Viruses

If you had DNA and RNA but could not make more copies of it

and

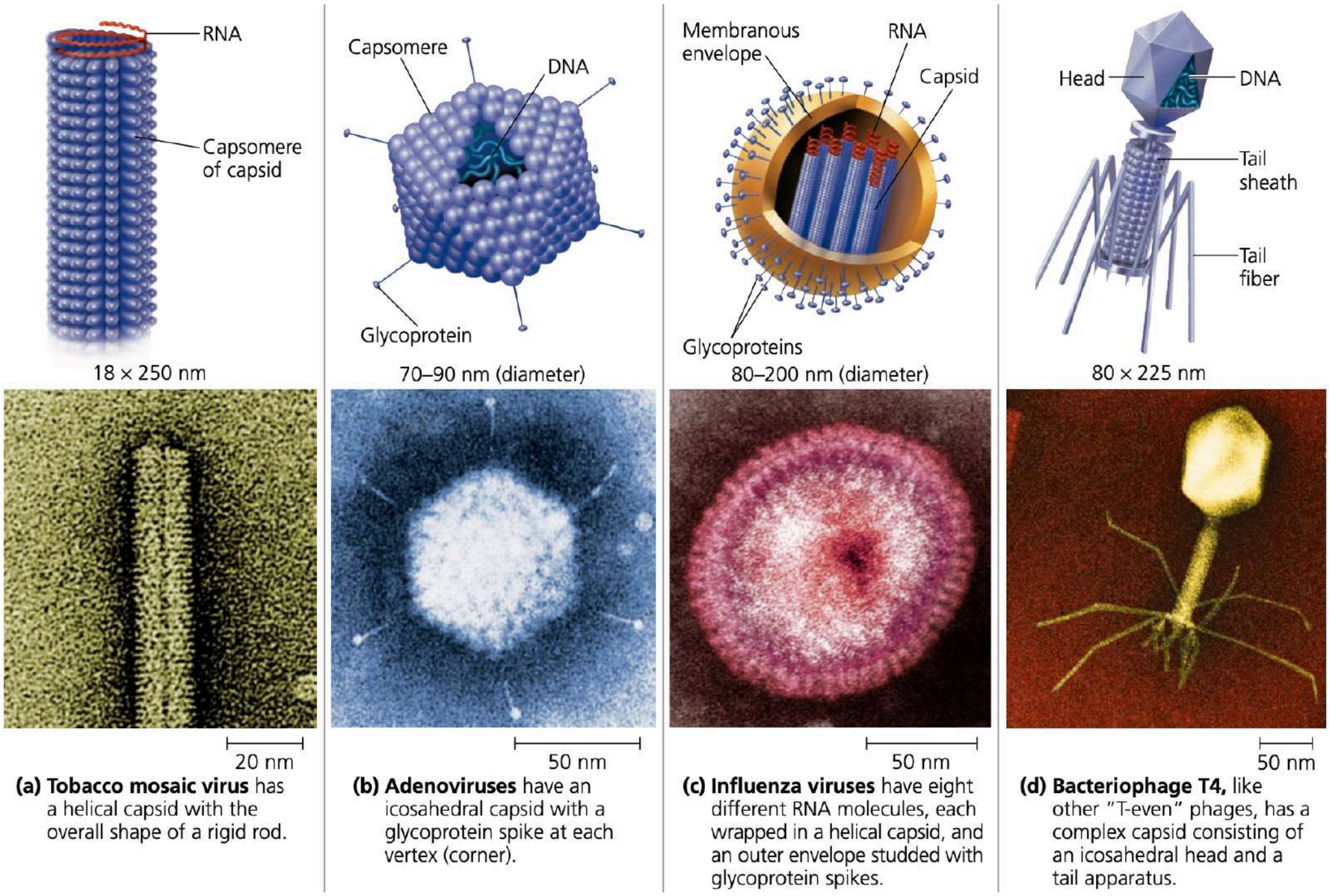
if your DNA and RNA codes for proteins

but

you cannot synthesize these encoded proteins

Are you “alive”?

Viruses



(a) Tobacco mosaic virus has a helical capsid with the overall shape of a rigid rod.

(b) Adenoviruses have an icosahedral capsid with a glycoprotein spike at each vertex (corner).

(c) Influenza viruses have eight different RNA molecules, each wrapped in a helical capsid, and an outer envelope studded with glycoprotein spikes.

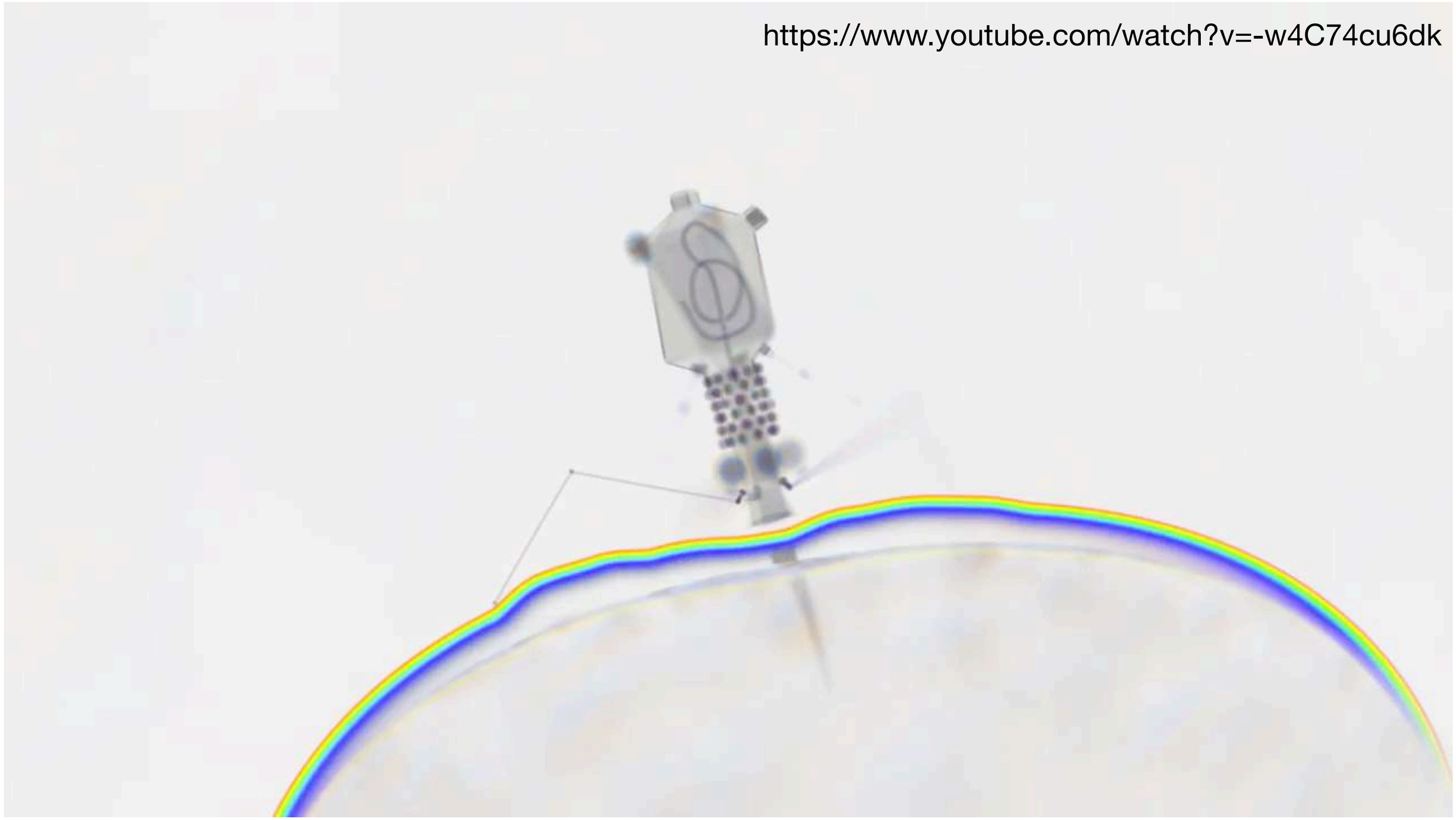
(d) Bacteriophage T4, like other "T-even" phages, has a complex capsid consisting of an icosahedral head and a tail apparatus.

- Viruses contain genetic information as DNA or RNA, which is made into proteins by the machinery of the host cell
- If viruses require a host cell to come into existence, which came first, the virus or the host cell?
- Viruses infect ALL life forms: bacteria, plants, animals

Figure 19.3 of Campbell's Biology: a global approach

Viruses

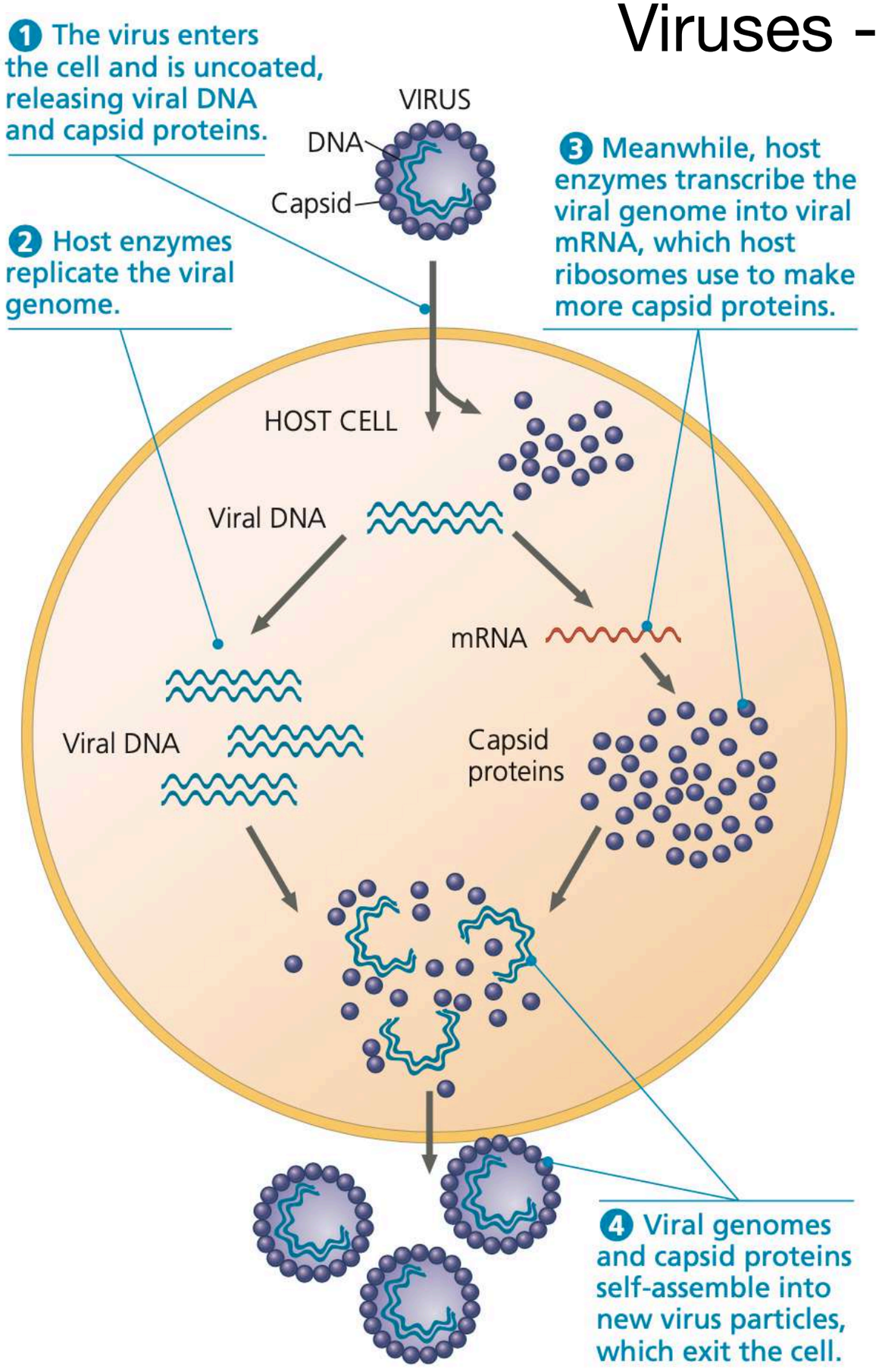
https://www.youtube.com/watch?v=-w4C74cu6dk



The video above is a schematised movie of the infection and lysis process. It is depicted in in a very broad manner to convey the general idea

This same process happens when a virus infects any host cell, regardless of whether it is a bacterial, plant or human cell

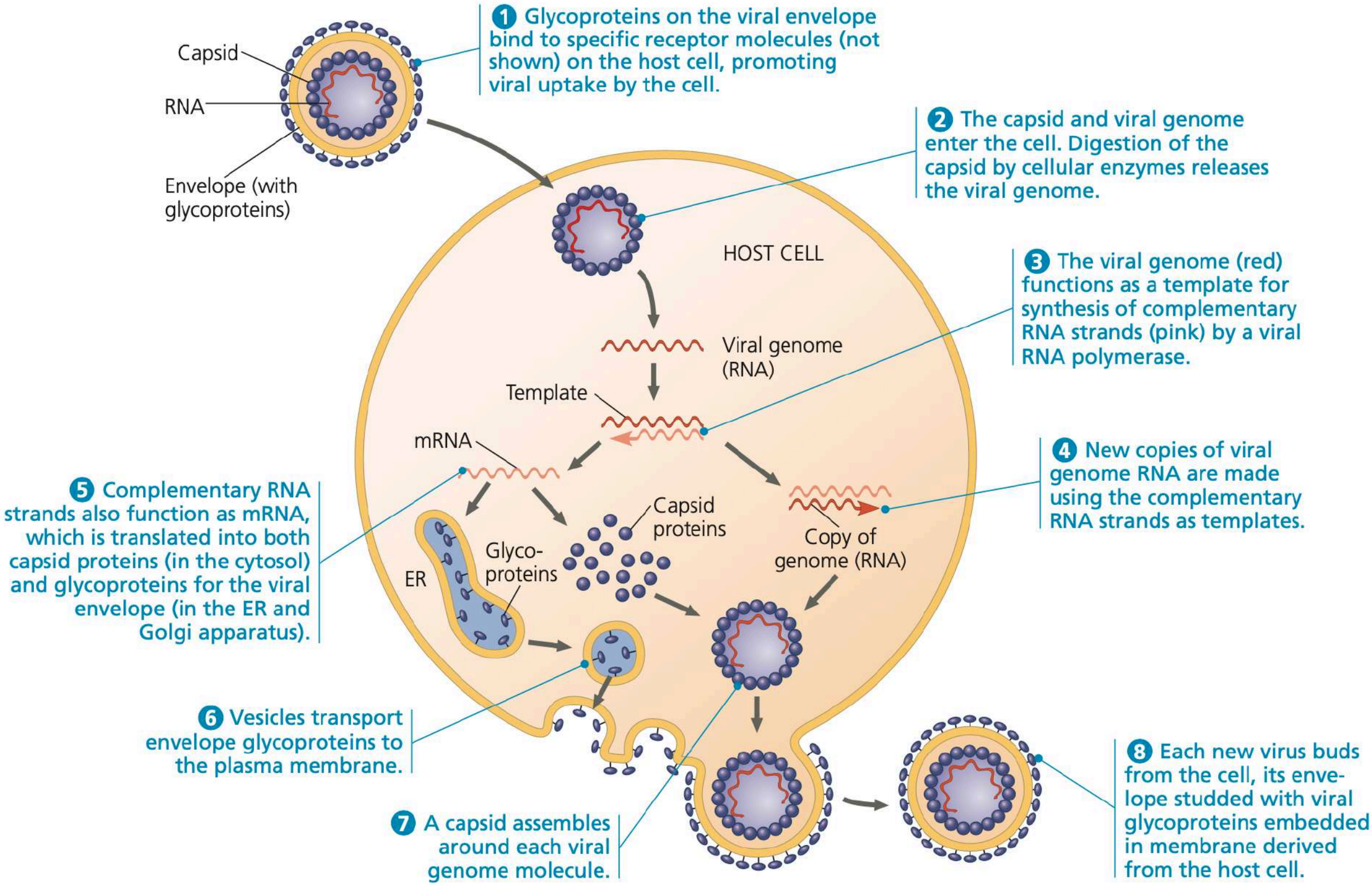
Viruses - no envelope



- To make more copies of itself, a virus particle known as a virion, has to enter a cell in which the machinery for genetic material replication and protein synthesis are functional.
- After it "hijacks" the host machinery and duplicates its genetic material, it uses the host machinery to package its genetic material.
- Then it ruptures the host cell and escapes into the host body, to find other cells into which it will enter and repeat the whole process.
- Viruses infect all life forms, but each type of virus can infect only a specific species of animal or a cell type in that animal.
- Excessive intrusion of humans into wild-life environments has caused an increase in the ability of viruses to cross species barriers.

Figure 19.4 of Campbell's Biology: a global approach

Viruses - with envelope



Genetic information in viruses can be DNA or RNA
DNA or RNA can be double or single stranded

Figure 19.7 of Campbell's Biology: a global approach

Viruses - vaccination

Prior infection with a milder variant provides immunity against subsequent exposure to more virulent strains

- Vacca = latin for cow
- Dr. Edward Jenner formally demonstrated that prior inoculation with cowpox provided protection from smallpox
- WHO declared smallpox as eradicated in 1980

Viruses - vaccination

- Louis Pasteur (1822–1895) initially showed attenuated pathogens provide protection against Cholera and Anthrax (bacteria) for farm animals

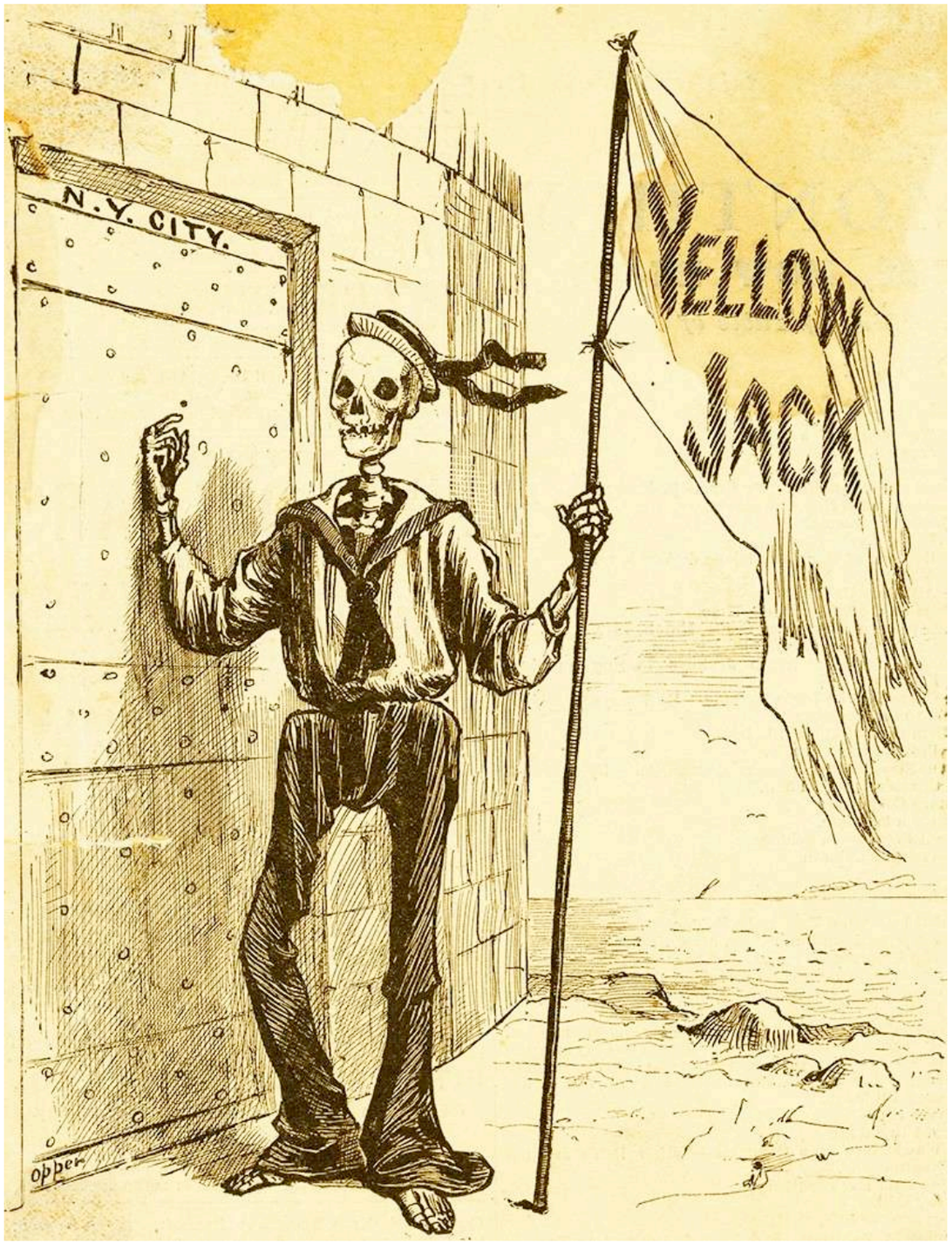
- In 1879, Pierre-Victor Galtier, a veterinary professor, serially transmitted rabies to rabbits from dogs
- ~1885, Pasteur used suspensions made from rabies-infected dried rabbit spinal cords to show that dogs could be protected from rabies infection

- Pasteur`s rabies strategy was post-exposure vaccine prophylaxis , still used today

- Pasteur found the strategy to vaccinate without ever isolating the causative agent, which would eventually be recognized in the twentieth century to be nonbacterial

Because rabies was 100% fatal, Pasteur went ahead and “vaccinated” humans bitten by rabid dogs without proper animal trials

Viruses - vaccination



- End of 19th century, yellow fever, caused by a flavivirus spread by infected female *Aedes aegypti* mosquitoes, had spread around the world due to slave trading and global markets

- Ships were required to fly the yellow quarantine flag - main symptom is jaundice

- South African virologist Max Theiler at the Harvard University School of Tropical Medicine, USA showed that the disease was viral

- Theiler showed that repeated passage in mouse brain cultures reduced the effect of the virus on most organs, but potentially increased its impact on the central nervous system, which could cause encephalitis

- Individuals could be vaccinated, but with risk of neurotoxicity

Viruses - vaccination

- In 1937, Max Theiler and Hugh Smith developed live attenuated yellow fever vaccine strain 17D: 176 serial passages in mouse embryonic tissue, then monkey serum, then minced whole chick embryo and then in chick embryo from which the brain and spinal cord had been removed.
- After these passages, 17D lost neurotropism, viscerotropism and mosquito competence, but still triggered an immune response in host

- Theiler showed that the Yellow Fever 17D vaccine prepared from infected whole chick embryos was safe and effective for human use without the addition of human serum - made it cost-effective and easier to produce in large quantities

- The Yellow Fever 17D vaccine was approved for human use in 1938 and provides lifelong protection with a single vaccine dose

- In 1951, Theiler was awarded the Nobel Prize in Physiology or Medicine for “discoveries concerning yellow fever and how to combat it”, the first and only time the prize has been awarded for a vaccine

Viruses - vaccination

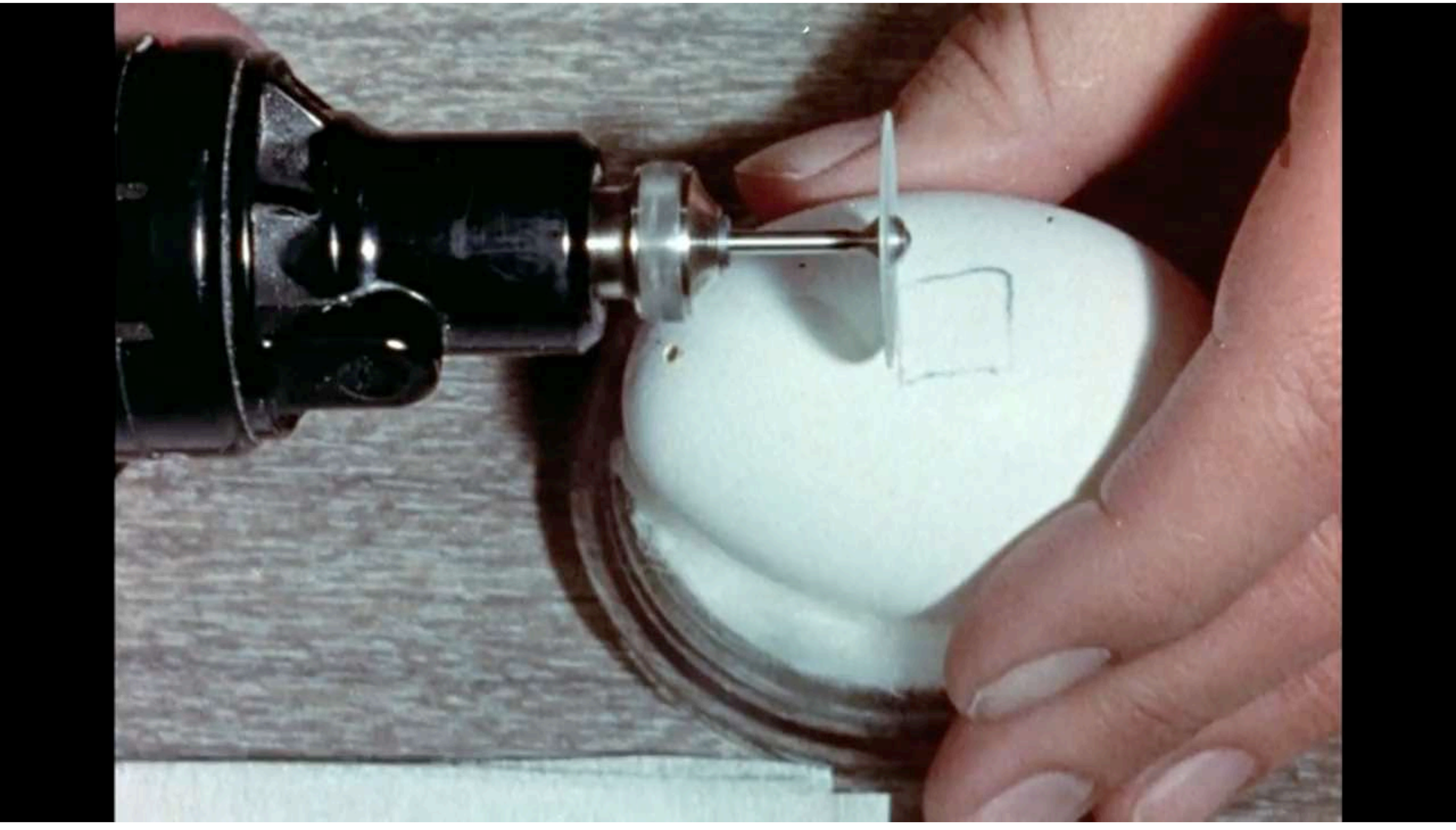
- Viruses are obligate intracellular pathogens - cannot multiply on their own, need a live host cell and its machinery to proliferate

- In the 1900s, live animals and/or animal embryos were used to culture viruses

- Later viruses were grown using animal cell cultures - still the most efficient method

Viruses - vaccination

In 1930s, Ernest Goodpasture, a US pathologist and medic, grew pure viruses in culture by infecting fertilized chicken eggs

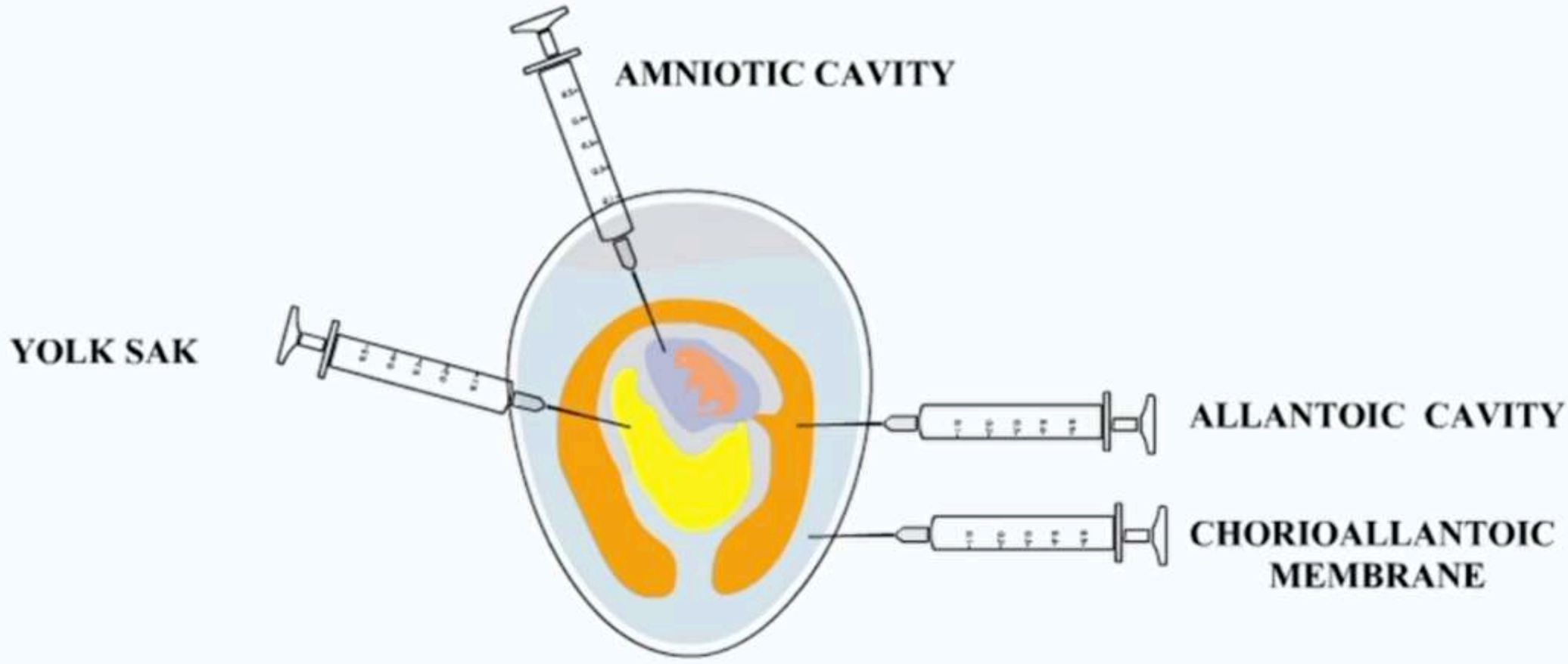


https://www.youtube.com/watch?v=NTUHX1mRfiE

Sreelaja Nair

Viruses - vaccination

Virus inoculation into chicken eggs - live embryo culture of viruses



https://medschool.vanderbilt.edu/md-admissions/an-egg-drill-and-its-connection-with-medical-discovery-and-advancement/

Viruses - vaccination

Poliomyelitis - caused by an enterovirus that in rare cases invades the nervous system and damages motor neurons, causing permanent disability, paralysis or death

In 1908, Karl Landsteiner and Irwin Popper showed that polio was spread by a virus:

- Injected monkeys with a suspension of spinal cord from a polio patient
- The suspension was bacteriologically sterile
- However, following inoculation the monkeys exhibited lesions in the spinal cord similar to those seen in humans with poliomyelitis
- Monkeys also developed paralysis of legs

Polio virus could not be grown in Chicken eggs:

- Albert B. Sabin and Peter K. Olitsky grew poliovirus in fragments of human embryonic brain
- Risk of using this for vaccine production was neurological damage in recipients

Viruses - vaccination

Thomas H. Weller, John F. Enders and Frederick C. Robbins
(1954 Nobel Prize in Physiology or Medicine)

Not for vaccine development, but "for their discovery of the ability of poliomyelitis viruses to grow in cultures of various types of tissue."

- grew the human Lansing strain of human poliomyelitis virus in skin, muscle and connective tissue from human fetal arms and legs
- also produced large quantities of human polio virus in cultures of fetal intestinal tissue
- succeeded in culturing human poliovirus in cells other than neurons

~1950s two polio vaccines developed:

- an injected vaccine containing inactivated virus, originally developed by Jonas Salk
- an oral vaccine containing live attenuated virus developed by Albert Sabin and colleagues

Viruses - vaccination

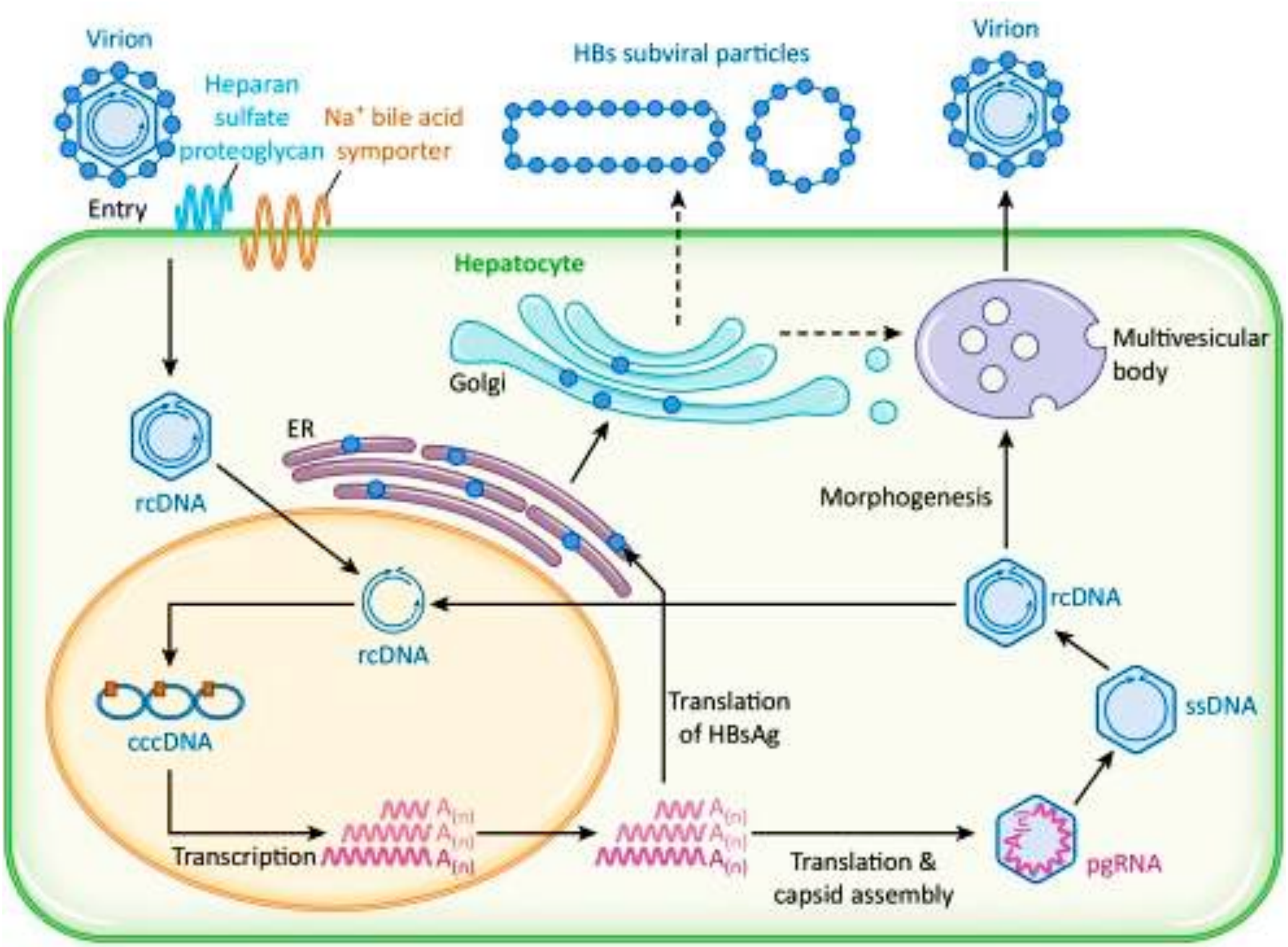
Whole virion, which has “forgotten” its original virulence features is used to trigger an initial immune response

This initial response is remembered by the host body as “immune memory” and stored in a dormant format

The stored, dormant “immune memory” reactivates when a new infection occurs with a virulent strain

Is the whole virion necessary to acquire immunity/ protection against viral infections?

Viruses - vaccination



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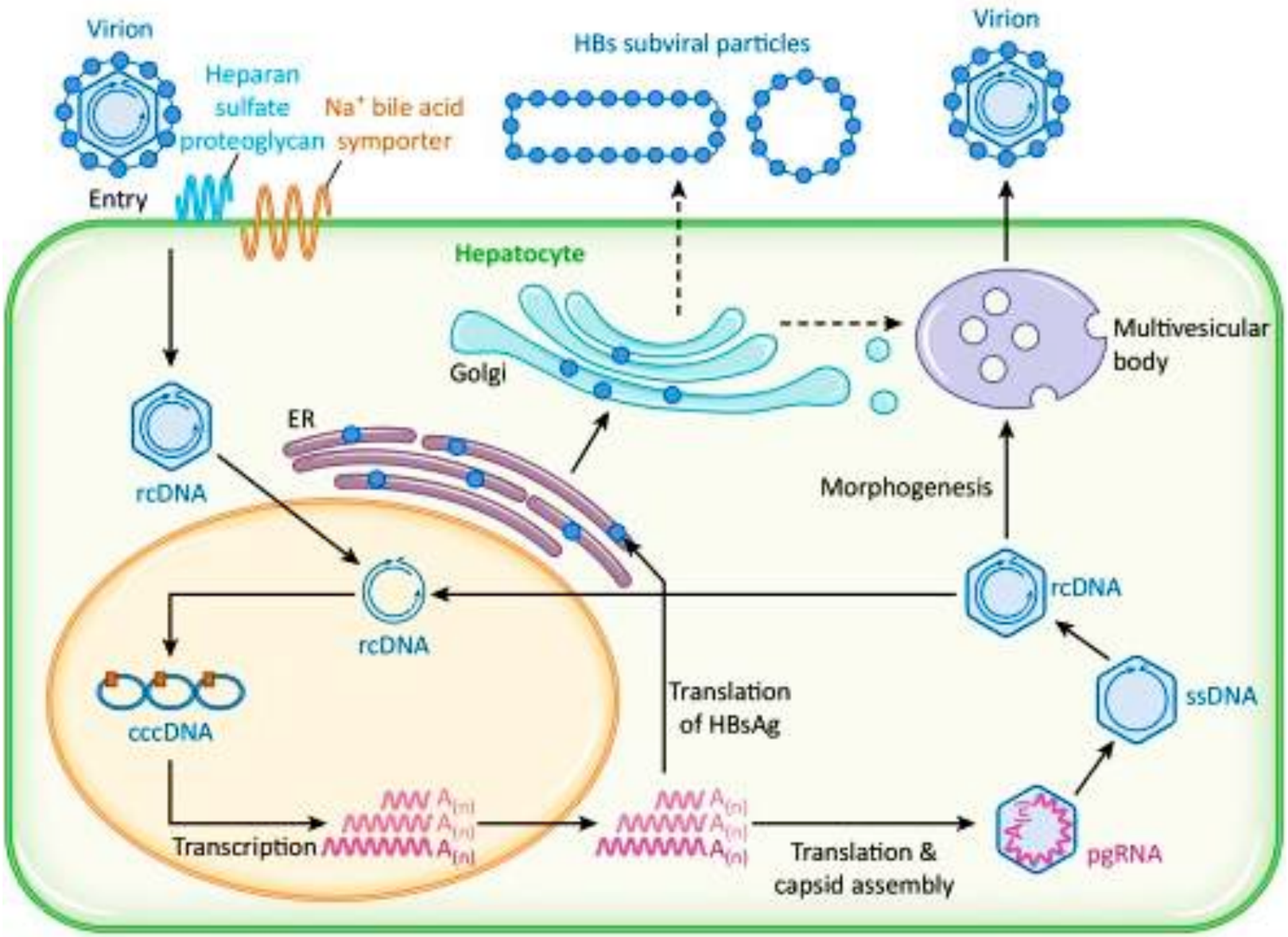
Viruses - vaccination

Recombinant DNA technology for vaccine production



- HBV infection results in production of intact spherical virions ~42nm Dane particles, and overproduction of 22nm particles of exclusively HBsAg

- HBsAg is encoded by gene S and the large form of HBsAg protein is the most abundant form found on the surface of infectious viral particles crucial for HBV binding to hepatocytes

- Cell-free production of immunogenic HBsAg in genome-free virus-like particles (VLPs) paved the way for genetic engineering for human health

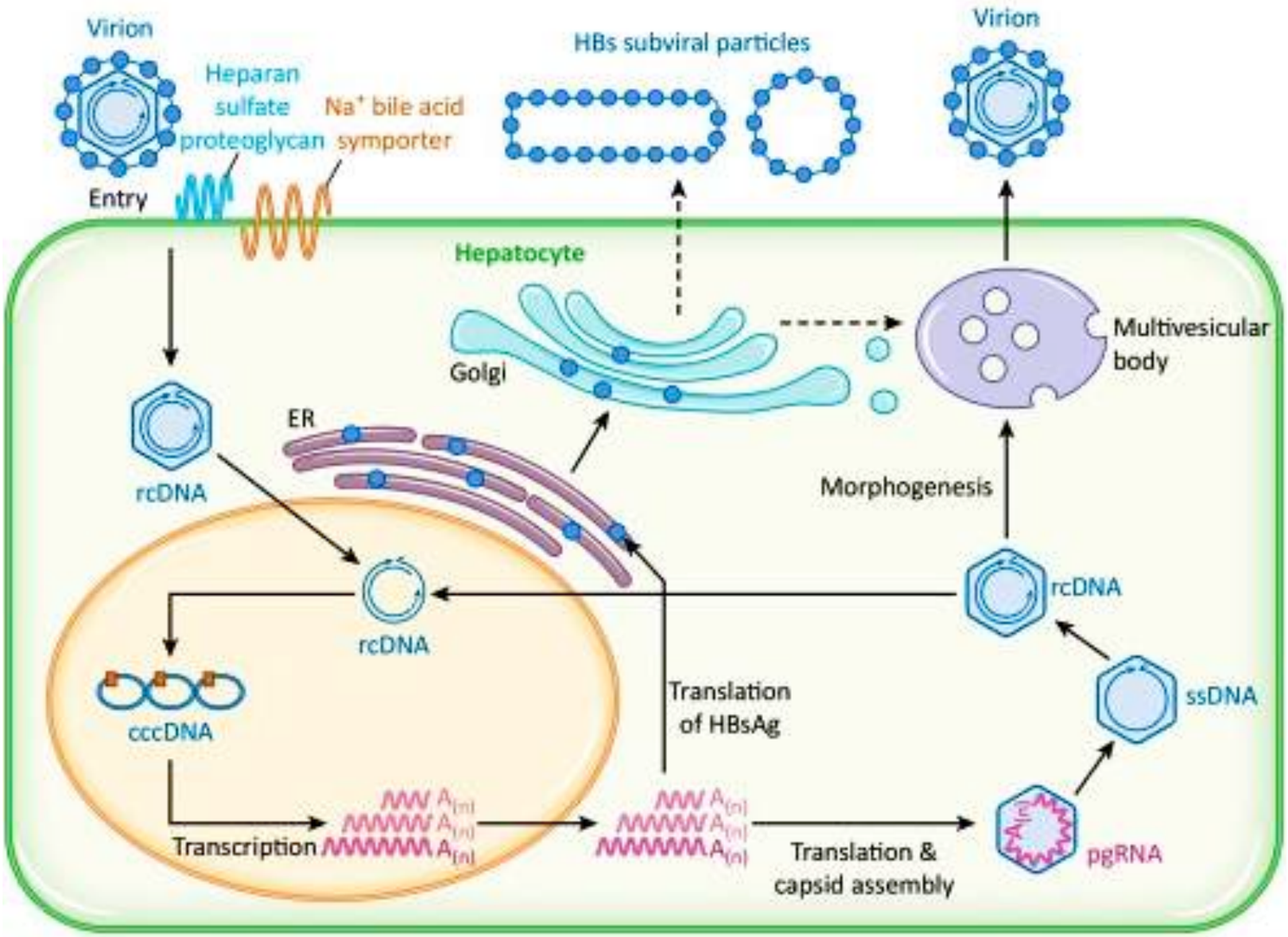
Empty shell of the virus, can trigger immune response in host, but cannot replicate and cause infection

PMID: 29500037

Sreelaja Nair

Viruses - vaccination

Recombinant DNA technology for vaccine production



- preventative vaccine made of human Hepatitis B virus capsid protein (HBsAg)
- possible because the gene for HBsAg was sequenced, cloned and protein produced in *E.coli* and Yeast

Empty shell of the virus, can trigger immune response in host, but cannot replicate and cause infection

PMID: 29500037

Viruses infect all forms of life

Organisms have evolved strategies to prevent or overcome infections caused by viruses

One such strategy is to trigger an immune response - requires a complex immune system

What about organisms that do not have an “immune system”?

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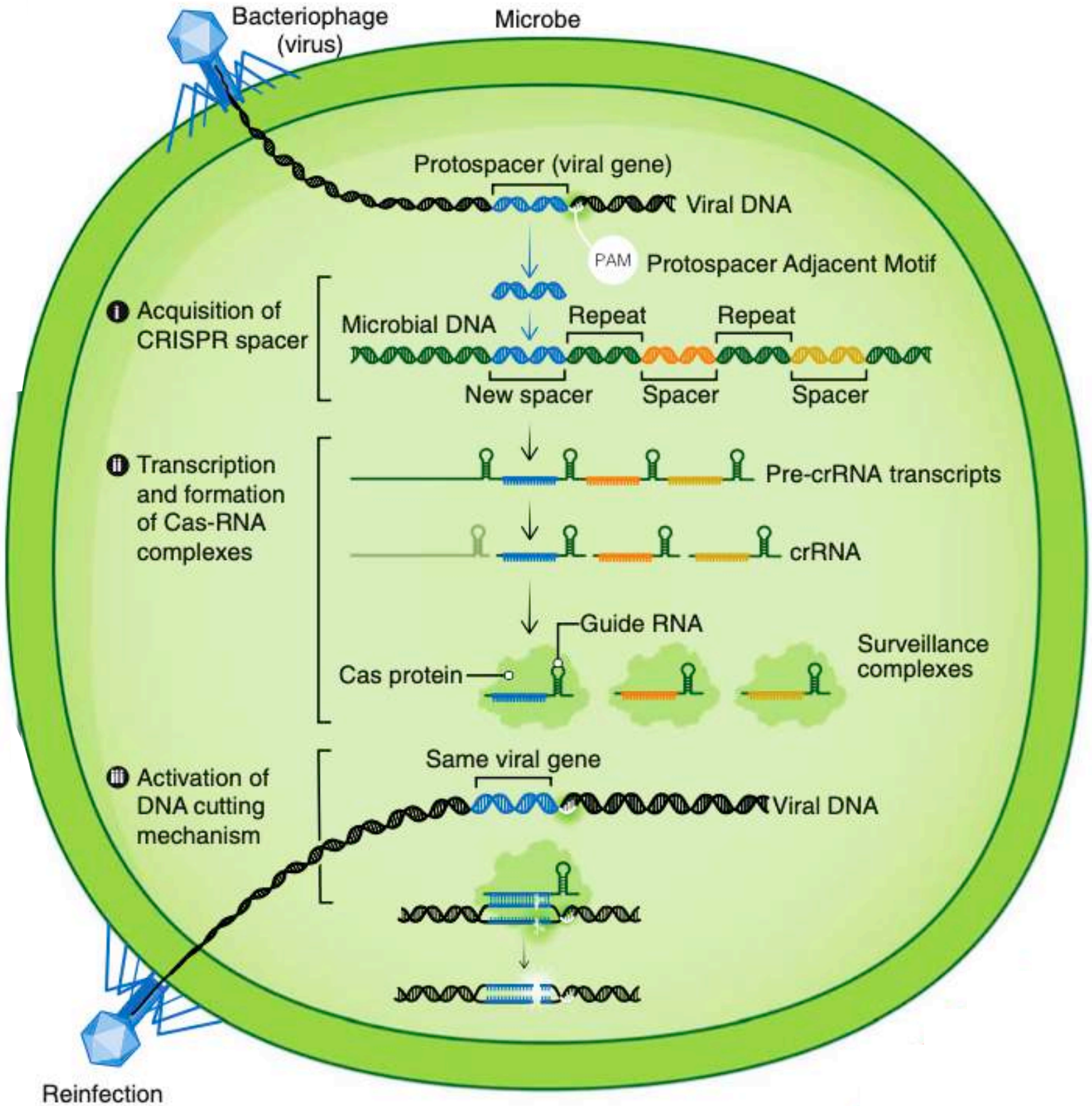
Viruses - how bacteria vaccinate themselves

Clusters of Regularly InterSpaced Palindromic Repeats



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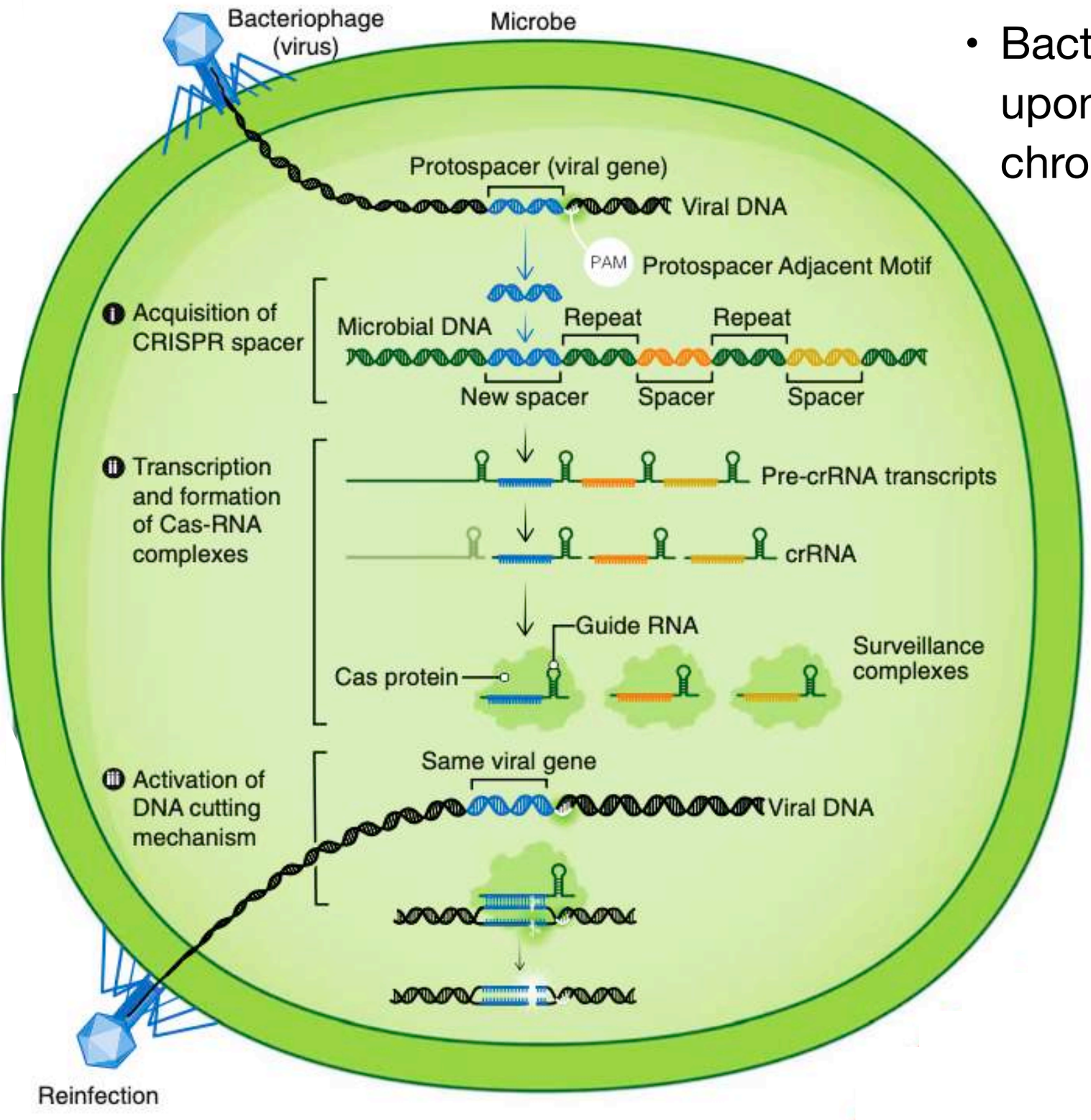
Viruses - how bacteria vaccinate themselves

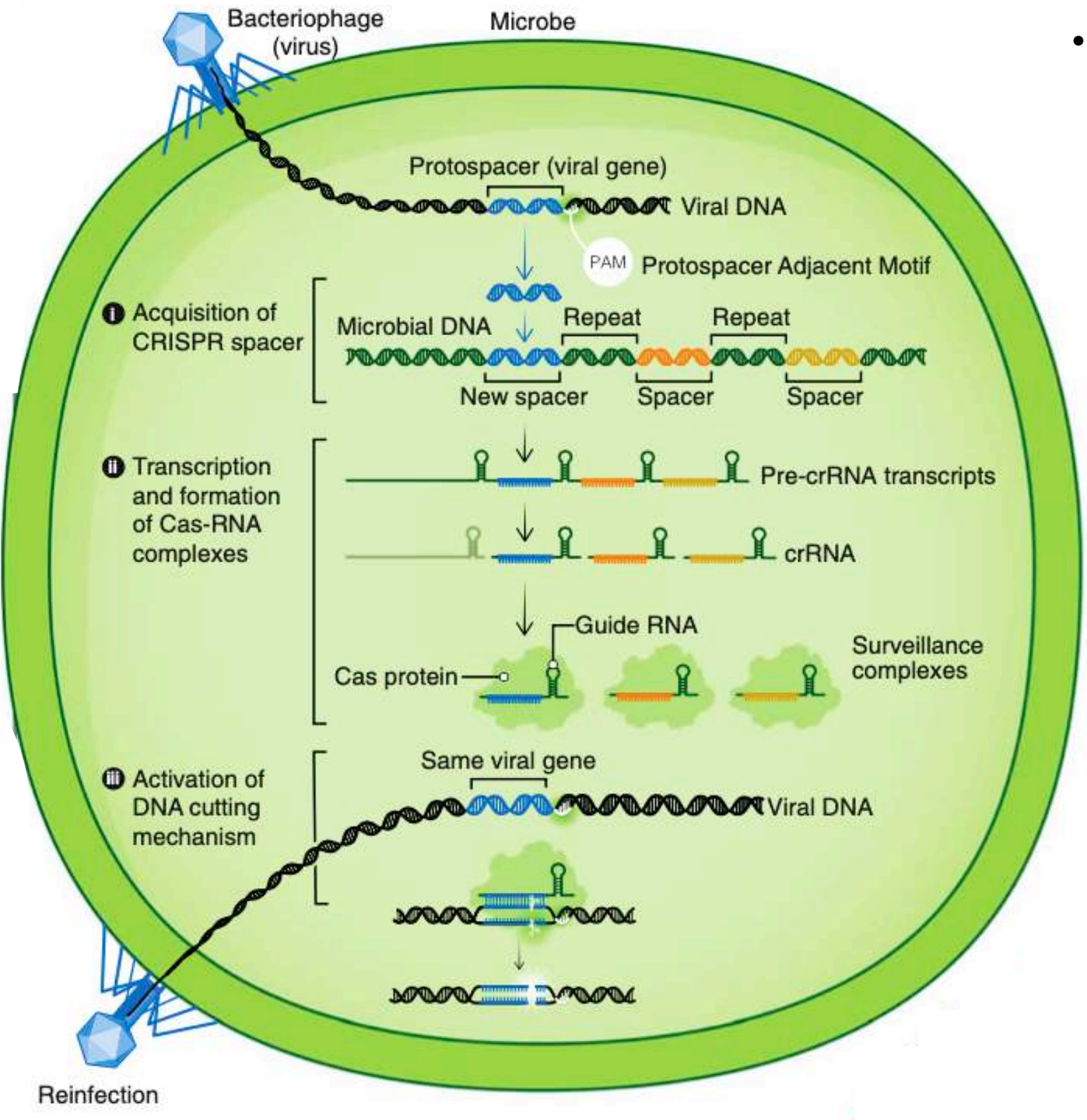
Clusters of Regularly InterSpaced Palindromic Repeats



- Bacteria acquire viral genome sequences upon infection and insert it into their chromosome as spacer sequences
- Spacer sequences from different viruses are separated by bacterial DNA repeats
- Spacers are the “memory” of a bacteria’s encounter with specific viruses - acquired during an unsuccessful infection
- This “memory” enables the recognition and neutralization of the invading genetic material upon subsequent infections

Viruses - how bacteria vaccinate themselves

Clusters of Regularly InterSpaced Palindromic Repeats



- Bacteria acquire viral genome sequences upon infection and insert it into their chromosome as spacer sequences
- CRISPR Associated genes (*cas* genes) convert the spacer+repeat into RNA
- Also makes cas protein, which is a nuclease (cuts DNA/RNA)
- The spacer+repeat RNA+cas protein complex “knows” where to target because of base complementarity between RNA sequence and DNA
- This complementarity recognizes the nucleic acid of the invading virus when encountered again

CRISPR - 2020 Nobel Prize in Chemistry

CRISPR discovery

Francisco Mojica
2005

CRISPR mode of action

Emmanuelle Charpentier
2011

Jennifer Doudna
2012

CRISPR application

Feng Zhang
2013

Francisco Mojica originally identified the spacer DNAs from viral genomes in archaea and bacterial genomes when he was a PhD student

Mojica coined the term CRISPR

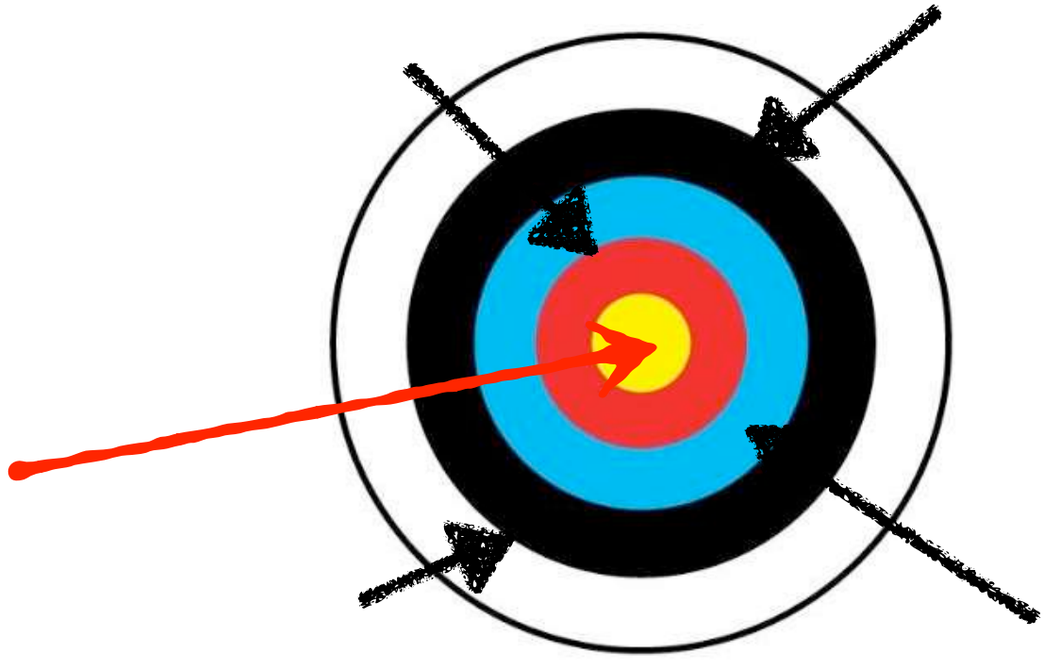
Why is CRISPR such a big deal?

- DNA can be “broken” in many ways
- Naturally occurring DNA mutations cause human diseases
- DNA mutations made in the lab in animals help mimic human diseases in model organisms
- Chemicals and radiations could “break” DNA and were engineered to be efficient

If DNA were a dart board, till 2012, we could ensure that the dart would hit somewhere on the board

The randomness of the hit meant that one could never target a specific site on the DNA

With CRISPR we can hit bulls eye



What makes CRISPR specific to a particular site?

If you know the sequence of DNA

↓

You can predict the sequence of the RNA

↓

You can make that RNA in vitro (guide RNA)

↓

Mix the RNA with cas9 protein

↓

The RNA-cas9 protein complex “knows where to go”

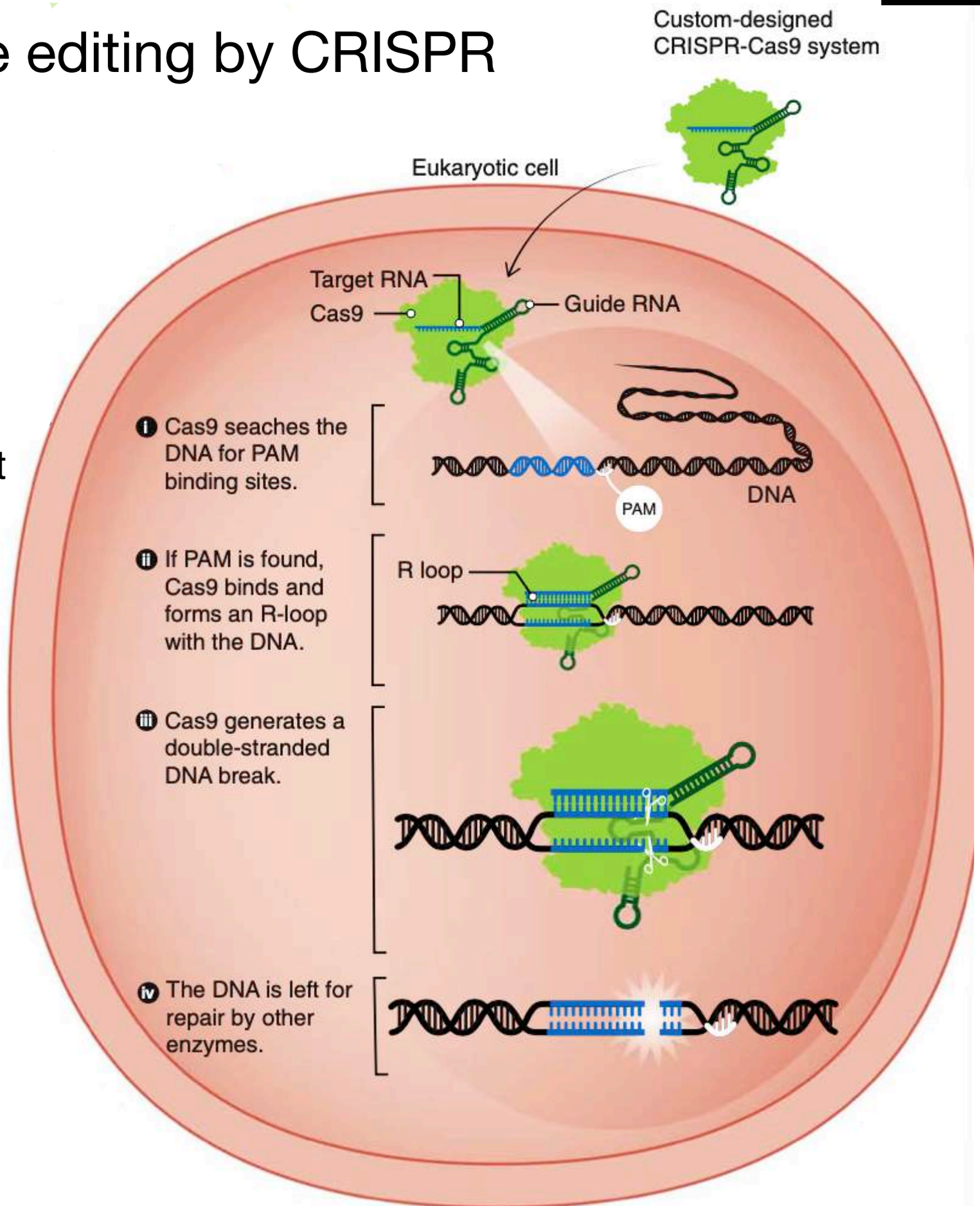
This occurs because of sequence complementarity between RNA and DNA

Allows a particular RNA sequence to target a specific DNA sequence

Gene editing by CRISPR

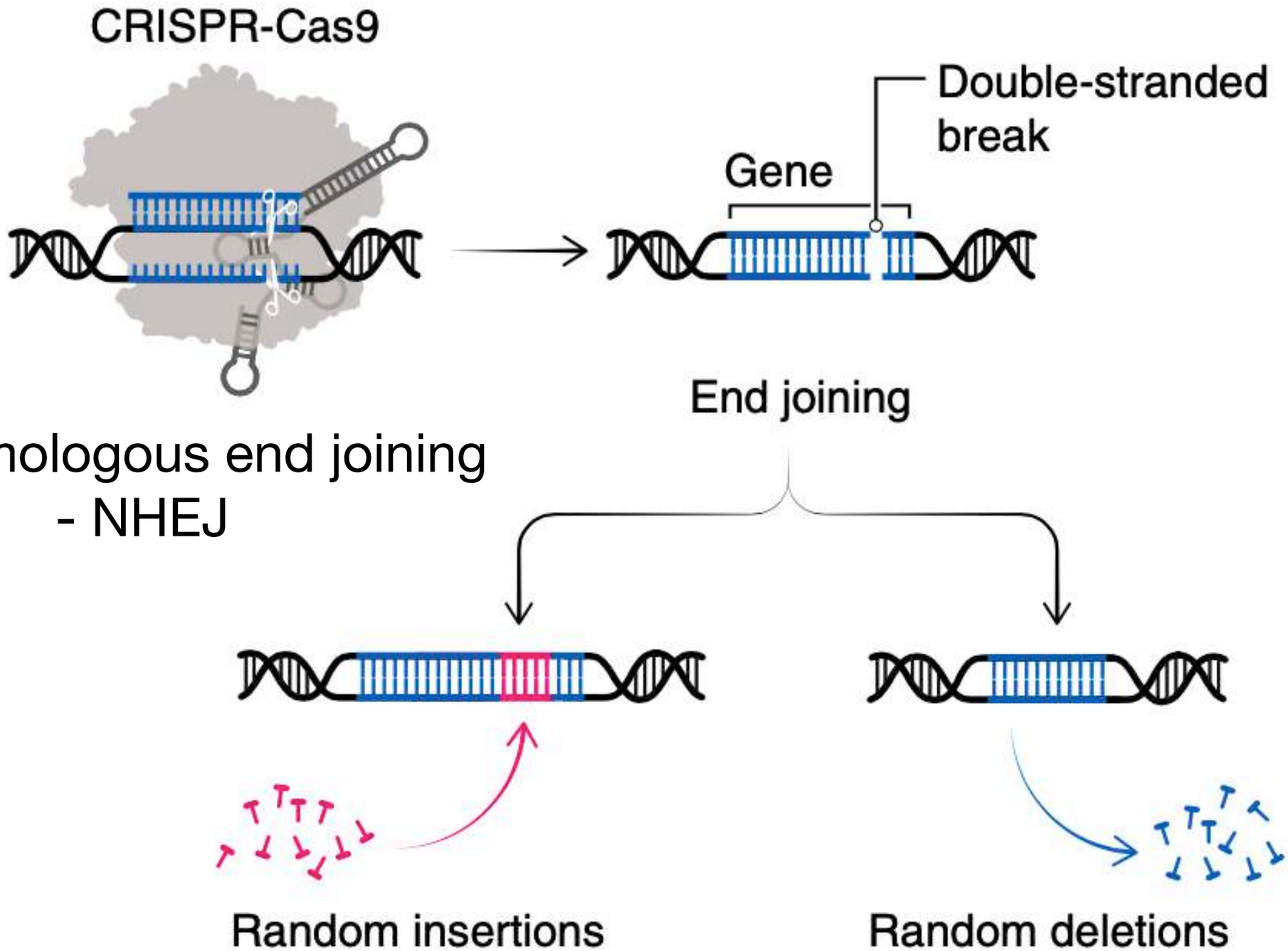
- Genome editing by CRISPR-Cas requires a single guide (sg) RNA to direct the Cas9 endonuclease to a specific region of the genomic DNA
- Requires a PAM sequence (Protospacer Adjacent Motif), next to the site of cleavage
- Once it reaches the site on the DNA, Cas9 cuts the DNA resulting in a double strand break

The cuts caused by cas9 need to be repaired



Gene editing by CRISPR - disrupt the gene

DNA breaks caused by cas9 are repaired without external information



Few bases can get added or removed at the cut site - alters the original information in a disruptive manner

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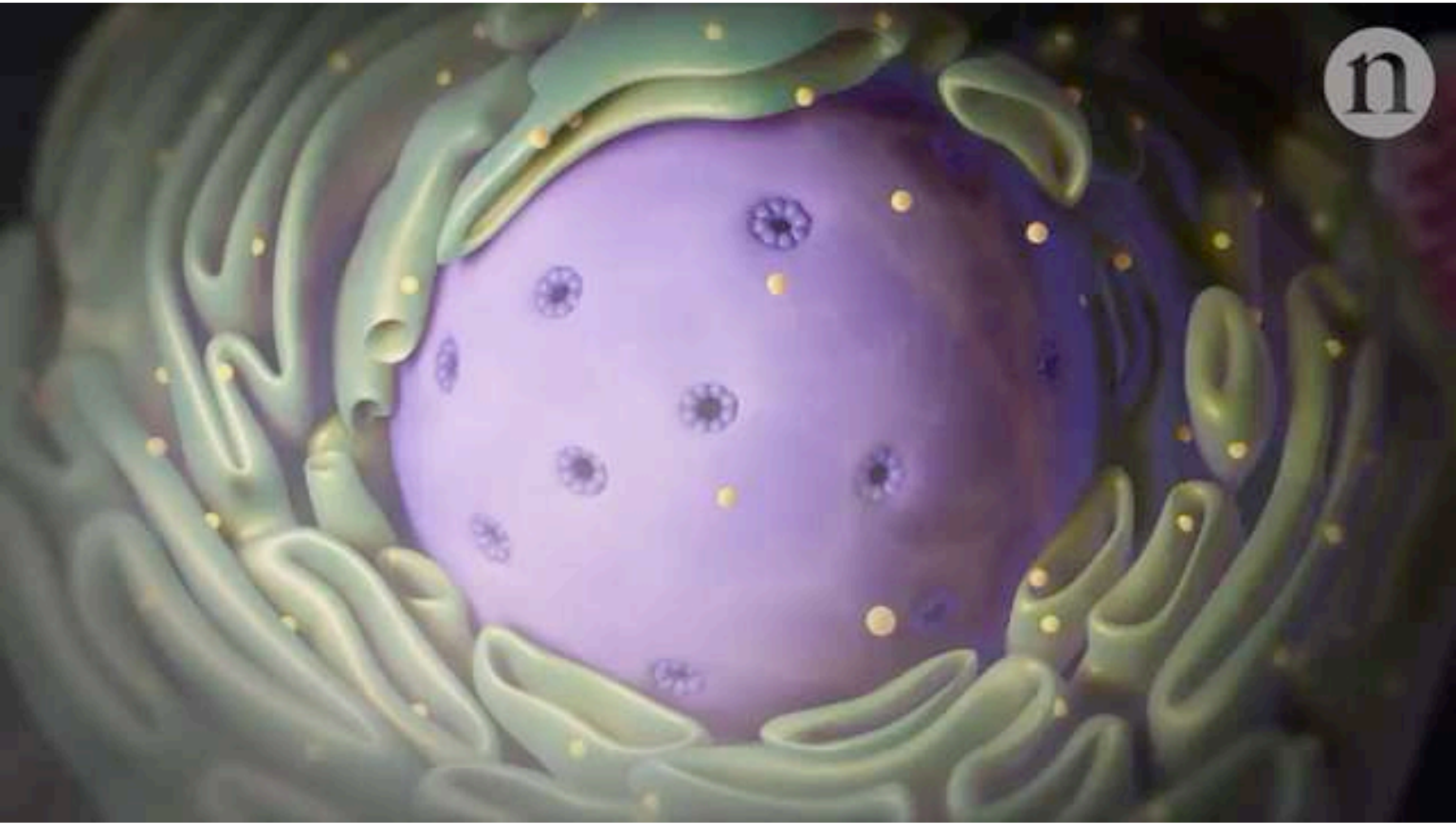
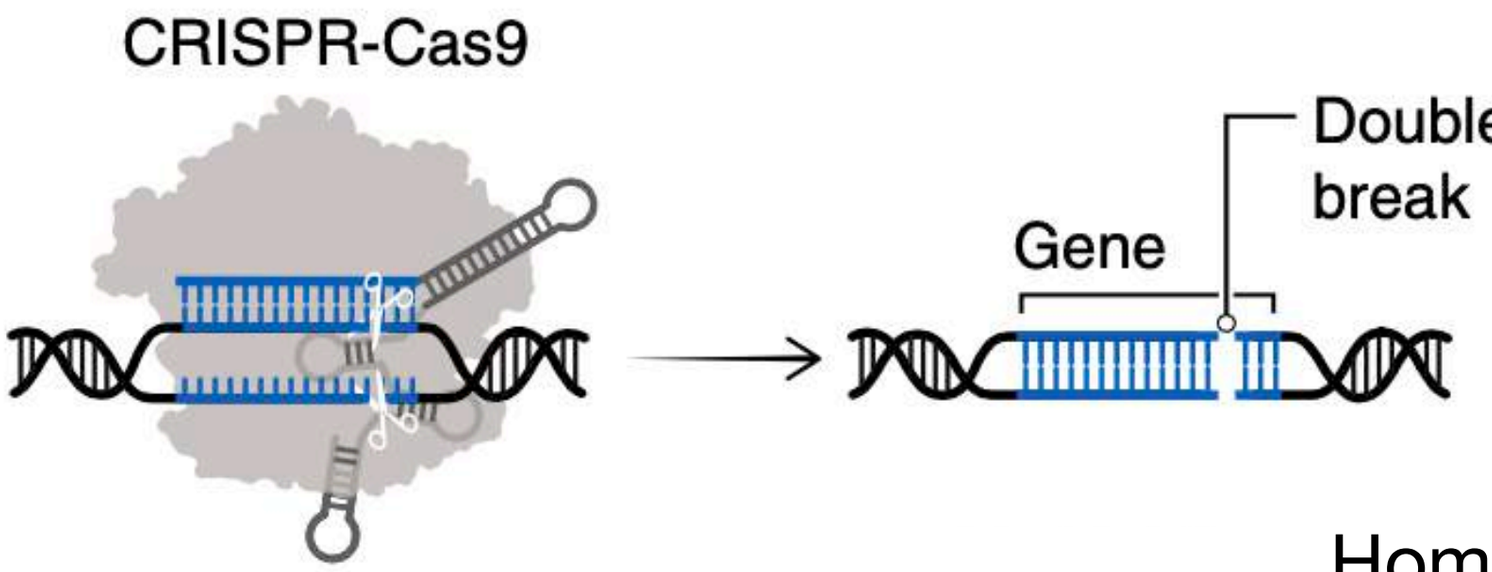
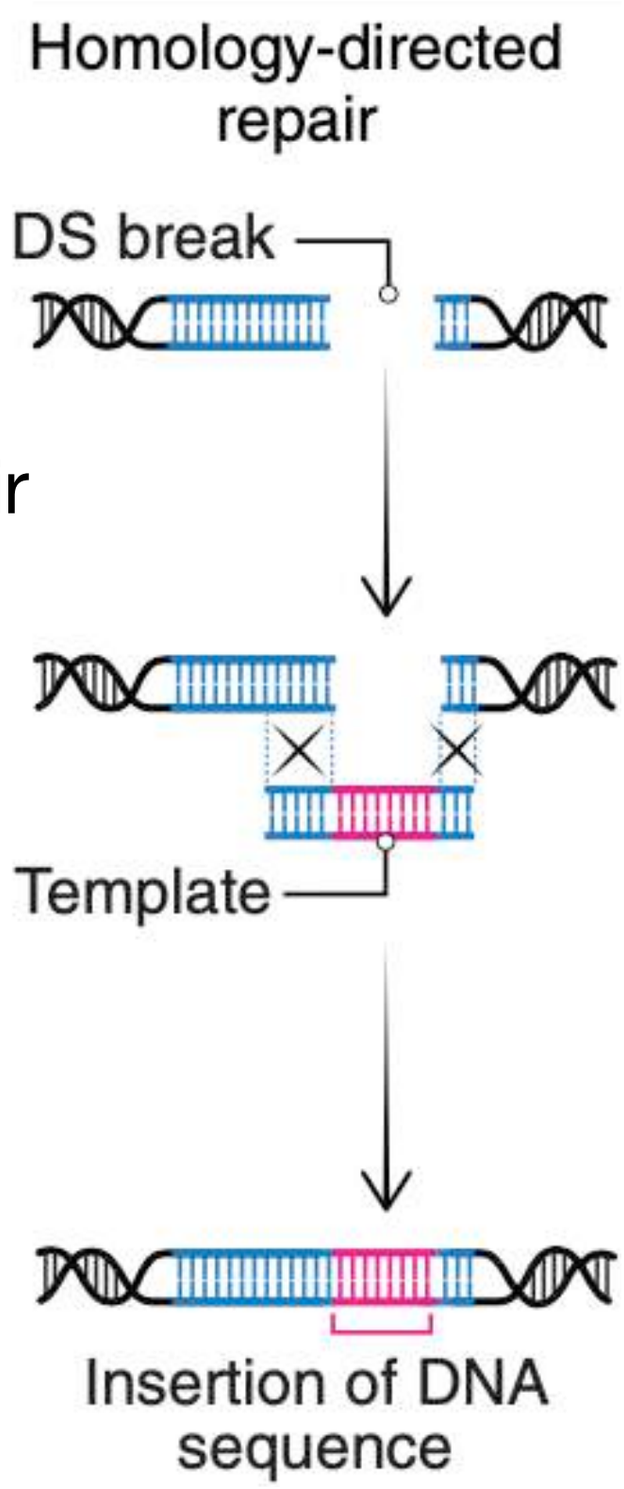


https://www.youtube.com/watch?v=4YKFw2KZA5o

Gene editing by CRISPR - repair the gene

DNA breaks caused by cas9 are repaired with the aid of external information





Homology Repair

- Requires a new piece of DNA
- The sequence of this new piece of DNA is the "correct" one
- The new DNA sequence is used as a template to recover the information lost when cas9 cut the DNA
- If cas9 cuts at two places on the DNA, the entire DNA segment in between the cuts can be fully replaced

Allows bad information on the DNA to be corrected

CRISPR is a tool which provides humans an unprecedented power to change the most important data

What does this power enable us to do as a species?

Should we do it?

The Immune System

1. Multiple layers of protection
2. Prevent
3. Eliminate

Innate immunity = all animals and plants

Adaptive immunity = only in vertebrates

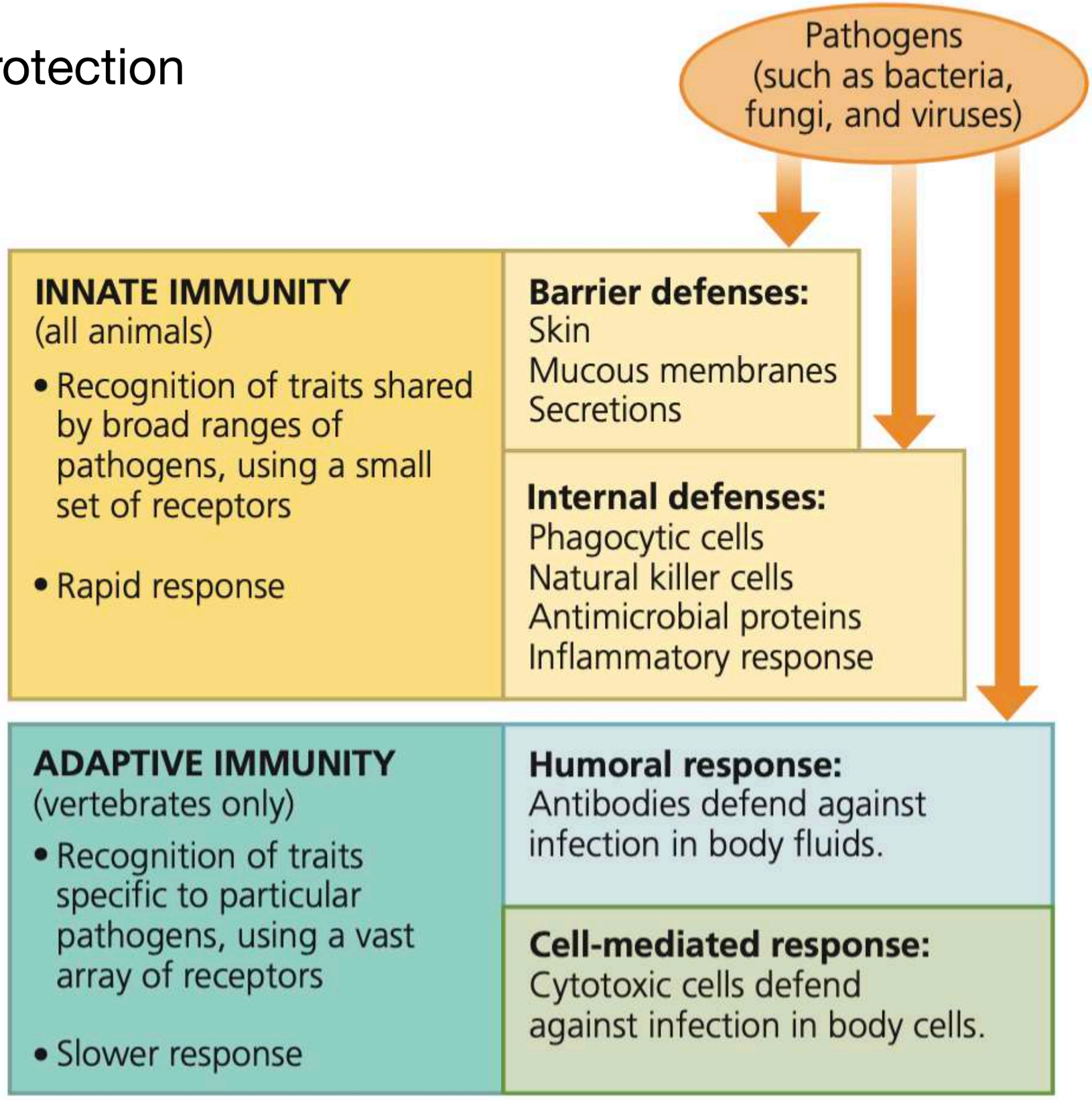
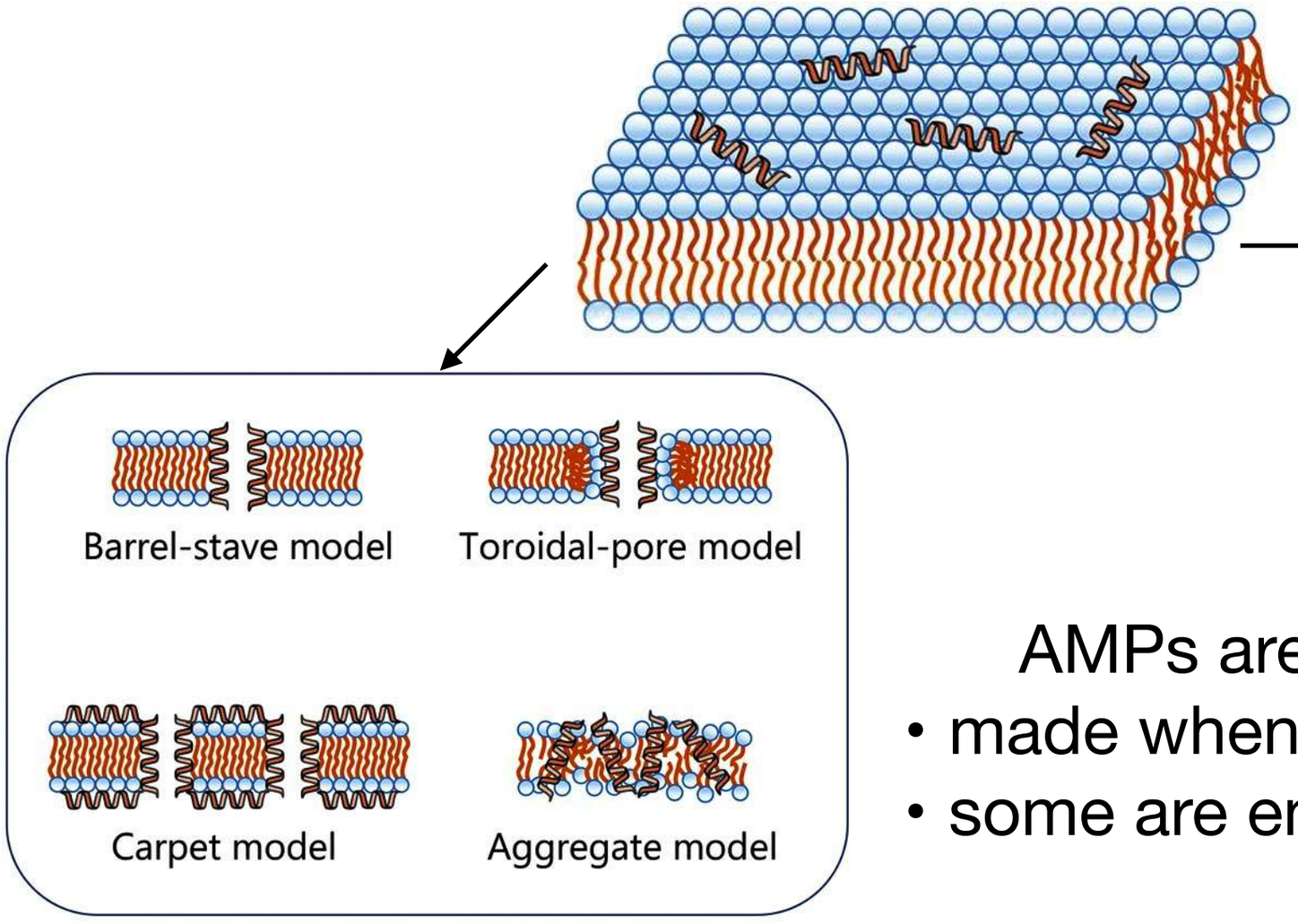
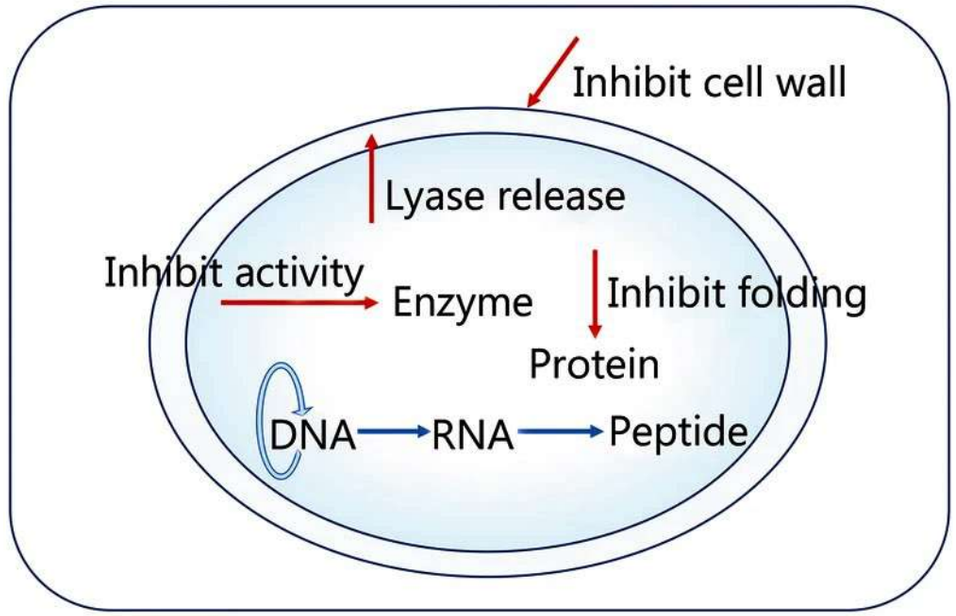


Figure 43.2 of Campbell's Biology: a global approach

Innate Immunity - Antimicrobial peptides (AMPs)





AMPs are short protein fragments -

- made when proteins are degraded
- some are encoded by genes

- Upon infection with a pathogen, AMPs penetrate into the phospholipid membrane and disrupt it by interacting with the lipid bilayer
- This creates holes in the membrane of the pathogen and causes it to lyse
- AMPs can also interact with cellular proteins and nucleic acids inside the pathogen and disrupt their function
- AMPs are also produced by bacteria - to eliminate competition for a “niche”
- Bacterial AMPs in human gut are important in our immune system

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Innate Immunity - Antimicrobial peptides (AMPs)

How specific are AMPs to one type of pathogen?

Experimental logic

Infect animals with a pathogen

↓

Detect the AMP produced

↓

Check that the AMP is able to neutralize that specific pathogen

Need the AMP to be genetically encoded

↓

Allows one to “tag” the resultant AMP and “see” it

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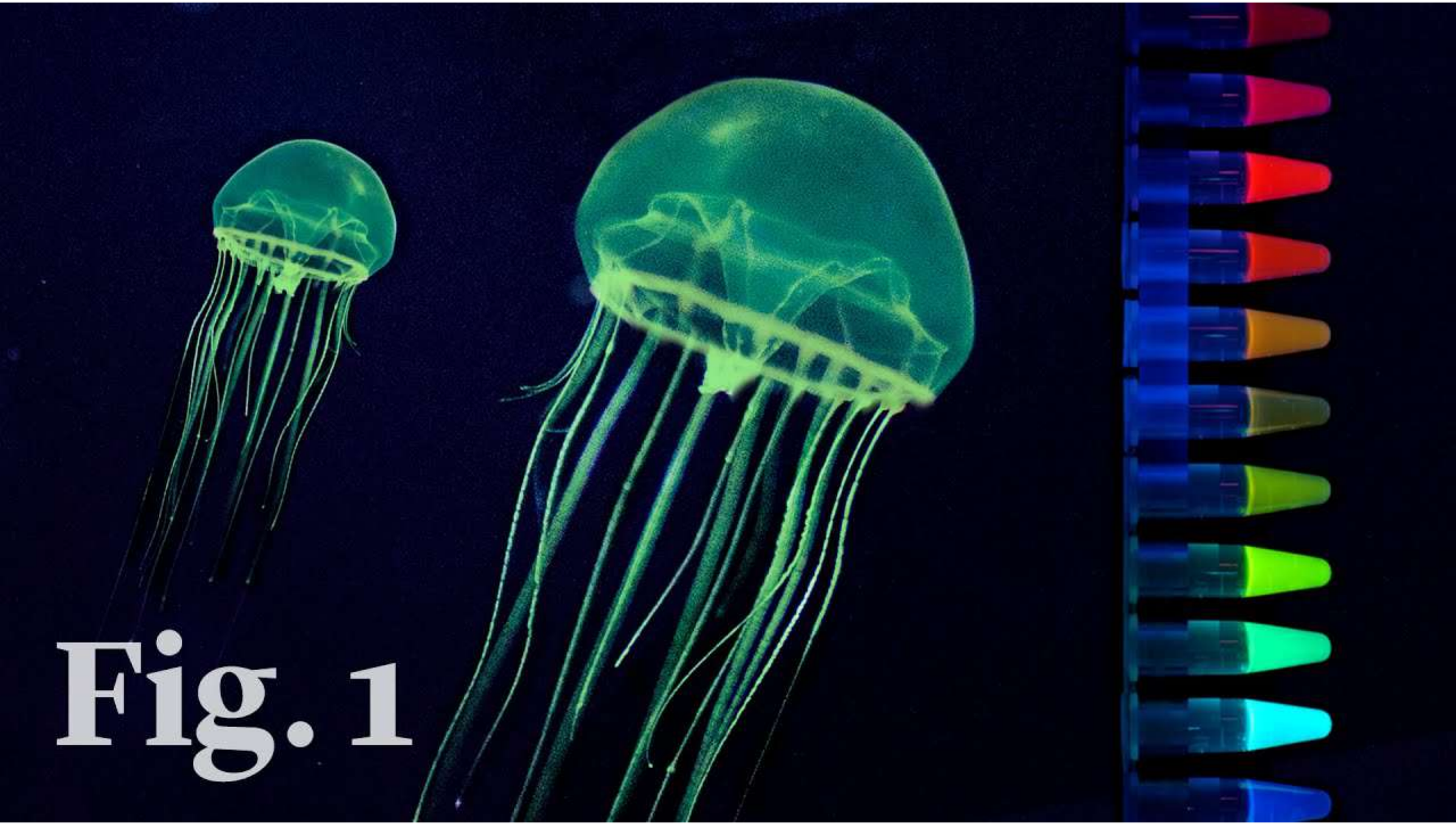
Green Fluorescent Protein and its variants

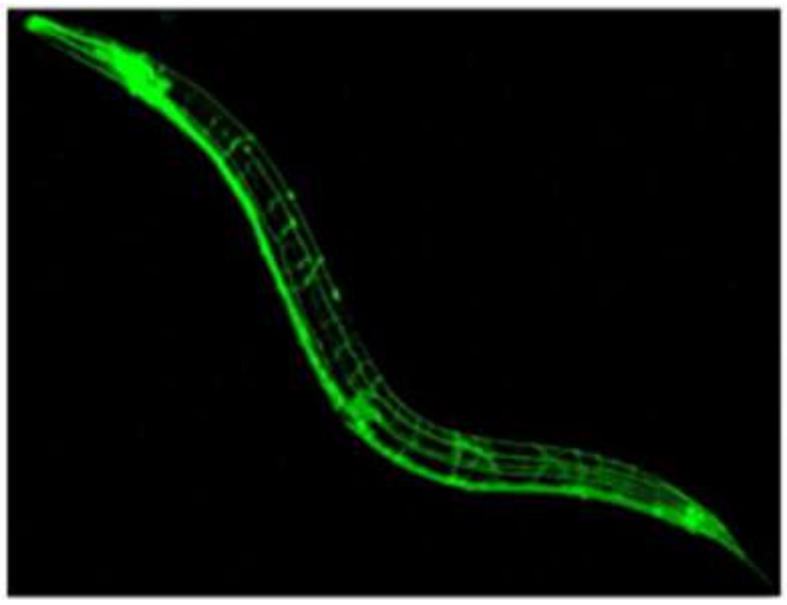
GFP is a genetically encoded fluorescent protein - has a high quantum yield
Variants can be made by mutating the original gene

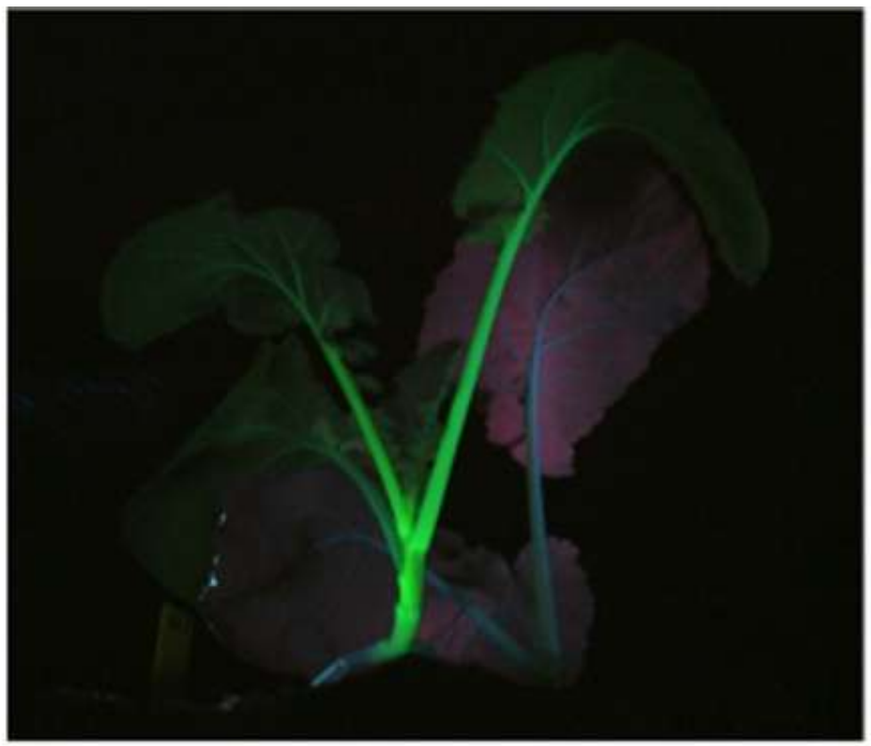


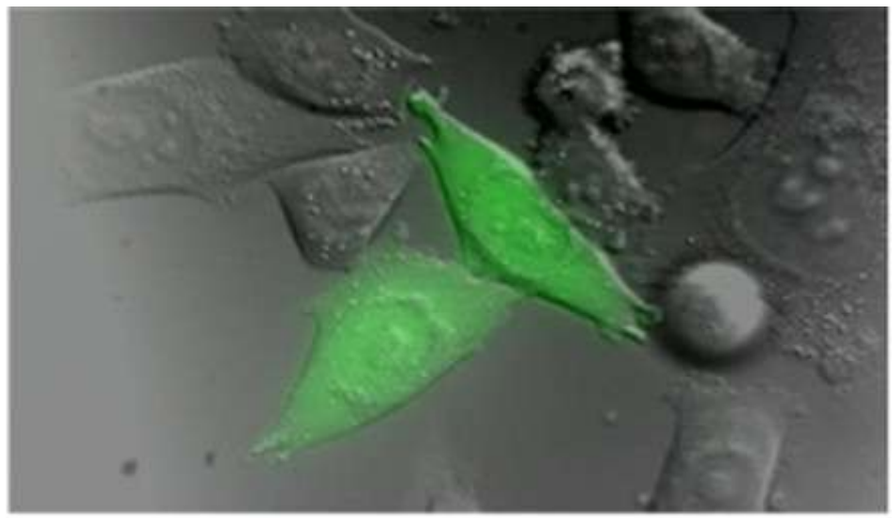
https://www.youtube.com/watch?v=Z4vJ8rQCNgg&t=1s

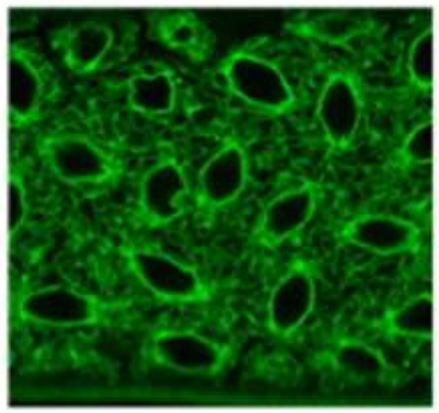
Sreelaja Nair

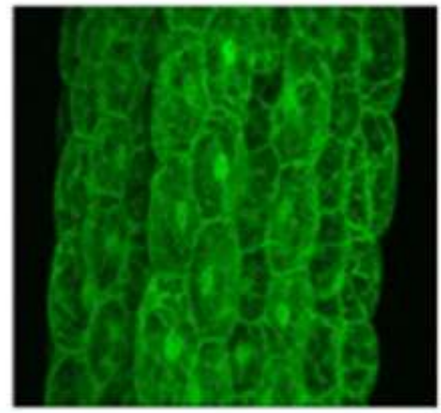
Fluorescently tagging proteins in cells and animals

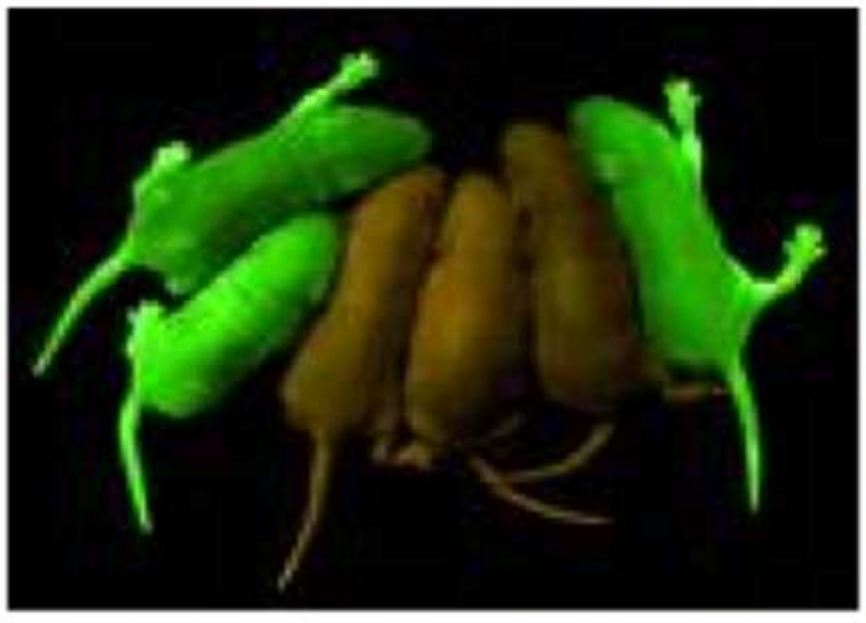


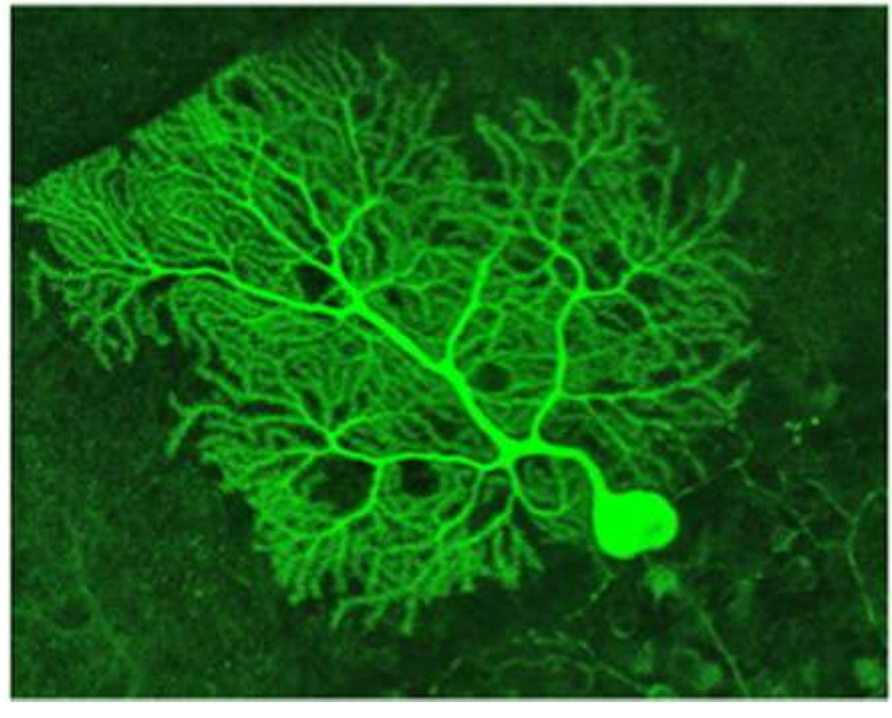


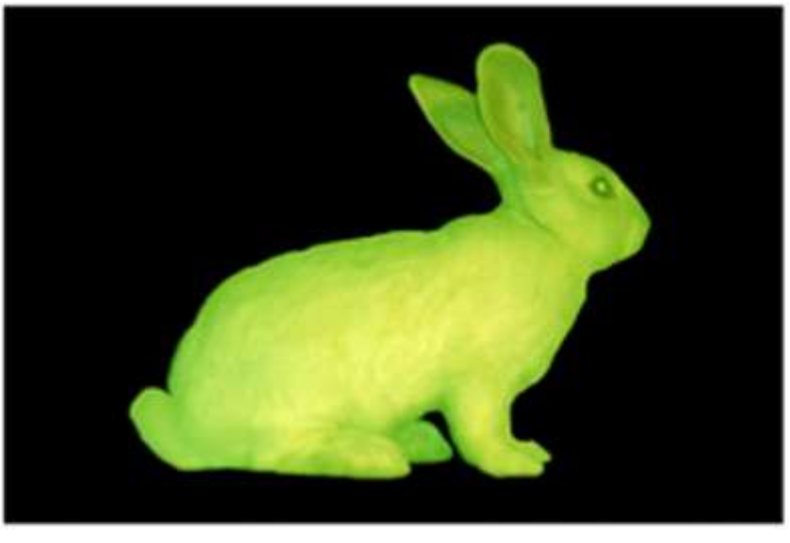


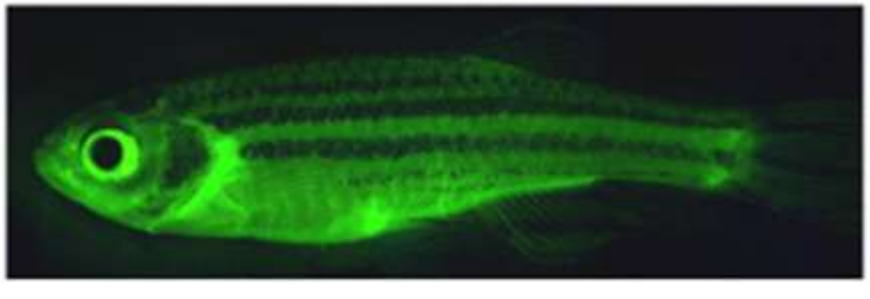












PMID: 19553219

Innate Immunity - Antimicrobial peptides (AMPs)

How specific are AMPs to one type of pathogen?

Experimental logic

Infect animals with a pathogen

↓

Fuse the AMP gene with GFP gene and “express” it in the animal

↓

AMP is produced fused with GFP protein and will fluoresce when translated by cells in the animal

↓

Infect the animal with pathogens

↓

Check if the cells are fluorescing

↓

If fluorescence is detected = cells in the animal produced that AMP against the pathogen it was exposed to

Innate Immunity - Antimicrobial peptides (AMPs)

How specific are AMPs to one type of pathogen?

Done in Drosophila (fruit flies), using two bacteria as pathogens and two AMP genes as “read outs”

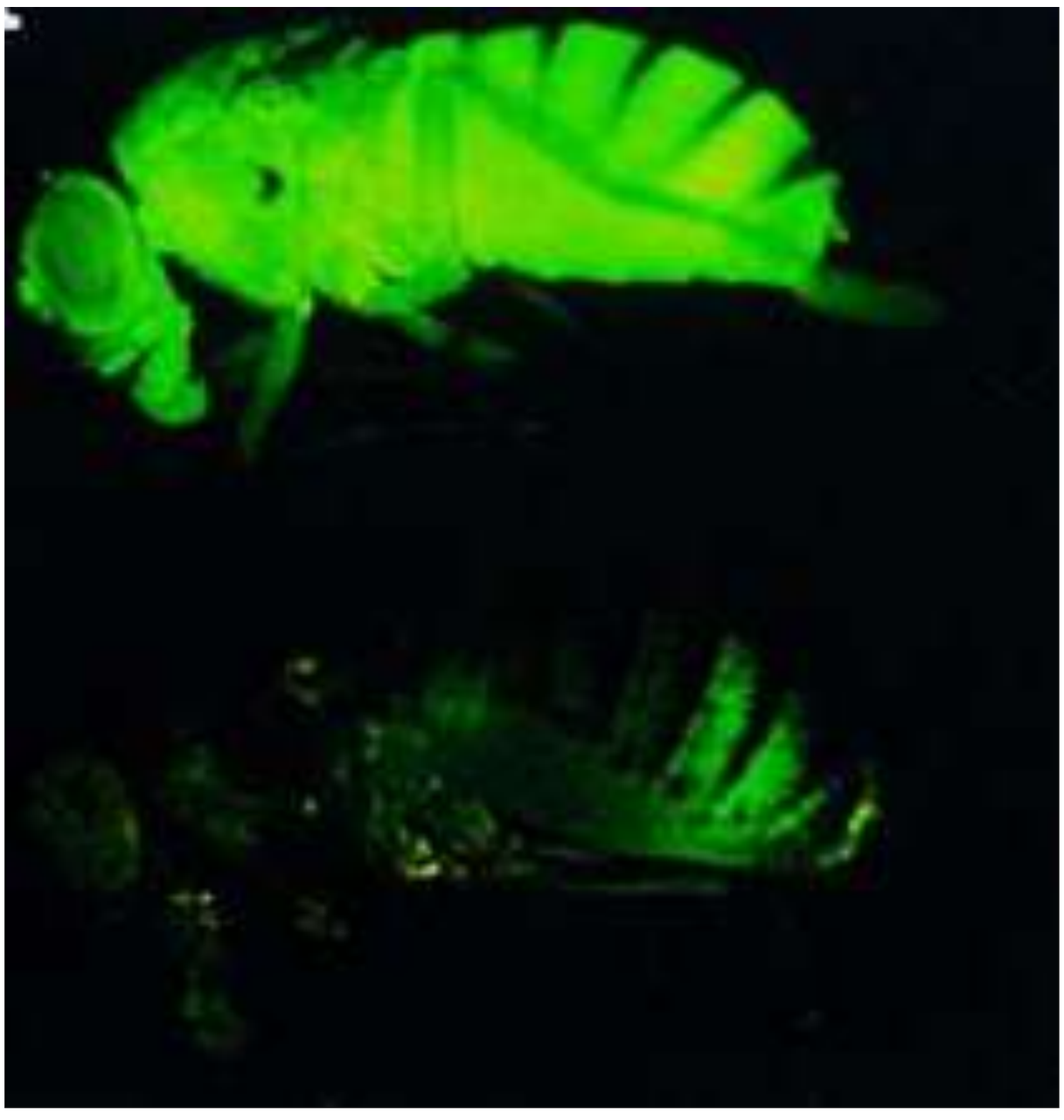


Figure 43.4 of Campbell’s Biology: a global approach

Pathogens used
fungus *Neurospora crassa*
bacterium *Micrococcus luteus*

AMP genes GFP tagged
drosomycin
defensin

Innate Immunity - Antimicrobial peptides (AMPs)

How specific are AMPs to one type of pathogen?

Experimental logic: must test two things - specificity and function

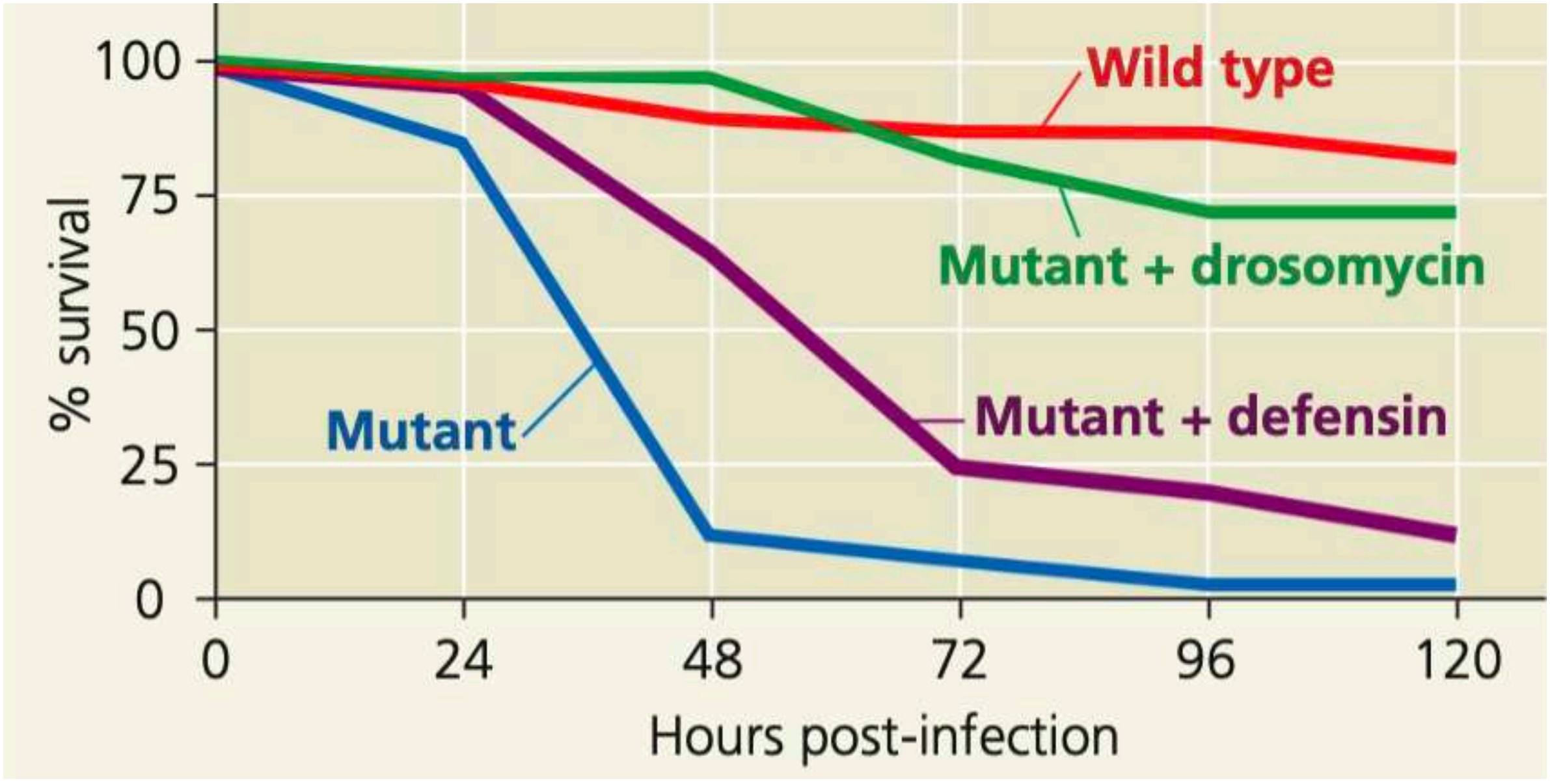
1. Generate mutant flies that cannot naturally produce AMPs - allows you to create a functional assay
2. The mutants will only make the AMPs you “express” in the flies, this will be the AMP::GFP fusion gene you have made - allows you to control production of one AMP at a time
3. Infect the mutant flies expressing one of the AMP::GFP fusion gene with one type of pathogen
4. AMP::GFP made in response to the pathogen can be seen by fluorescence - allows you to check if the AMP made is unique for a pathogen
5. Allow the infected flies to live. Survival of the flies will be the assay to test if the AMP produced can kill the pathogen - this is the functional assay

Logic loop complete

Innate Immunity - Antimicrobial peptides (AMPs)

How specific are AMPs to one type of pathogen?

Flies infected with fungus *Neurospora crassa*

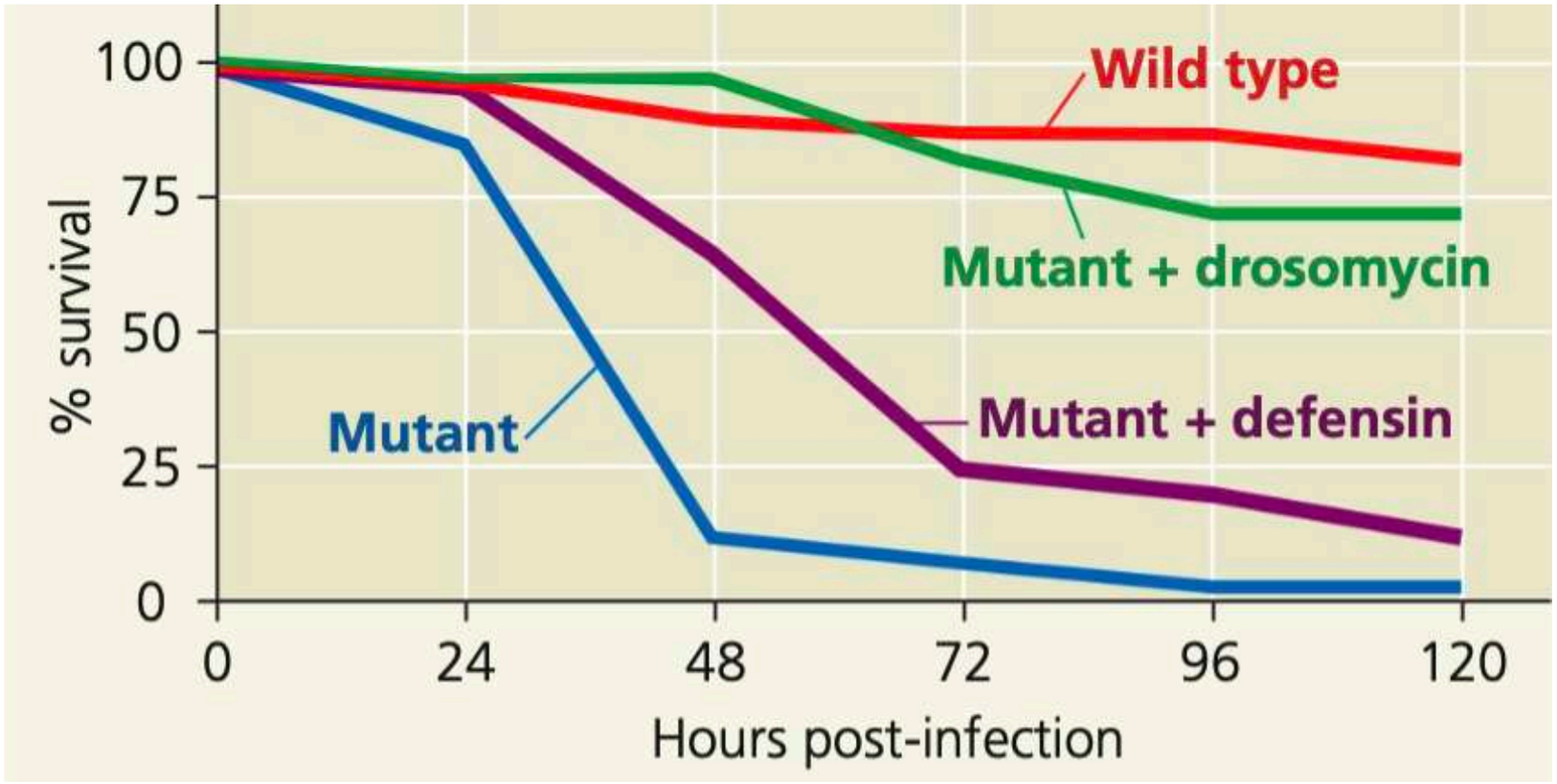


What can you interpret and conclude from the graph?

Innate Immunity - Antimicrobial peptides (AMPs)

How specific are AMPs to one type of pathogen?

Mutant flies expressing drosomycin::GFP infected with fungus *Neurospora crassa*



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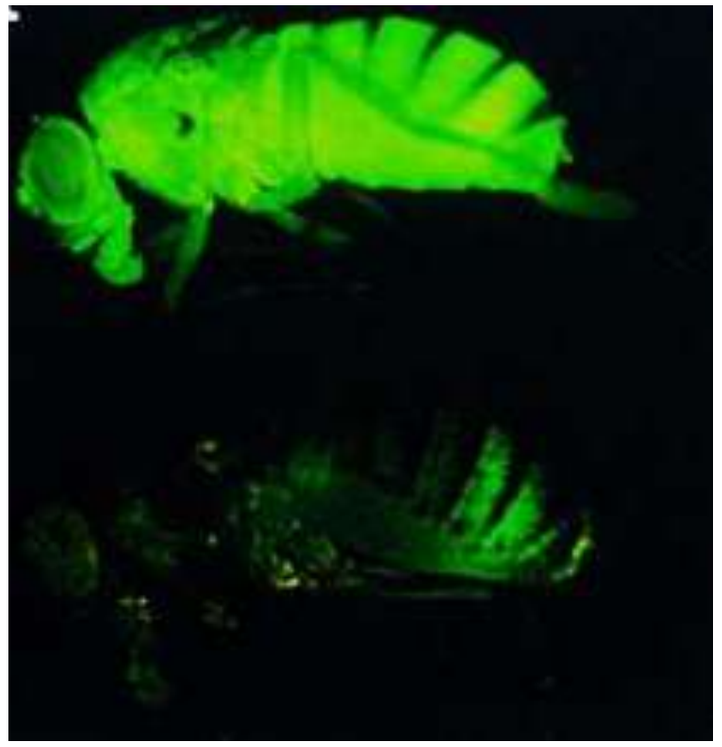


Interpretation for drosomycin:GFP experiment

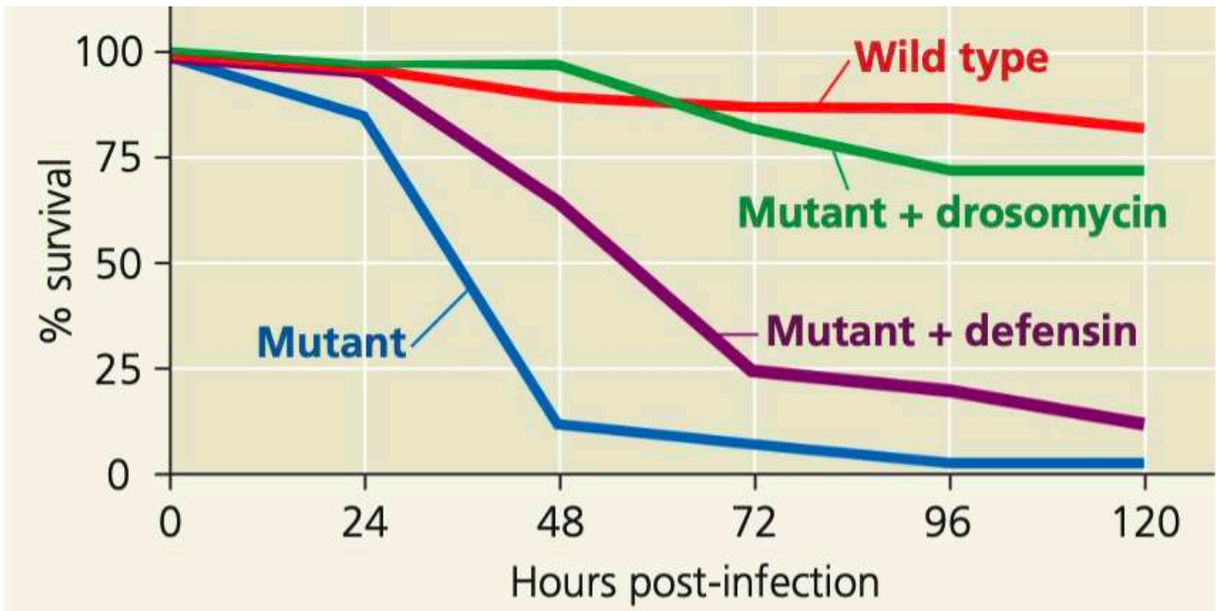
1. ~80% wildtype flies infected with *N. crassa* live 120 hours and beyond
2. ~90% mutant flies infected with *N. crassa* begin dying at ~48 hours
3. ~75% mutant flies infected with *N. crassa* and expressing drosomycin::GFP survive to 120 hours and beyond
4. ~75% mutant flies infected with *N. crassa* and expressing defensin::GFP begin dying at ~72 hours

Figure 43.5 of Campbell's Biology: a global approach

Innate Immunity - Antimicrobial peptides (AMPs)

How specific are AMPs to one type of pathogen?

Mutant flies expressing drosomycin::GFP infected with fungus *Neurospora crassa*



Interpretations

1. ~80% wildtype flies infected with *N. crassa* live 120 hours and beyond
2. ~90% mutant flies infected with *N. crassa* begin dying at ~48 hours
3. ~75% mutant flies infected with *N. crassa* and expressing drosomycin::GFP survive to 120 hours and beyond
4. ~25% mutant flies infected with *N. crassa* and expressing defensin::GFP begin dying at ~72 hours

Some relevant conclusions:

1. Drosomycin production upon infection with *N. crassa* allows flies to fight the infection and survive - fact
2. Drosomycin may be produced specifically against *N. crassa* (need the fluorescence data as well) - inference subject to additional experiments - logical leap based on facts

How will you test this inference?

Figure 43.5 of Campbell's Biology: a global approach