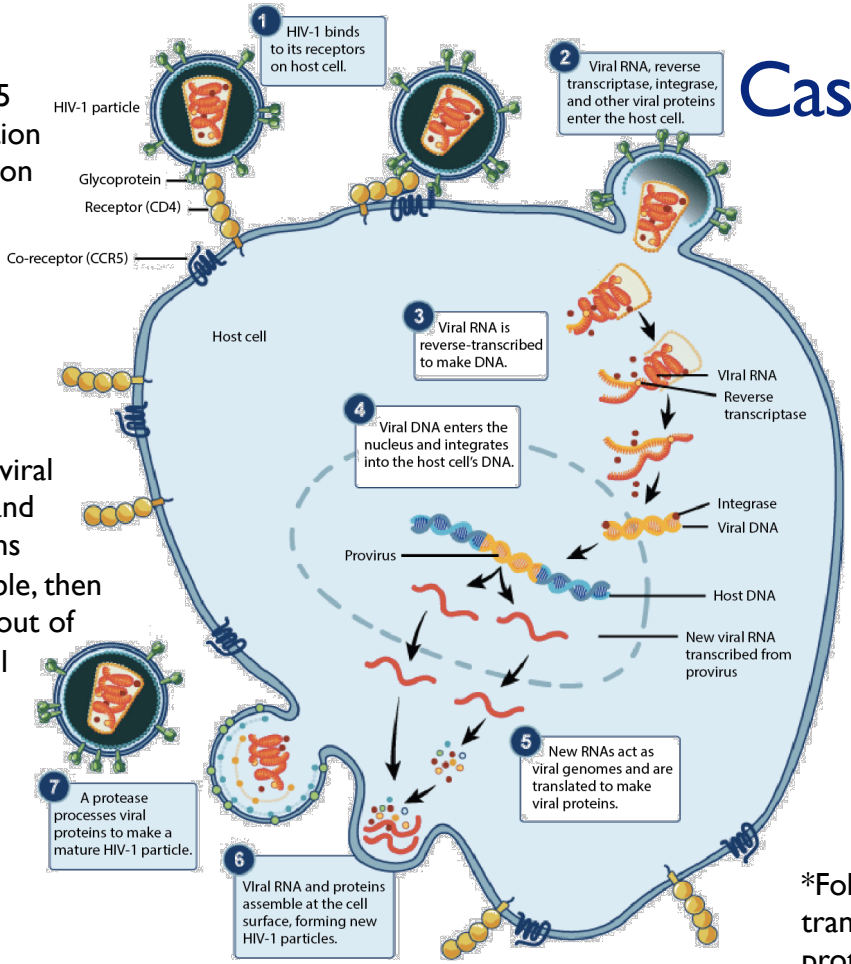
- Orthopoxviridae, variola major virus
- dsDNA virus
- Enveloped virus, with TWO envelopes!
  - The outer envelope is present only in the extracellular state
  - The outer surface or the core membrane, which surrounds the core of the virus, contains lipids and proteins
- **"Fun" facts –**
  - Before vaccines, people were inoculated with… pus or scabs of smallpox survivors 🤢
  - In 2003, a librarian in New Mexico opened a book from 1888, and found an envelope in the book. The book's owner, a doctor, decided to save these "very useful" smallpox scabs in that envelope...
  - In 1796, Edward Jenner invented the worlds first vaccine – against smallpox!

Viruses and cancer - oncoviruses

- Epstein-Barr Virus (EBV)
  - Strong association with Burkitt's lymphoma, Hodgkin's lymphoma, PTLD, Nasopharyngeal carcinoma
  - Likely because the integration of the virus disrupts the genome at some crucial locations
- Hepatitis B Virus (HBV), Hepatitis C Virus (HCV)
  - Strong association with liver cirrhosis and hepatocarcinoma
  - More likely a combination of genetic factors and immunological factors, e.g. chronic inflammation of the liver when virus reactivates repeatedly
- Human Papillomavirus (HPV)
  - Cervical, anal, penile, vaginal, oropharyngeal cancer
  - Viral protein interferes with cell function; integration causes dysregulation of viral protein production

Case study: HIV

*CCR5 reception mutation



*New viral RNA and proteins assemble, then break out of the cell

*The genetic material of the virus encodes for many proteins necessary for the virus to survive and replicate

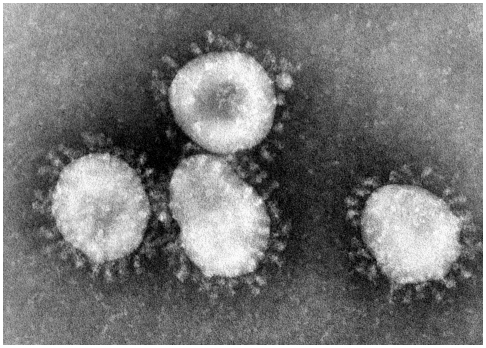
*HIV has reverse transcriptase

*Not all viruses integrate into the genome – integrase needed

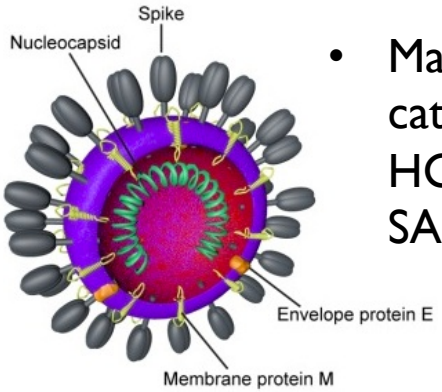
*New viral RNA is transcribed from the provirus, by host polymerases!

*Followed by translation into viral proteins

Case study: Coronavirus



EM image; By CDC/Dr. Fred Murphy

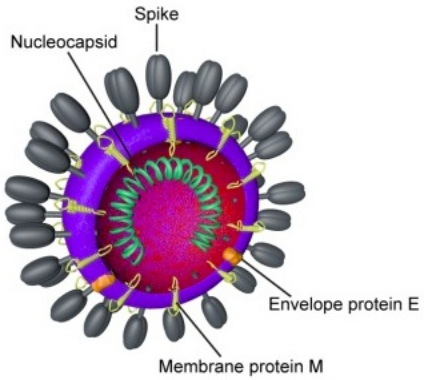


Schematic; By Belouzard, et al - https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3397359

- **Enveloped, +sense, ssRNA**
- The viral genome is 26–32 kb
- Surface has large (~20 nm) projections ("peplomers"/"spikes")
- Generally infect humans and birds (avian)

- Many viruses fall under this category – common cold-causing HCoV-229E; SARS-CoV; MERS; SARS-CoV-2

Case study: Coronavirus



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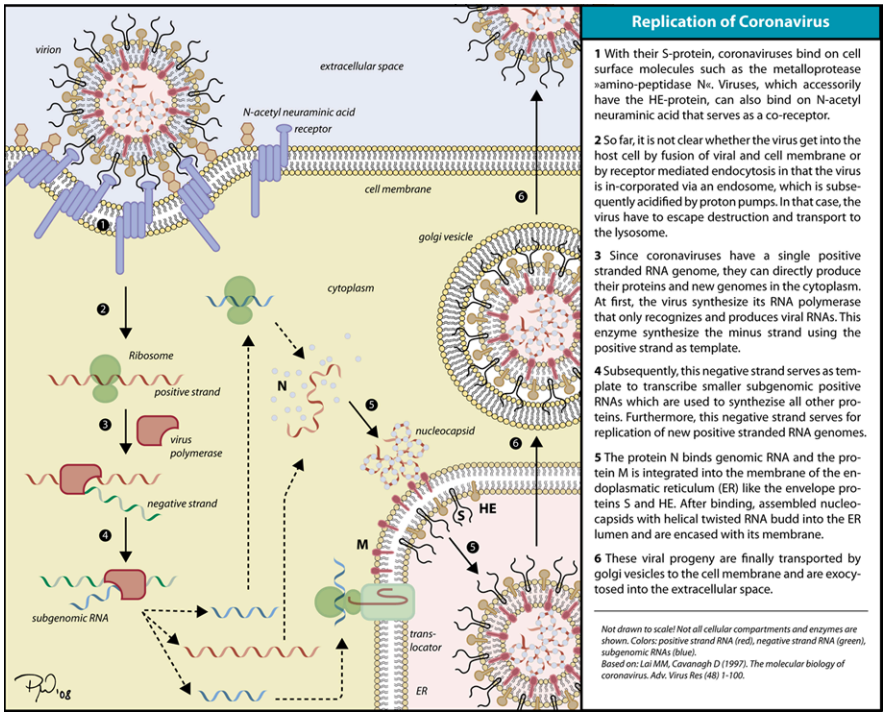
- FIVE key proteins are made:
  - S – spike
  - E – small envelope
  - M – membrane
  - N – nucleocapsid
  - *HE – hemagglutinin-esterase (only some subtypes have; it is a spike-like protein)
- The genome also makes some non-structural proteins, e.g.
  - RdRp – RNA-dependent RNA polymerase

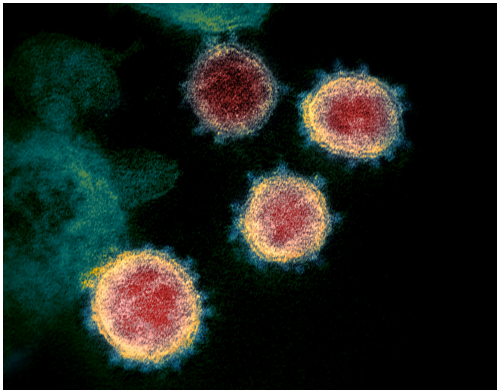
S protein has a domain that facilitates cellular entry by binding with cellular receptors

Case study: Coronavirus



By Crenim at English Wikipedia, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=26529404

Case study: SARS-CoV and SARS-CoV-2



SARS-nCoV-2 EM image; By NIAID Rocky Mountain Laboratories (RML), U.S. NIH

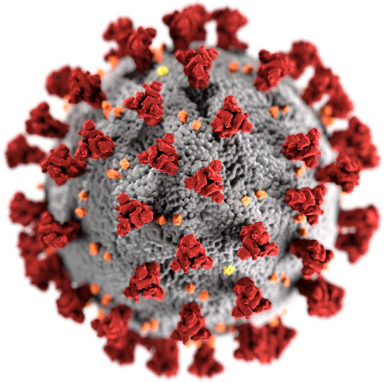
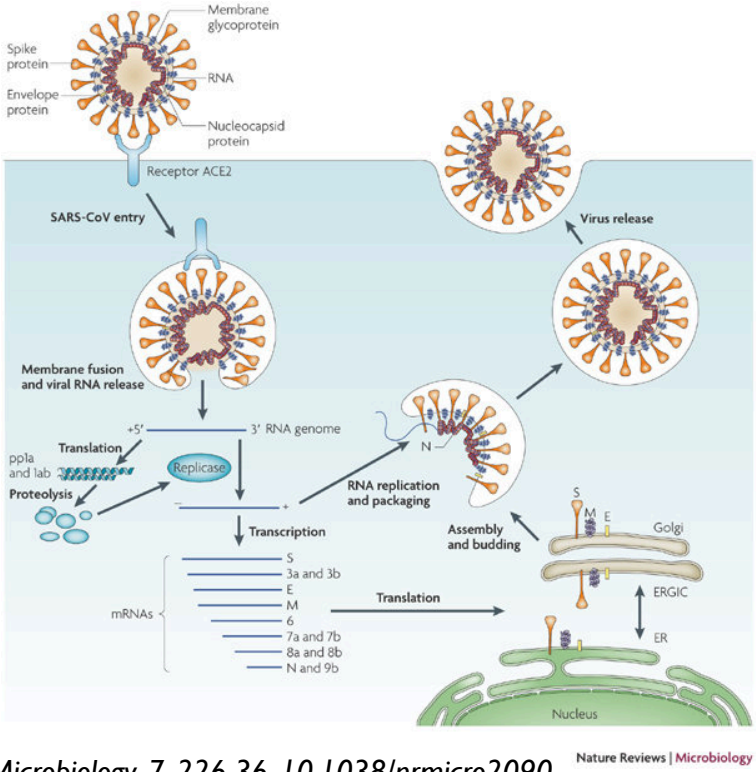


Illustration of SARS-nCoV-2 virion; By CDC/ Alissa Eckert, MS; Dan Higgins, MAM

Side note: COVID-19 is the name of the disease caused by the SARS-CoV-2 virus

- CoV-2 has 96% sequence similarity to a bat coronavirus; widely suspected to originate from bats
- Primary receptor for both SARS-CoV and SARS-CoV-2 is angiotensin-converting enzyme 2 (ACE2)
- ACE2 is found in: lung, gastrointestinal tract, heart, kidneys

Case study: SARS-CoV and SARS-CoV-2



Du et al., Nature reviews. Microbiology. 7. 226-36. 10.1038/nrmicro2090.

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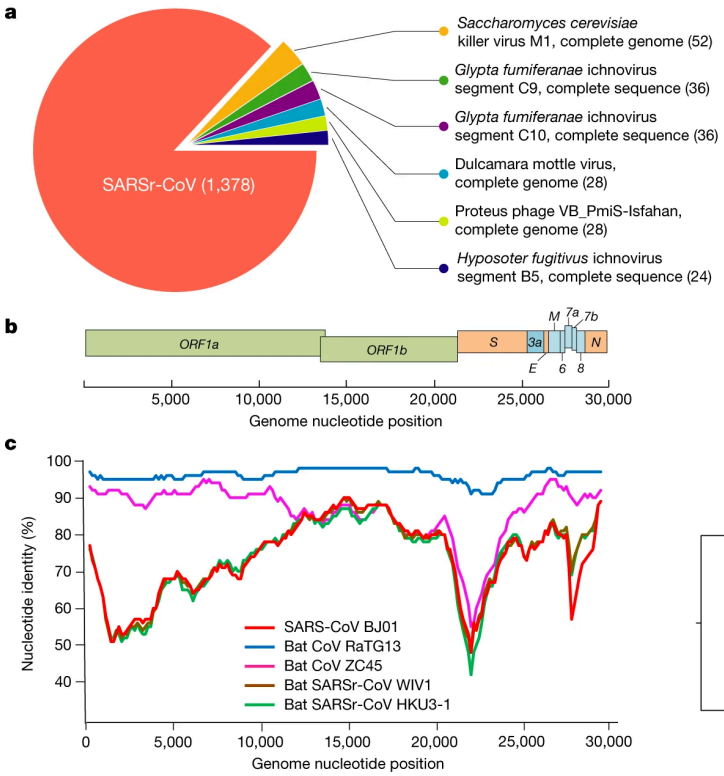
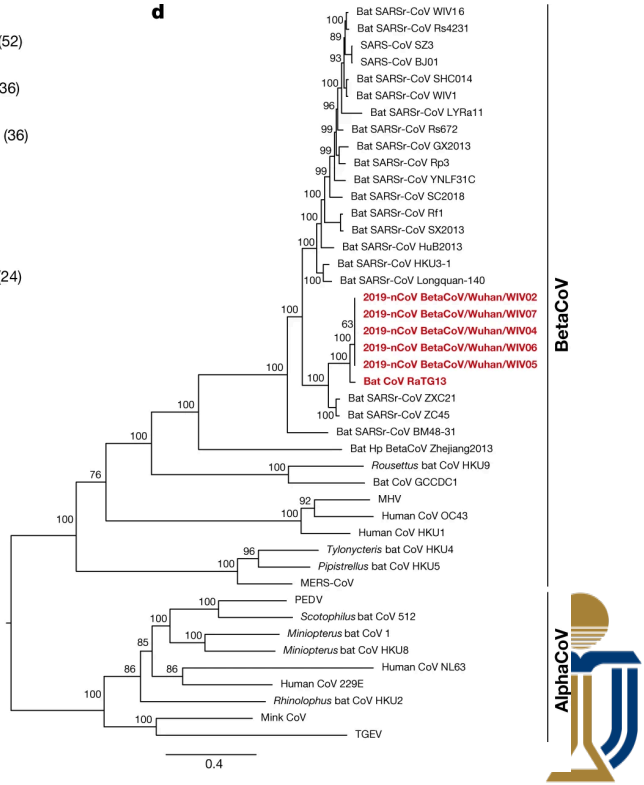




Further reading

- *SARS-Coronavirus* ancestor's foot-prints in South-East Asian bat colonies and the refuge theory: https://www.sciencedirect.com/science/article/pii/S1567134811002346?via%3Dihub
- Evolutionary Relationships between Bat Coronaviruses and Their Hosts: https://wwwnc.cdc.gov/eid/article/13/10/07-0448_article
- Good article for lay-person: Bats Carry Many Viruses. So Why Don't They Get Sick? https://www.npr.org/sections/goatsandsoda/2020/02/09/803543244/bats-carry-many-viruses-so-why-dont-they-get-sick
- SARS-CoV-2 sequence similarity to bat coronavirus: https://www.nature.com/articles/s41586-020-2012-7
- Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding: https://www.sciencedirect.com/science/article/pii/S0140673620302518?via%3Dihub
- Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses: https://www.nature.com/articles/s41564-020-0688-y
- Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation: https://science.sciencemag.org/content/early/2020/02/19/science.abb2507

Treatments

- Anti-virals
  - Target before entry
  - Target replication/transcription
  - Target viral particle assembly
- Stimulate the immune system
  - Interferons – they call over immune cells to attack, eat, and kill virus infected cells
  - Antibodies – neutralize the viral particles that get released, so that it doesn't infect more cells, and maybe helps with being less contagious, and also signals for immune cells to destroy the viruses (by eating it)
- Resistance to treatment
  - Rate of viral evolution: some viruses get one or more points mutations per genome per round of replication!

Discussion

- What conditions favor the inactivation of this virus? Think about what you can do to prevent infection – why do they work?

- Lopinavir/ritonavir is the current preferred treatment for HIV/AIDS. It is a nucleoside analog.
- Remdesivir is a drug being developed and tested for Ebola and Marburg virus (Gilead). It is also a nucleotide analog.
- Both of these are currently being explored as promising treatments for COVID-19. **Why might these drugs be effective?**

- What are the challenges of diagnosing/detecting this virus?
  - Think about the samples that can be taken…

There will now be a short intermission…

http://stories.barkpost.com/wp-content/uploads/2013/05/sleepingpuppy4.jpg

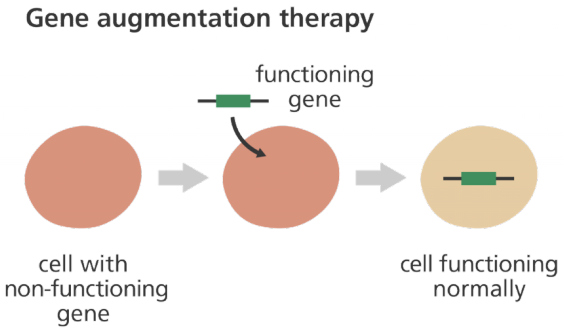
What is gene therapy?

1. Introduce a plasmid into the cell nucleus to replace missing or defective gene (GAT – gene augmentation therapy)
2. Introduce a plasmid into the cell nucleus to provide a new, beneficial protein (e.g. cancer-specific antibodies)
3. Inactivate or knock down a mutated gene by RNA interference
4. Replace the defective gene by genome editing

Remember that modifications to the genome can be either:
HERITABLE, if changes are made to the germline cells (sperm/egg); or
NOT INHERITABLE, if changes are made only to somatic cells

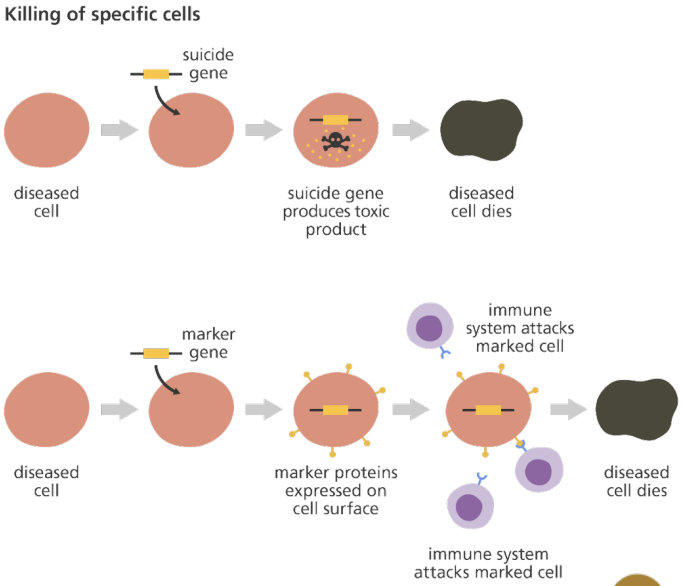
Gene augmentation: Fixing a defective gene

- We discussed how plasmids can be introduced into a cell
- Plasmid → mRNA → protein
- The goal is to have the new gene consistently being expressed, so:
  - Plasmid has to make it to the nucleus
  - Plasmid must contain necessary components to transcribe into mRNA
  - Plasmid needs to be replicated when the cell divides! (origin of replication)



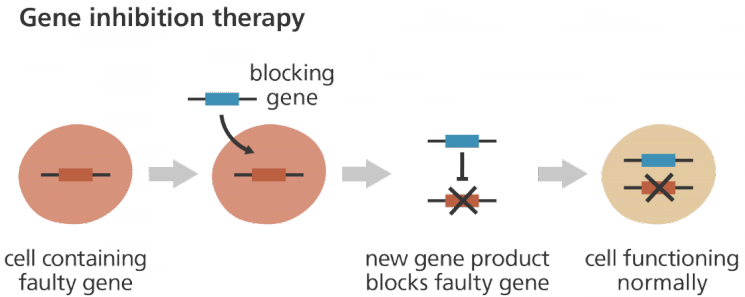
Gene augmentation: Targeted cell killing

- Adding genes that encode for toxins or "suicide" protein
- Adding genes that make the expressing cell more sensitive to a specific drug
- Adding genes that get expressed at the cell surface and induce immune response to kill the target cell

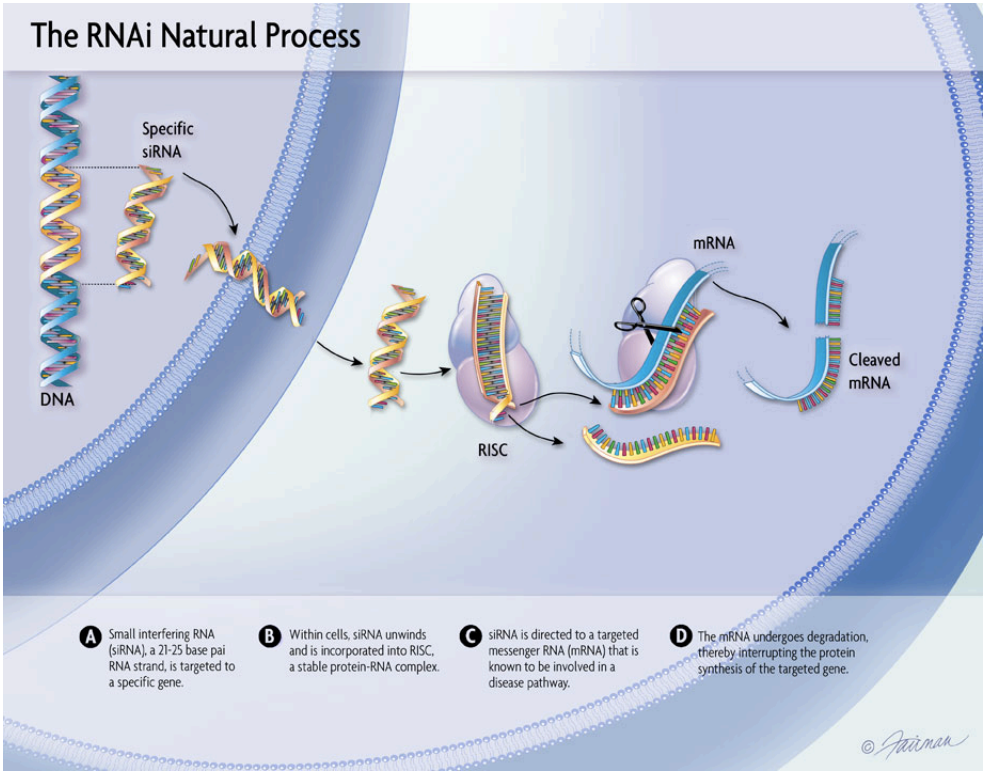


Inhibition of gene expression

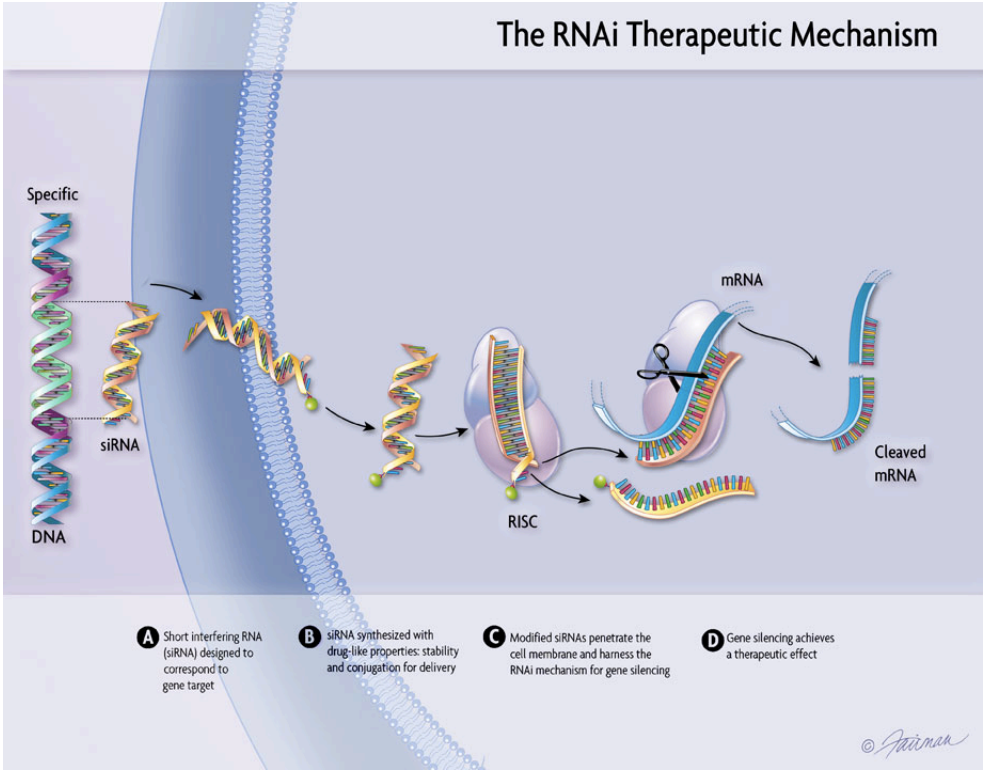
- New gene makes a protein that blocks/inhibits, or breaks down the faulty gene
- New gene makes small interfering RNA (siRNA) that causes the target mRNA to be degraded (RNA interference, or RNAi)



RNA interference (RNAi)

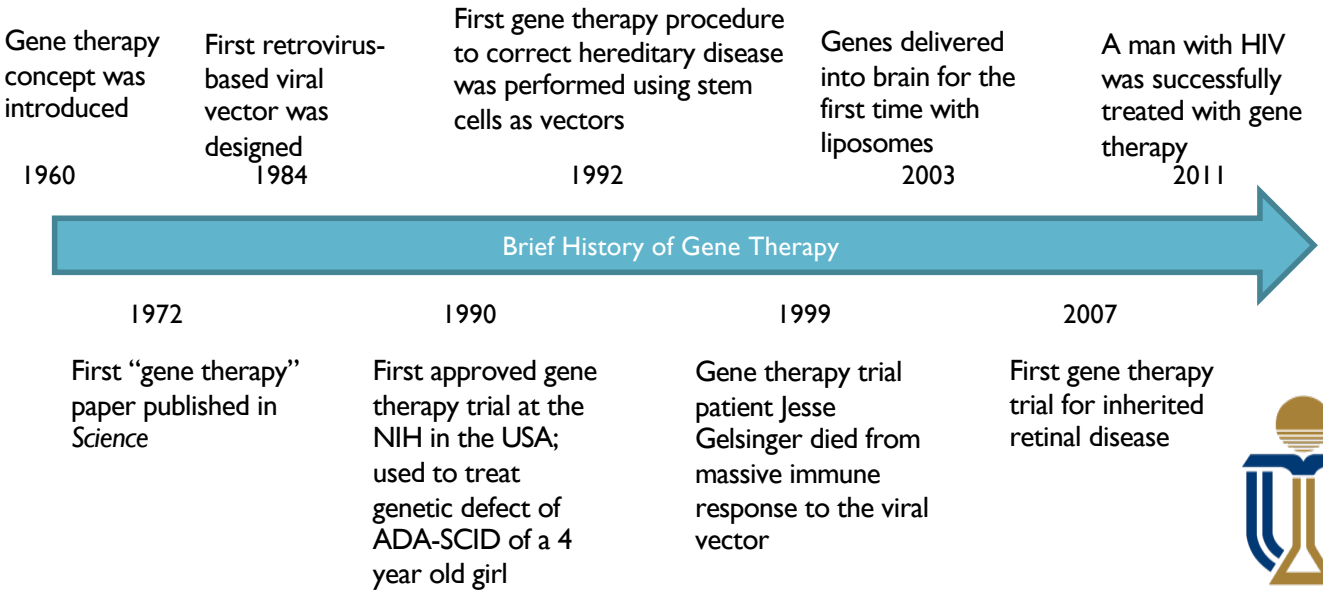


RNA interference (RNAi)



A brief history of gene therapy

- If gene therapy is so promising, and we have molecular biology tools to apply it, why is it not more prominently used today?



The bubble girl (1990)

This is David Vetter, who also had SCID. He wore a special 'spacesuit' to protect him from infections. Image credit: NASA Johnson Space Center

- Ashanthi, a 4 y.o. girl, with ADA-SCID
  - A form of severe combined immuno-deficiency caused by lack of adenosine deaminase (ADA) enzyme
  - Body cannot make any white cells
- A good target for gene therapy:
  - Effects of the disease are reversible
  - Disease results from loss of function of a single gene
  - ADA levels vary widely in the normal population so tight control of the introduced gene is not important
  - *ADA* gene is very small and easy to manipulate
  - Target cells are lymphocytes which are accessible, easy to grow and easy to put back into the body of a patient
  - Alternative treatments hazardous/non-existent (no marrow donor)

The bubble girl (1990)

- Again using viral vector as delivery
- Ex-vivo procedure
- Gene therapy on Ashanthi was initially successful:
  - Within six months her white blood cell count had risen to normal levels, and over the next two years she continued to improve
  - During trial, she continued receiving ADA supplement to ensure safety, which diminished significance of gene therapy result
  - When ADA supplement was discontinued briefly, her symptoms returned
- Since 2002, new methodology for performing this same treatment was developed and trial patients have seen success
  - Introduced procedure to partially ablate patient's own marrow

Jesse Gelsinger (1999)

- Gelsinger suffered from ornithine transcarbamylase deficiency (OTCD), a genetic disease of the liver
  - Liver cannot metabolize ammonia (byproduct of protein breakdown)
  - Usually fatal at birth, but Gelsinger had a less severe version – some of his cells were normal, enabling him to survive on a restricted diet and special medications
- The gene therapy was delivered using adenoviral vector (AAV), directly injected (in-vivo)
- He died 4 days later from multiple organ failure and brain death, as a massive immune response was triggered by the AAV

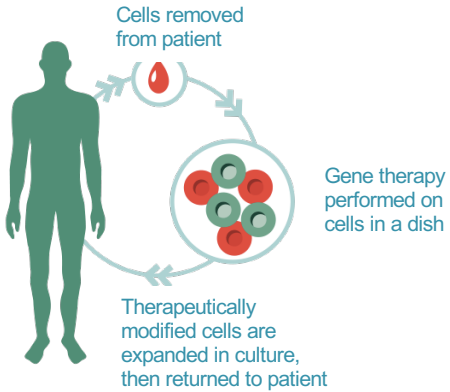
Delivery of therapeutic genes

- https://youtu.be/Ez560GnkSrE
- What?
  - What are the things that need to be delivered? (Single plasmid? Multiple plasmids? Viral vector? RNA? Protein?)
  - What are the cell type(s) it needs to target?
- Where?
  - Where in the body should it be targeted?
  - Where should it absolutely NOT go?
  - Where should the procedure take place, inside or outside the body?
- How?
  - How to deliver the payload? Viral? Non-viral?
  - How to introduce the vector? Injection? Cream? Incubation?

Delivery approaches

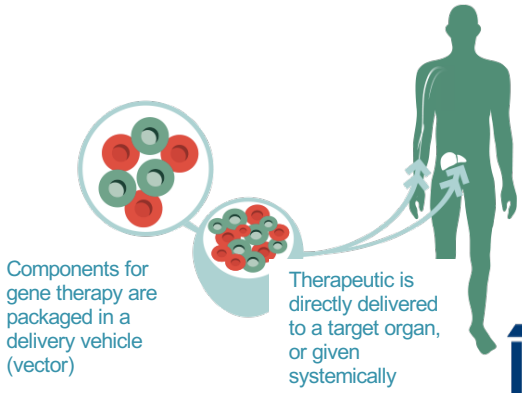
Ex-vivo delivery

- Target cells treated **outside** body
- Reduces safety risk; can screen for tumorigenic cells before giving to patient; but cannot be applied for many cell types



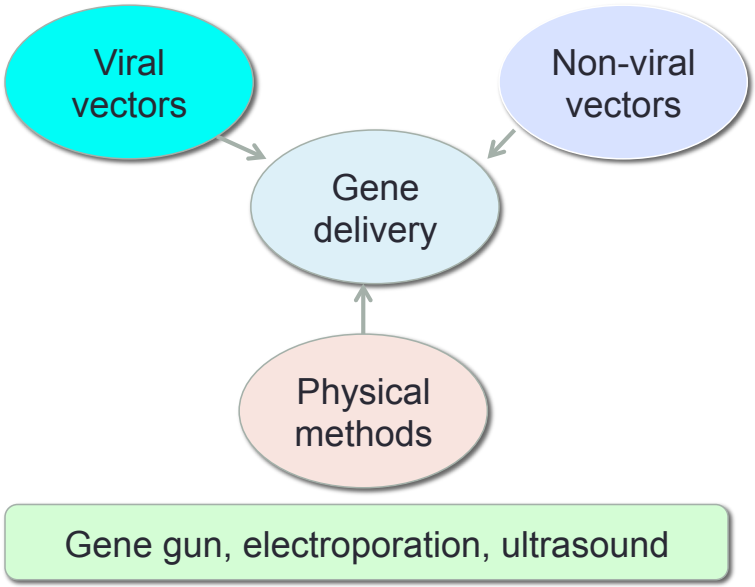
In-vivo delivery

- Target cells treated **inside** body
- Useful if target cells are hard or impossible to culture (e.g. brain); but cell-specific targeting is hard

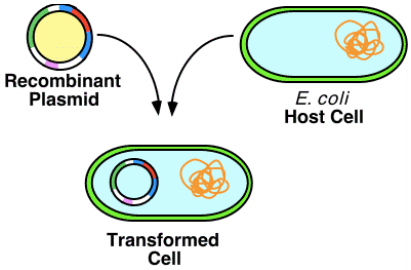


Case study – Macular degeneration CRISPR trial, first in-vivo

Delivery approaches



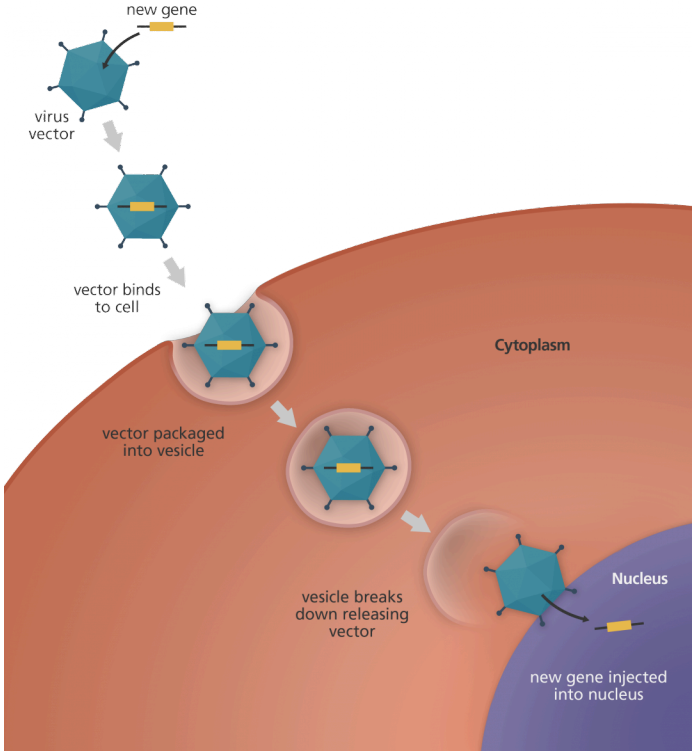
Delivery vectors



- Criteria of delivery vectors:
  - Target the right cells
  - Able to transfer and integrate genes into cells
  - Minimal harmful side effects
- Examples of types of vectors:

Adenovirus	Naked/plasmid DNA (gene gun)
Retrovirus	Lipid complex
Vaccinia virus	Liposomes
Poxvirus	Peptides/proteins
Adeno-associated virus	Polymers
Herpes simplex virus	Other non-viral vehicles
Lentivirus	

Viral vectors



Choosing a viral vector

Table 1 | **The main groups of viral vectors**

Vector	Genetic material	Packaging capacity	Tropism	Inflammatory potential	Vector genome forms	Main limitations	Main advantages
Enveloped							
Retrovirus	RNA	8 kb	Dividing cells only	Low	Integrated	Only transduces dividing cells; integration might induce oncogenesis in some applications	Persistent gene transfer in dividing cells
Lentivirus	RNA	8 kb	Broad	Low	Integrated	Integration might induce oncogenesis in some applications	Persistent gene transfer in most tissues
HSV-1	dsDNA	40 kb* 150 kb‡	Strong for neurons	High	Episomal	Inflammatory; transient transgene expression in cells other than neurons	Large packaging capacity; strong tropism for neurons
Non-enveloped							
AAV	ssDNA	<5 kb	Broad, with the possible exception of haematopoietic cells	Low	Episomal (>90%) Integrated (<10%)	Small packaging capacity	Non-inflammatory; non-pathogenic
Adenovirus	dsDNA	8 kb* 30 kb§	Broad	High	Episomal	Capsid mediates a potent inflammatory response	Extremely efficient transduction of most tissues

*Replication defective. ‡Amplicon. §Helper dependent. AAV, adeno-associated viral vector; dsDNA, double-stranded DNA; HSV-1, herpes simplex virus-1; ssDNA, single-stranded DNA.

Thomas, Ehrhardt & Kay, Nature Reviews Genetics, 2003

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Main application: short term gene expression, for proof of concept studies

Thomas, Ehrhardt & Kay, Nature Reviews Genetics, 2003

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*Replication defective. ‡Amplicon. §Helper dependent. AAV, adeno-asso stranded DNA.

Main application: long term expression of small genes

Note: AAV is not known to cause disease in humans, therefore lower immune risk

Thomas, Ehrhardt & Kay, Nature Reviews Genetics, 2003

Choosing a viral vector

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Main application: long term expression of small and large genes; ex-vivo applications

Note: lentivirus vs retrovirus – dividing cells

Thomas, Ehrhardt & Kay, Nature Reviews Genetics, 2003

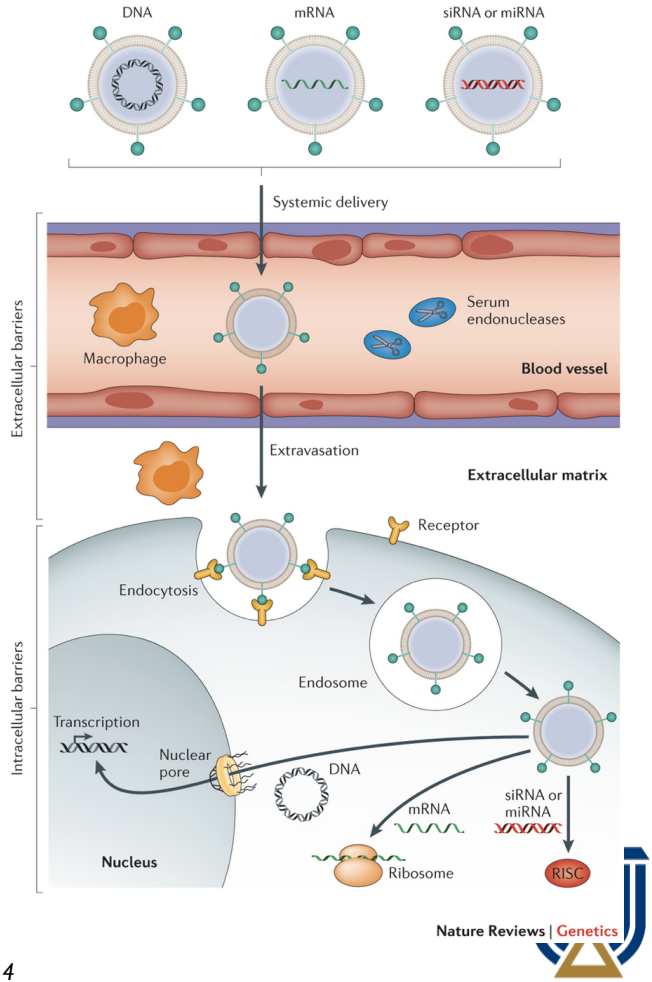
Choosing a viral vector

		Adenovirus	Adeno-associated virus	Alphavirus	Herpesvirus	Retrovirus / Lentivirus	Vaccinia virus
Particle characteristics	Genome	dsDNA	ssDNA	ssRNA (+)	dsDNA	ssRNA (+)	dsDNA
	Capsid	Icosahedral	Icosahedral	Icosahedral	Icosahedral	Icosahedral	Complex
	Coat	Naked	Naked	Enveloped	Enveloped	Enveloped	Enveloped
	Virion polymerase	Negative	Negative	Negative	Negative	Positive	Positive
	Virion diameter	70 - 90 nm	18 - 26 nm	60 - 70 nm	150 - 200nm	80 - 130 nm	170 - 200 X 300 - 450nm
	Genome size	39 - 38 kb	5 kb	12 kb	120 - 200 kb	3 - 9 kb	130 - 280 kb
	Gene Therapy Net .com						
	Family	*Adenoviridae*	*Parvoviridae*	*Togaviridae*	*Herpesviridae*	*Retroviridae*	*Poxviridae*
Gene Therapy Properties	Infection / tropism	Dividing and non-diving cells	Dividing and non-diving cells	Dividing and non-diving cells	Dividing and non-diving cells	Dividing cells*	Dividing and non-diving cells
	Host genome interaction	Non-integrating	Non-integrating*	Non-integrating	Non-integrating	Integrating	Non-integrating
	Transgene expression	Transient	Potential long lasting	Transient	Potential long lasting	Long lasting	Transient
	Packaging capacity	7.5 kb	4.5 kb	7.5 kb	> 30 kb	8 kb	25 kb

http://sgugenetics.pbworks.com/f/1301871554/virus%20classification.jpg

Non-viral vectors

- Non-viral vectors can be used to deliver DNA, mRNA and short double-stranded RNA
  - siRNA and miRNA mimics must be loaded into the RNA-induced silencing complex (RISC)
  - mRNA must bind to the translational machinery
  - DNA has to be further transported to the nucleus to exert its activity

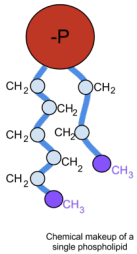


Yin et al., "Non-viral vectors for gene-based delivery", Nature Review Genetics, 2014

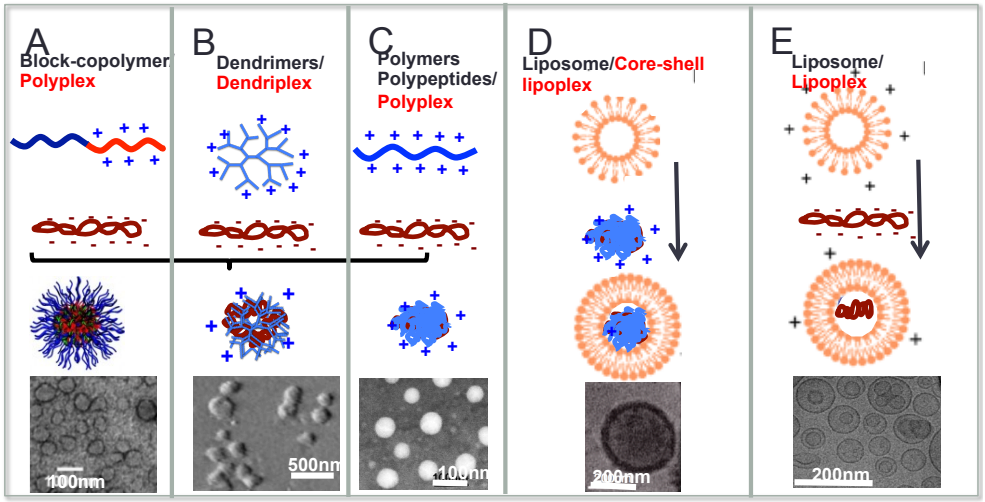
Creation of non-viral vectors

- Non-viral vectors form due to charge interactions
- https://youtu.be/RBjVwlnq3cA?t=10s
- https://youtu.be/04SP8Tw3htE?t=2m10s

A phospholipid with a hydrophilic head and a hydrophobic tail



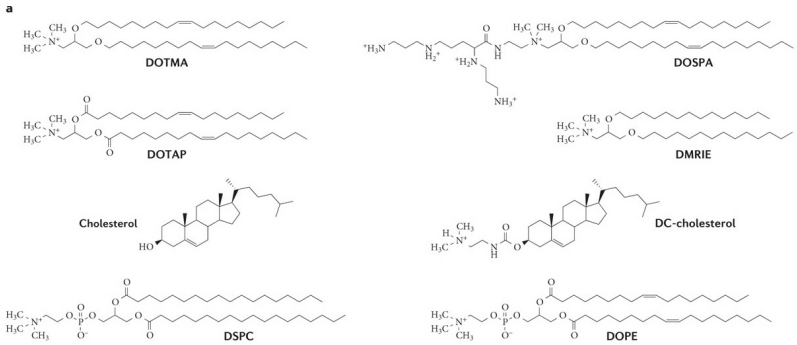
Chemical makeup of a single phospholipid

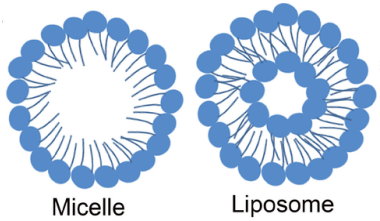


Lipoplex; liposome; lipid nanoparticle; micelle

Lipid-based vectors

- Lipid-based vectors are among the most widely used non-viral gene carriers.
- Limitations of cationic lipids include low efficacy (poor stability and rapid clearance), and tendency to generate inflammatory or anti-inflammatory responses





Yin et al., "Non-viral vectors for gene-based delivery", Nature Review Genetics, 2014
Wang et al, "Lipid Nanoparticles for Ocular Gene Delivery", J. Funct. Biomater. 2015

Polymeric vectors

- Cationic polymers are attractive due to their immense chemical diversity and potential for functionalization

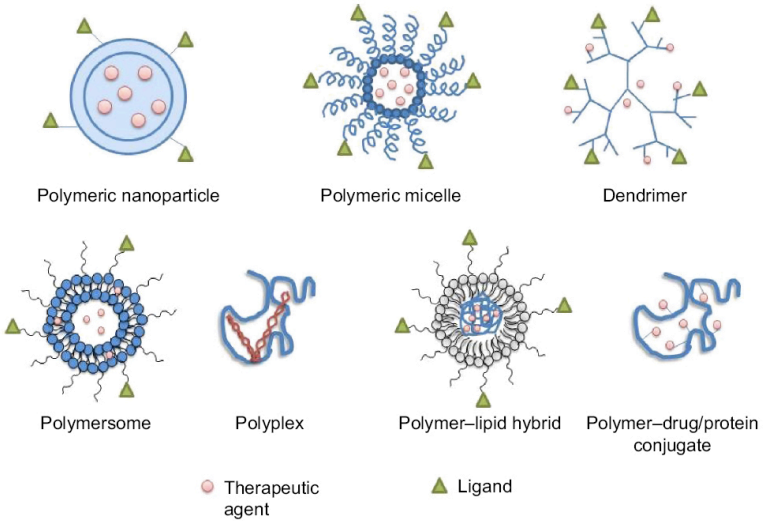
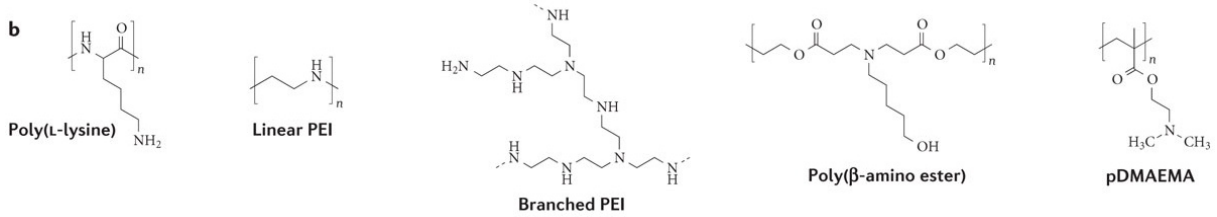


Figure 1 Schematic illustration of polymeric nanoparticle platforms.
Note: Blue color represents the polymeric platform.

Prabhu et al. "Polymeric nanoparticles for targeted treatment in oncology: current insights", Intl J Nanomedicine, 2014

Polymeric vectors

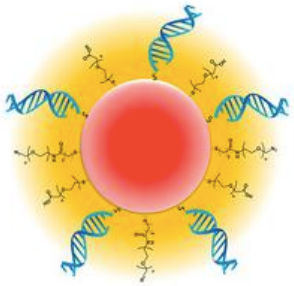
- Early examples of polymeric vectors: poly(L-lysine) (PLL) and polyethylenimine (PEI) – PEI and its variants are among the most studied polymeric materials for gene delivery
- A nitrogen atom at every third position along the polymer means PEI has a high charge density at reduced pH, which seems to aid in condensation of DNA and endosomal escape
- PEI can actually induce cytotoxic, so requires chemical modifications to improve biocompatibility and biostability



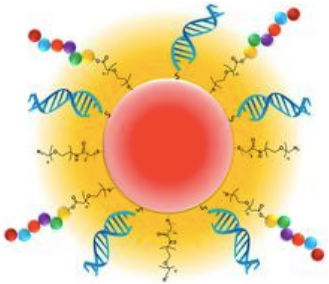
Yin et al., "Non-viral vectors for gene-based delivery", Nature Review Genetics, 2014

Inorganic and mechanical delivery

- Gold nanoparticles/nanoshells
  - Au-S bond covalently linked nucleic acids - cargo can be released from the particle by light-inducible mechanisms (e.g. pulse laser)
- Direct injection of naked DNA plasmid into the cell/tissue
- Electroporation
  - Uses short pulses of high voltage to temporarily form pores in the cell membrane so DNA can pass through



Au-siRNA



Au-Tat-siRNA

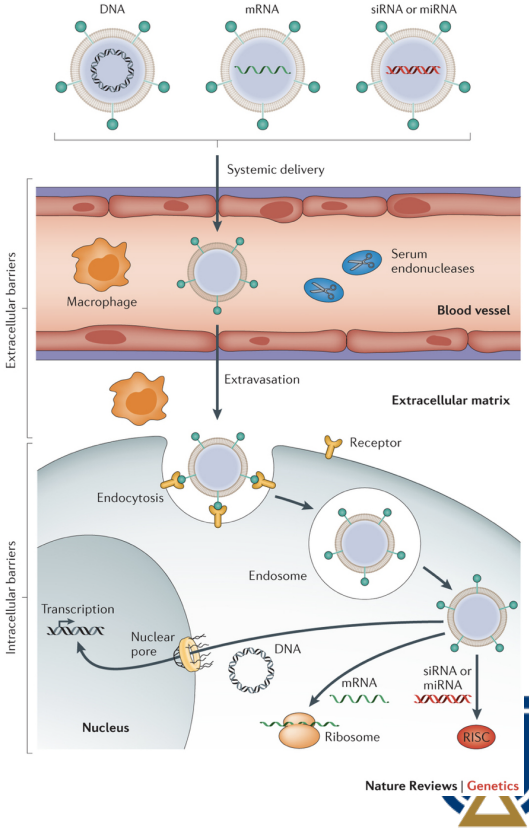
Child et al. "Gold Nanoparticle-siRNA Mediated Oncogene Knockdown at RNA and Protein level, with associated Gene effects", Nanomedicine (Lond.), 2015

Inorganic and mechanical delivery

- Gene gun
  - DNA is coated onto gold particles and loaded into a device which generates a force to achieve penetration of the DNA into the cells
- Sonoporation
  - Uses ultrasound to deliver DNA into cells. The process of acoustic cavitation is thought to disrupt the cell membrane and allow DNA to move into cells
- Hydrodynamic delivery
  - Rapid injection of a high volume of a solution containing DNA/RNA into vasculature; elevated hydrostatic pressure helps molecules enter the cell

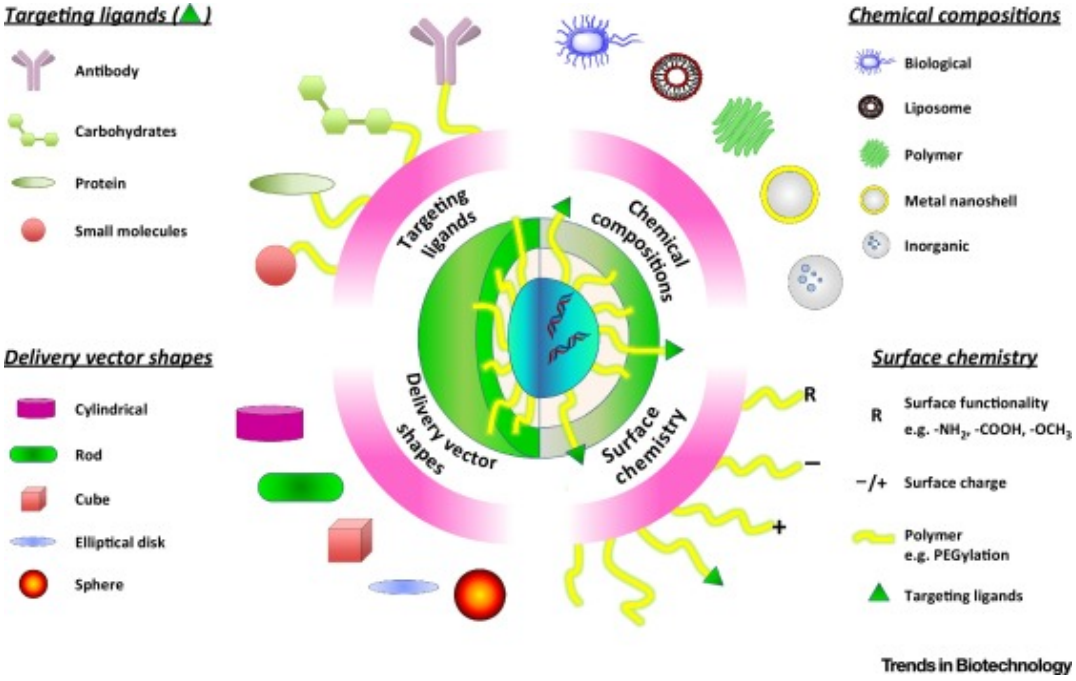
Designing non-viral vectors

- To survive from outside to cell target, non-viral vectors need to:
  - Avoid degradation by serum endonucleases and evade immune detection, e.g. by chemical modifications of nucleic acids/encapsulation of vectors
  - Avoid renal clearance from the blood and prevent nonspecific interactions, e.g. using polyethylene glycol (PEG) or through specific characteristics of particles
  - Extravasate from bloodstream to target tissues, e.g. by using certain characteristics of particles and specific ligands
  - Mediate cell entry and endosomal escape, e.g. by specific ligands and key components of carriers



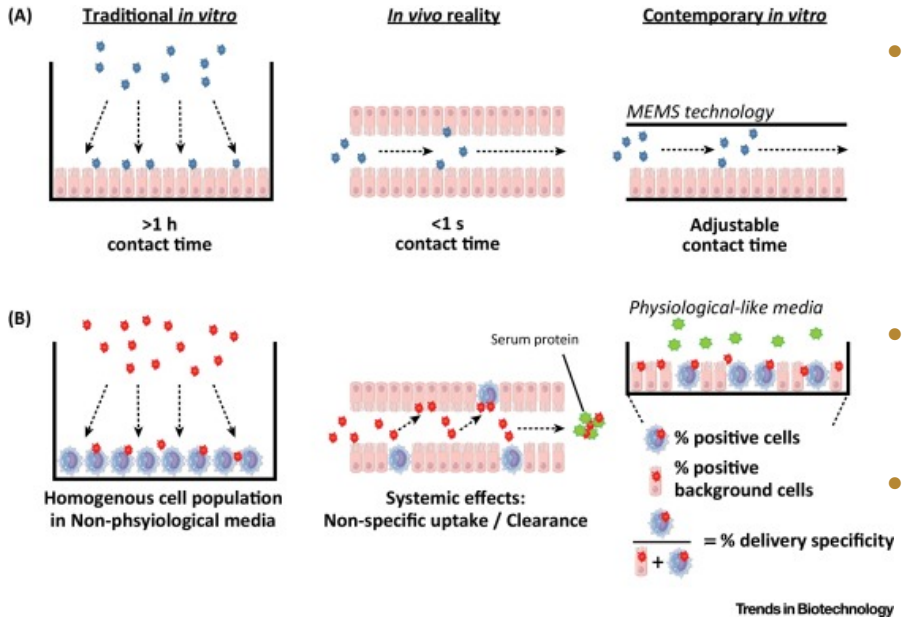
Yin et al., "Non-viral vectors for gene-based delivery", Nature Review Genetics, 2014

Designing non-viral vectors



Hill et al. "Overcoming Gene-Delivery Hurdles: Physiological Considerations for Nonviral Vectors", Trends in Biotechnology, 2015

Challenges in designing non-viral vectors



- Balancing protecting vs. releasing the cargo
- Endosome escape
- Nuclear entry (DNA)

Hill et al. "Overcoming Gene-Delivery Hurdles: Physiological Considerations for Nonviral Vectors", Trends in Biotechnology, 2015

Pros and cons of viral vs. non-viral vectors

Viral vectors

Pros

1. They are very efficient, and the rate of successful gene expression is very high
2. Naturally, we can select viruses to target specific cell-types

Cons

1. Size of cargo is restricted
2. They can cause immune response in patients which reduce treatment effectiveness, or worst case case death
3. Integration mechanism could cause mutations/cancer

Non-viral vectors

Pros

1. Low immune risk
2. No cargo size limitation
3. Can design intelligently according to needs
4. More cost-effective and available because they are easier to make

Cons

1. Efficiency is much lower than viral systems
2. Difficult to design parameters precisely/accurately and difficult to model in-vivo dynamics