Viruses in the world of bacteria

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January 22, 2020

When you think of viruses, you probably think of diseases that affect you and other people: influenza, measles, rubella and many more. However, humans are not the only victims of viruses. In fact, the first virus ever discovered did not cause a disease in humans but in tobacco plants. Today, we know that viruses are ubiquitous and infect all forms of life – humans, birds, plants, insects, etc. There is no living species that is not affected by viruses; not even bacteria are safe from them! Some viruses kill bacteria, others bring benefits to their bacterial hosts. Let's take a closer look at this miniature world of attack and defense!

Bacteria have populated essentially every environment on earth. They live in soil, in water, and in and on plants, animals, and humans. At any given time, you have about as many bacterial cells as human cells! They play innumerable roles in our planet's ecosystem and in human health and disease. By infecting bacteria, viruses enter the scene wherever bacteria are. Viruses that infect bacteria are called bacteriophages or phages, which is the Greek word for 'bacteria eaters'.

Bacteria are complex, single-celled organisms that can reproduce on their own. They are clearly living organisms. Viruses are pretty simple in comparison, containing only a few DNA or RNA molecules wrapped in a protein shell. They cannot reproduce on their own and are thus usually not considered to be alive. In particular, to reproduce, they must infect a host cell and hijack its reproduction machinery, which often harms the cell.

Bacteria and viruses

Bacteria and viruses have something in common: they can cause pretty nasty infections. Both can make you cough and sneeze, give you stomach problems or a fever, or even kill you. Infections caused by both viruses and bacteria can be acute or chronic, mild or severe. They even spread in the same ways: through contact with infected people via touch, kissing or sex, through contact with infected animals such as pets, livestock, or insects, or through contact with contaminated surfaces, food, or water. However, despite all these similarities, bacteria and viruses are completely different.

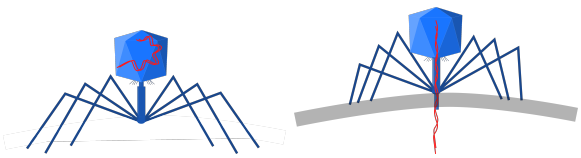
bacteria	viruses
larger: 200-2000 nm	smaller: 20-200 nm
single-celled organism	DNA or RNA molecules in a protein shell
reproduces independently by cell division	needs the machinery of the host cell to reproduce
infections can be treated with antibiotics	infections cannot be treated with antibiotics

In the following sections, you will learn about viral infections in bacteria; the roles of bacteriophages in experimental evolution, oceans' eco-systems, and in disease treatment; how bacteria defend themselves; and how scientists have turned the bacterial immune system into a revolutionary technology.

Viral attack

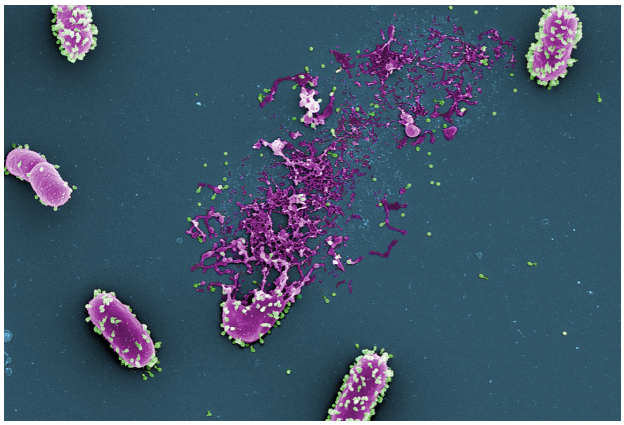
How phages infect bacteria

While human or animal viruses enter host cells as a whole, bacteriophages inject their DNA or RNA into the cell. Many phages have tails with which they pierce the bacterial cell wall and membrane. The DNA/RNA is packed under high pressure within the capsid, and for many bacteriophages, it is the release from this pressure that "shoots" the DNA/RNA into the bacterial cell. Sometimes, only part of the DNA enters at first, say, 70% of the strand. However, when the bacterial RNA polymerase starts transcribing the DNA, it pulls it entirely inside the cell. The empty capsid remains outside on the surface of the cell.



Left: Free virion – the genetic material (in red) is wrapped in the capsid. Right: The phage injects the DNA/RNA into a bacterial cell.

Generally, phages have one of two possible life cycles.[1] *Lytic* phages use the host cell machinery to replicate, and once a sufficient number of virions has been produced, they destroy (that is, "lyse") the cell. In this way, they are released into the environment where they can infect new cells. *Temperate* phages, on the other hand, integrate into the chromosome of the bacterial cell. Thus, when the bacterial DNA is replicated, the viral DNA is replicated as well and passed along to the daughter cells together with the bacterial chromosome, propagating the phage. This life cycle is called *lysogenic*, and the viral DNA is called a "prophage" during this stage. At some point, triggered by environmental conditions, the phage may switch to a lytic life cycle, cause the bacterial to produce virions, and finally lyse the cell.



Electron microscopy image of bacteriophages (green) attacking and killing bacteria (purple). Image by courtesy of M. Rohde and C. Rohde (Helmholtz Centre for Infection Research, Braunschweig/Leibniz Institute DSMZ, Braunschweig, Germany) and colorized by Dwayne Roach (Institut Pasteur). ©M. Rohde and C. Rohde

Prophages can be useful to bacteria. They often provide the bacterial cell with immunity against infection by certain other types of phages. This can, for example, happen through modification of the cell surface. Temperate phages also sometimes carry genes that prove beneficial to the bacterial host cells. For instance, they help the bacteria to infect humans or animals (see Box on Cholera). Prophages can thus provide their carriers with an evolutionary benefit.

R/Evolutionary insight from bacteriophages

By the middle of the 20th century, the Mendelian ideas of inheritance and mutation were generally accepted, though DNA had not yet been fully understood. However, scientists did not know whether mutations occur randomly, or as a reaction to the environment.

[1] Other life cycles are possible. For instance, some phages cause a chronic infection: they are continuously produced, leaving the cell one by one without killing it.

Cholera: A bacterial disease with a viral culprit

Cholera is a diarrhoeal disease that leads to severe dehydration and can even lead to death within a few hours in severe cases. It is transmitted via contaminated food or water and is thus particularly prevalent in regions with poor hygienic conditions. It affects an estimated 3-5 million people worldwide, causing 28,800-130,000 deaths a year.
Cholera is caused by a bacterium called *Vibrio cholerae*. But here is the twist: the toxin that makes us sick is actually encoded by a virus that was integrated into the bacterial genome. It is passed on from the mother cell to the daughter cells during cell division but can also be vertically transmitted to neighbouring bacterial cells. Variants of the *Vibrio cholerae* bacterium that do not carry this virus do not cause disease. (On the other hand, carrying the virus is insufficient to make bacteria pathogenic. To be pathogenic, they also need to possess genes that allow them to attach to the intestinal wall.)

Therefore, to find this out, Salvador Luria and Max Delbrück designed an ingenious experiment that made use of bacteria and phages.

When a bacterial population grows in a liquid that contains nutrients to feed the bacteria, the liquid will turn cloudy within a few hours due to the increasing numbers of bacteria. When you add phages, it will become clear again very quickly as the phages kill the bacteria. Yet, after some time (hours or days), the liquid will go back to being cloudy. This means that some bacterial variants were not harmed by the phage and were able to multiply. The offspring of these bacteria can withstand the phage as well. This shows that bacteria can – somehow – become resistant to phages. However, is this resistance due to some sort of mutation that happened *before* the bacteria encountered the phage (and hence occured independently of the phage)? Or did the interaction with the phage induce some heritable change in the bacteria?

Salvador Luria let bacteria grow in a liquid in the absence of phages. Then he spread some of it onto a Petri dish containing a large number of phages. Most of the bacteria died, but the resistant ones formed colonies that he could count. He then repeated this procedure several times.

Salvador Luria and Max Delbrück considered what would happen in both cases – mutations induced vs. occurring independently – and formed respective hypotheses.

Hypothesis 1: If mutations are induced by the environment, we expect that a certain proportion of bacteria mutate, and the others do not. Let's say approximately 1 % of the bacteria that encounter the stimulating environment mutate. All these mutations happen (or do not happen) at the time of encounter with the phage. This makes it very unlikely that a plate contains a large number of resistant colonies. If the experiment is repeated many times, the number of colonies in each experiment should be similar. In other words, we expect a relatively low variance in the number of colonies.

Hypothesis 2: If the mutations are independent of the environment, they may arise at any time: a long time before the encounter with the phages, during the encounter, or not at all. The early mutations are like 'jackpots'. The mutated bacteria will have left many descendants by the time of phage encounter. Hence, if a 'jackpot mutation' occurred, we will count many resistant colonies on the plate. If it did not, there will be very few, or no colonies at all. Repeating the experiment many times, we should observe some cases with many colonies, some with none – that is, we expect a relatively high variance in the number of colonies.

Max Delbrück calculated the distribution of colonies that one would expect under each hypothesis, and compared these theoretical predictions to the experimental results.

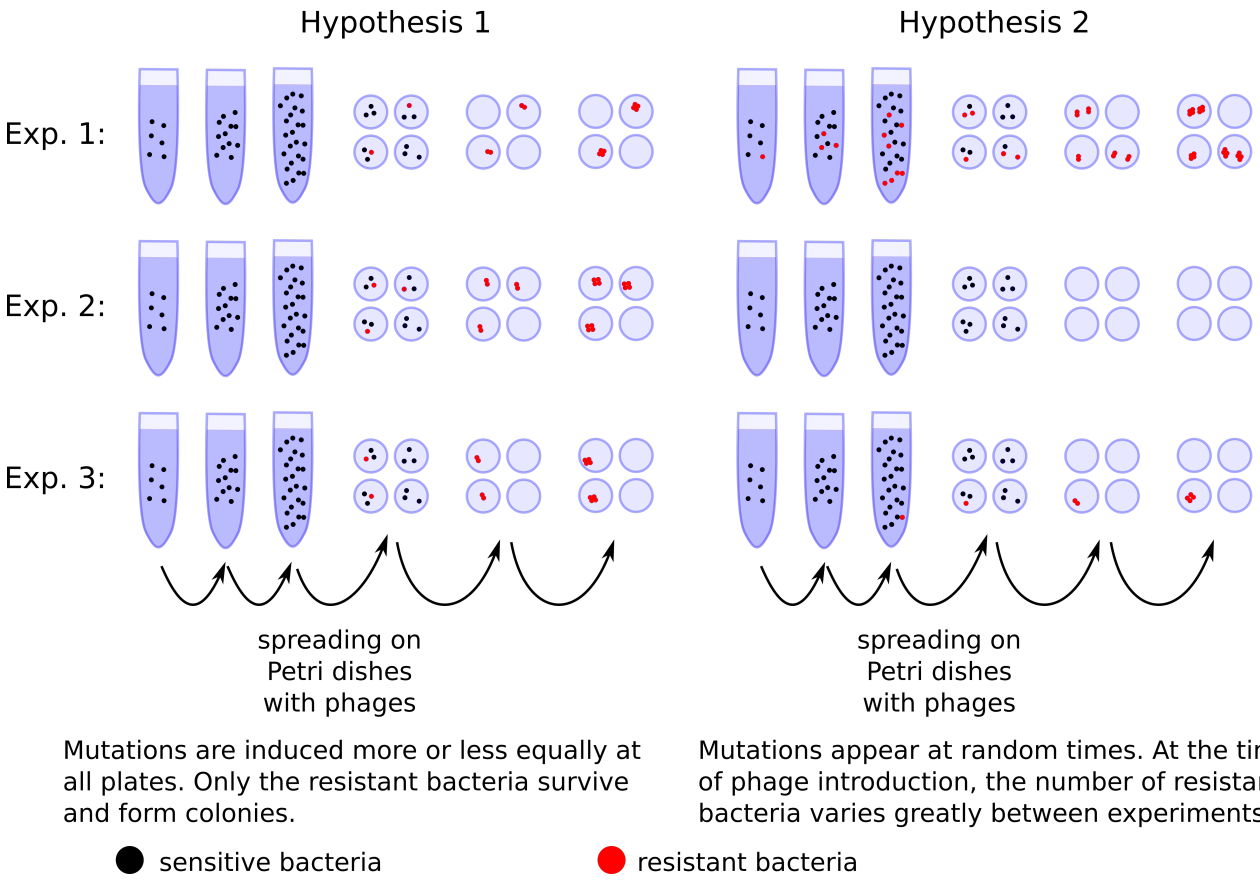


Illustration of the Luria-Delbrück experiment and the two hypotheses for how mutations occur.

The experimental observations that Salvador Luria made were consistent with the second hypothesis. Hence, the puzzle was solved: Resistance mutations happen spontaneously and not as a result of some interaction with the phage. (Much later, researchers found that stress increases the mutation rate in bacteria. But as far as we know, it increases the rate of *all* mutations, not specifically those mutations that confer resistance.)

The Luria-Delbrück experiment is very important for a second reason. The colony count allows us to estimate the mutation rate of bacteria. For this purpose, similar experiments are still used today.

Viruses in the ecosystem

Viruses play a major role in the ecosystems of the Earth. As an example, let's look at the world's oceans. Overall, the world's oceans contain on the order of 10^{30} viruses. If we put all these viruses into a line, it would stretch 100 times the distance across our galaxy. If we take one liter of seawater from the surface of the ocean, we would find more viruses than there are humans on earth (10^{10} versus $7.7 \cdot 10^{9}$). Every single second, approximately 10^{23} viral infections happen in the oceans of this world. All ocean life, be it whales, fish, plants, algae, bacteria, or other microorganism, is susceptible to infection by viruses. Clearly, this has an immense effect on life in the ocean. Let us look at the viruses in the ocean that infect bacteria.

To begin, phages play a big role in nutrient cycling. About 10-20 % of bacteria living in the ocean are lysed by viruses every day. Through the lysis of bacteria and other microbes, nutrients such as carbon or phosphorus are released. This is called *viral shunt*, because it makes these nutrients directly available to other microorganisms to uptake. If the microbes were eaten by other, larger organisms rather than being lysed, these nutrients would be transported upwards to higher levels in the food web.

But phages also have less obvious effects

on the ecosystem. For example, you probably know that plants perform photosynthesis. Basically, during photosynthesis, water and carbon dioxide are converted into oxygen and sugar using energy provided by sunlight. The sugar can then be used as an energy source for other organisms, and of course, we rely on the oxygen for breathing. In fact, about half of all oxygen in the atmosphere is produced not on land but in the ocean, mainly by algae and bacteria (the so-called cyanobacteria). Some of the viruses that infect such cyanobacteria also carry photosynthetic genes, and this can help stabilize photosynthesis in the bacteria. In particular, without prophage infections, photosynthesis in the bacteria is inhibited at high light intensities; the system breaks down. However, with the help of the phage, photosynthesis continues.

Viral thieves and Robin Hoods

Sometimes, when the viral DNA is packed into the capsid to be released into the environment, some bacterial DNA is packed as well. In this way, viruses can acquire new genes. This happened, for example, with the photosynthetic genes in cyanobacteria: phages actually picked up these genes from the bacteria, and now both viruses and bacteria carry them. Moreover, viruses can transfer the acquired genes to their new host, a process called *transduction*. Researchers estimate that in the ocean, about 10^{24} genes are moved from viruses to hosts each year via transduction – yet another way viruses affect life and evolution in the sea.

Phage therapy

As we mentioned before, bacteria can also be pretty nasty. Many diseases – tuberculosis, for example – are caused by bacteria. Bacteria are also responsible for most hospital-acquired infections. We have antibiotics to treat bacterial infections, but antibiotic resistance in bacteria is a huge threat to effective healthcare. 25,000 patients die every year in the European Union due to resistant bacteria. Now, as you have heard, phages kill bacteria. Why not use phages instead of (or in combination with) antibiotics to treat bacterial infections? The foe of our foe is our friend, right? Félix d'Hérelle, a doctor, had this precise idea around 100 years ago. First, he swallowed phages himself to see whether it was safe to swallow phages, and then he gave them to patients suffering from dysentery, cholera, and plague. According to his reports, the phage-treatment helped. With the discovery of antibiotics, however, the idea of phage therapy essentially disappeared. Antibiotics worked more reliably. Moreover, doctors felt more comfortable treating their patients with chemicals than with viruses. However, a scientific institute, founded in 1923 in Tbilisi, that specialized in phage therapy has persisted and continued research and clinical trials. The institute still exists today, and with the rise of antibiotic resistance, interest in phage therapy is increasing again. A few years ago, doctors used phage therapy as a last resort to save the life of a patient with a multidrug-resistant infection.

Of course, bacterial resistance to phage attack (as observed by Salvador Luria and Max Delbrück) is a threat to successful phage therapy. Spontaneous mutations can, for example, alter the receptors at the bacterial cell surface to which the phages attach. And resistance through spontaneous mutation is not the only way in which bacteria can escape viral attacks.

Bacterial defence

You might wonder if bacteria also have defences against viruses, as humans have immune systems. Indeed, bacteria also possess immune systems (though they are, of course, extremely different from ours).

Innate immunity

One component of the bacterial immune response is the so-called restriction-modification systems. The main building blocks of these systems are a restriction enzyme and a methyltransferase. Let's see what these two building blocks do and how the system works. The restriction enzyme cuts DNA at specific sequences, destroying it. In this way, it protects the cell from incoming DNA such as viral DNA. Different restriction enzymes recognize different sequences, and often the cell contains more than one type. But how does the bacteria prevent the enzyme from cutting its own DNA? After all, it is quite likely that the bacterial genome contains the same (usually short) sequences that the restriction enzymes bind to. This is where the methyltansferase becomes important. The methyltransferase modifies these exact sequences by binding a molecule (a methyl group) to the DNA. With this molecule in place, the restriction enzyme is unable to bind to the DNA, and cannot take action. It is thus a question of who arrives first: the restriction enzyme or the methyltransferase. Bacterial DNA is immediately modified after replication and is therefore protected. In contrast, incoming DNA most likely encounters the much more abundant restriction enzyme first, and so is cut and degraded. Restriction-modification systems are part of the innate immune response of bacteria to phage infection: they are readily available but not flexible – they either fit the virus or they don't.[2]

Adaptive immunity

This is different for so-called CRISPR-Cas systems. These constitute the adaptive immune system of bacteria (but not all bacteria have it). The CRISPR locus contains short repetitive sequences interspaced by so-called spacers that are themselves short DNA sequences. The repetitive sequences are (partially) palindromic, i.e., they read the same forwards and backwards. This structure gave the locus its name: CRISPR stands for 'clustered regularly interspaced short palindromic repeats'. When this locus was first discovered in a bacterial genome, its purpose was unclear. Later, researchers noticed that the spacers (the DNA sequences between the repeats) match the DNA sequences of phages (and plasmids). Now, we know why they are there: they serve as a memory of prior phage infection and help the cell fight the same virus in the future. Let us look at the entire process. There are many different CRISPR-Cas systems acting against DNA phages but the general principle is the same for all of them.

The build-up of immunological memory requires the acquisition of phage DNA; this step is called 'adaptation'. It is accomplished by Cas (CRISPR associated) proteins. These bind to short nucleotide sequences in the phage DNA that are specific to the Cas protein. These short sequences are called *PAM sequences* (protospacer adjacent motif). The Cas proteins then cut out a DNA segment from the phage genome – the *protospacer* – and incorporate it into the CRISPR locus as a new spacer. Of course, this process is only possible if the phage does not kill the cell, e.g. because restriction enzymes have already neutralized it.

Next, the cell needs to get ready to fight the phage upon re-infection. For this, the CRISPR locus is transcribed and crRNA (CRISPR RNA) is generated in a step called "biogenesis". The crRNA consists of a single spacer flanked by partial repeat sequences. Together with a Cas protein, the crRNA forms a complex that patrols the cell in search of matching foreign DNA. The role of the crRNA is to guide the Cas protein to the DNA sequence in the phage genome that matches the spacer. If this sequence is next to a PAM sequence, the complex binds and the Cas protein (the "scissor") cuts and destroys it. This final step is called "interference".

CRISPR-Cas systems thus provide a form

[2] Another response to phage infection is abortive infection, where phage-infected bacterial cells commit suicide to prevent phage replication and spread to sister cells. This does not save the cell itself but may save the population.

of acquired immunity. In this respect, they resemble our own adaptive immune systems that memorize pathogens with the help of memory B cells. But there is a fundamental difference: since in spacer acquisition the genome is modified, immunity is passed on to the daughter cells and is hence heritable.

New spacers are usually added to the same side of the CRISPR locus. This is exciting as the sequence of the CRISPR locus therefore reveals the history of phage infection of the cell lineage. You might then wonder if the bacterial genome could become intolerably large as ever more spacers are added. Yes, it would indeed, but spacers cannot only be acquired, they can also be lost. Normally, bacteria contain less than 50 spacers but in exceptional cases, there can be several hundred of them.

What about autoimmunity?

It is very important that the bacterium does not use its CRISPR-Cas system against itself. Otherwise, it would harm itself. First, to avoid autoimmunity, the cell should not use its own DNA as a protospacer, and although this sometimes happens, bacteria have evolved ways to minimise the risk. Second, the cell needs to be able to distinguish the spacer in the CRISPR locus from foreign DNA. This works because the CRISPR locus does not contain the PAM sequence to which the Cas protein binds.

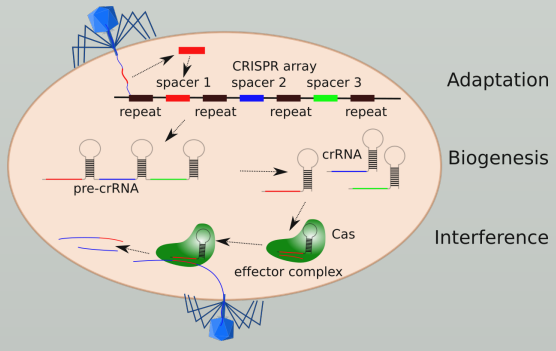
While CRISPR-Cas systems are a great way to fight viruses, they also have disadvantages. One is the risk of self-harm (see Box on autoimmunity). Moreover, as discussed above, phages are not always bad for bacteria; they can also carry beneficial genes and develop a symbiotic relationship with their bacterial hosts. In that case, a CRISPR-Cas system would be deleterious to the bacterial cell since it would destroy the beneficial phage. This is perhaps why not all bacteria contain CRISPR-Cas systems. So far, they have been found in about 50% of all sequenced bacterial genomes (note that there is a bias in which genomes we sequence: genomes of bacteria that we can cultivate in the lab are much more likely to be sequenced; the number of 50% may thus not be representative for environmental bacteria).

Summary: The three stages of CRISPR-Cas immunity

Adaptation: With the help of Cas proteins, DNA is cut out of the infecting phage and incorporated as a spacer into the CRISPR locus.

Biogenesis: The CRISPR locus is transcribed and crRNA is generated that essentially consists of the RNA of a single spacer.

Interference: The crRNA forms an effector complex with the Cas protein and guides it to the matching DNA sequence of an invading phage, where the Cas protein – the scissor – cuts it.



The stages of CRISPR-Cas immunity.
Figure based on Loureiro A. and da Silva G.J. (2019) CRISPR-Cas: Converting a bacterial defence mechanism into a state-of-the-art genetic manipulation tool. Antibiotics 8(1), 18. (License: CC BY)

The arms race between bacteria and viruses

In the main text, we saw how bacteria evolve resistance or defend themselves against phages. However, viruses can also evolve; for example, the viral sequences where the restriction enzymes cut or the PAM sequences where the Cas proteins bind can mutate. Then the bacterial defense becomes ineffective or weakened. Moreover, the viral protospacer sequences can accumulate mutations such that the spacer no longer matches. However, in the latter case, some CRISPR-Cas systems are able to react quickly. While they do not provide good immunity to the mutated phage, the old crRNA-Cas complexes with the almost-matching spacer are used to speed up the acquisition of new perfectly matching spacers. This is termed 'primed adaptation' in contrast to the 'naive adaptation' that occurs when the bacterial cell has not encountered any version of the phage before.

Glossary

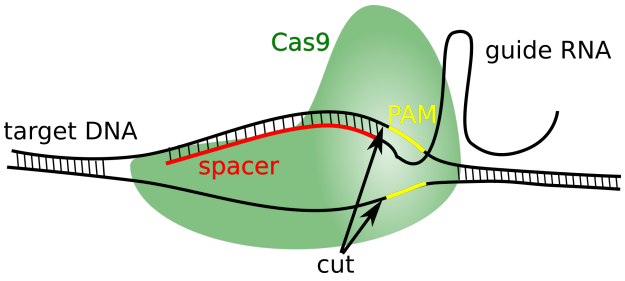
CRISPR: locus in the genome of many bacteria that contains short repeat sequences and spacer sequences and is part of the adaptive immune system
spacer: sequence in the CRISPR locus that corresponds to a DNA fragment of a previously encountered phage (or plasmid)
protospacer: DNA fragment in the viral genome that is selected as a spacer
Cas: proteins that are involved in spacer acquisition and in the cutting of invading phage DNA
Cas9: specific Cas protein
crRNA: RNA sequence of a spacer that is used to guide the Cas proteins to the corresponding position in the viral genome
transcrRNA: additional RNA molecule in the CRISPR-Cas9 system needed to build the CRISPR-Cas complex
PAM: short DNA sequence that is required for attachment of the Cas proteins to the viral genome
gRNA: RNA fragment in the synthetic system that corresponds to the crRNA of the natural system
sgRNA: gRNA + transcrRNA

From the bacterial immune system to genome editing

You have likely heard of CRISPR-Cas9, which is a new, extremely powerful tool for gene editing that allows scientists to edit the genomes of almost any organism, by inserting, deleting and modifying genes. You have probably already guessed that there is a connection to the bacterial immune system. Indeed, one of the bacterial CRISPR-Cas immune systems (that uses a Cas protein called Cas9) is the basis of the CRISPR-Cas9 technology. In this specific system, a second RNA molecule, known as trans-activating crRNA (transcrRNA), is needed in addition to the crRNA. TranscrRNA contains the sequence of the repeats of the CRISPR locus. Together, crRNA, transcrRNA and Cas9 form the complexes that find and cleave the target DNA. This structure and mechanism were discovered by Emmanuelle Charpentier, Jennifer Doudna, and their collaborators, who published their findings in a seminal article in 2012 and also recognized the potential to turn this into a gene-editing technology. Although the technology derives from bacteria, it also works in almost all other cells, in particular in eukaryotic (including human) cells, which is quite amazing. How are genes edited with CRISPR-Cas9?

Instead of the crRNA of the natural system, scientists synthesize an RNA fragment of about 20 nucleotides – the gRNA ("guide RNA") – that corresponds to the fragment in

the DNA that they want to edit. They combine it with the transcrRNA to obtain the sgRNA ("single guide RNA"). Last, the sgRNA forms a complex with the Cas9 protein that is delivered into the cell whose genome is supposed to be edited. The 'g' stands for 'guide' since the RNA molecule guides the complex to the corresponding gene in the genome, where it cuts the DNA. If you remember from above, for this to work in the bacterial cell, there needs to be a PAM sequence next to the protospacer. This is the same here: scientists need to choose DNA sequences for editing that are close to a PAM sequence. This restricts the possible sequences to some extent, but not a great deal, as PAM sequences appear frequently in the genome. Now that the DNA is cut, the cell will repair it. Normally, this will not work perfectly, and the gene is broken. In this way, CRISPR-Cas9 can be used to knock out genes. However, scientists can also provide the cell with a DNA repair template that contains additional or altered DNA and matches the broken genome pieces at the ends. In this way, scientists can introduce mutations or add entire DNA sequences into the genome.



Scheme of the CRISPR/Cas9 complex.

The CRISPR-Cas9 system was the first one to be turned into a genome-editing tool. Since then, additional technologies that are based on other bacterial CRISPR systems with different Cas proteins have been developed. For instance, a close cousin of the Cas9 protein, Cas13, is able to edit RNA instead of DNA. As you know, during protein production, DNA is transcribed to RNA, which is then translated into a protein. By editing RNA, CRISPR-Cas13 can be used to modify the expression of genes without the permanence of a DNA edit.

CRISPR-Cas genome editing is a revolutionary technology that does not only lay the ground for future scientific discoveries but also for innumerable applications in agriculture and disease treatment. One of its advantages is that it is cheap, quick, and easy (compared to other gene editing techniques).

Applications of CRISPR-Cas genome editing

Even though the discovery of the CRISPR-Cas mechanism and the development of the technology are pretty new, scientists have already performed numerous studies to push the application of this technology in agriculture and disease treatment. For instance, it has been used to alleviate genetic deafness in mice and to make mushrooms brown less easily. We hope that CRISPR-Cas will help us to develop crops that are more nutritious, delicious, drought-tolerant, and/or resistant to pests. For example, scientists are working to apply CRISPR-Cas to make bananas resistant to a fungal disease that is threatening banana production all over the world. Moreover, CRISPR-Cas shows great potential to treat genetic diseases such as Huntington's disease, cystic fibrosis, and sickle cell anemia. To accomplish this, respective genes in the concerned tissues are edited. E.g. to treat sickle cell anemia, the genomes of stem cells in the bone marrow (from which the red blood cells are continuously generated) are modified to produce healthy hemoglobin. This was applied in a patient last year, and seems to have been successful so far. Importantly, these treatment strategies do not involve modifications of DNA in the germline, meaning that the modifications are not inheritable. Most researchers consider editing genomes in the germline to be unethical. Another medical application is the treatment of infections caused by human viruses that incorporate their DNA in the human genome – such as HIV. In these cases, CRISPR-Cas could be used to cut the viral DNA out of the human genome. In in vitro experiments, scientists have managed to

remove the HIV genome from T-cells obtained from infected patients.

Questions of safety and of ethics

As with all new technology, safety is a big concern. Moreover, ethical questions may arise – how should we be allowed to use it?

Crops. While CRISPR-Cas can be used to insert entirely new genes, other applications rely on the modification of existing genes that could also occur naturally, as in traditional breeding. In traditional breeding, mutations occur randomly, and irradiation is often used to increase the number of new mutations and hence the genetic variation. In contrast, with CRISPR-Cas, specific mutations can be purposefully introduced, tremendously speeding up the development of new crop variants. Although these crop variants are no different from those that could arise naturally, the Court of Justice of the European Union decided in July 2018 that *all* CRISPR-Cas edited crops fall under the regulations for genetically modified organisms. This means that they must undergo a long and tedious approval process before they can be cultivated.

Humans. While the CRISPR-Cas technology is normally precise, some small fraction of edits happen at the wrong site. Moreover, some studies have shown that CRISPR-Cas editing may select for cancer-prone cells. Another problem is potential immune reactions to the editing tool. All these risks need to be assessed and minimised before CRISPR-Cas technology can be used routinely in disease-treatment. Furthermore, especially when it comes to editing of cells in the germline or of human embryos, where the future individual will carry the edits in many or all the cells of its body, one huge risk is that we do not understand the function of a gene well enough. The edit might therefore well have deleterious side effects that were unforeseeable.

Apart from these risks, there are also concerns that editing of embryonic cells may not only be used for disease prevention, but also to 'design' babies (although we are still far from being able to do this). This leads to very fundamental ethical questions: What are we allowed to do? Will we value some human life (with the desired characteristics) higher than others? What does this imply for the humans that are born? Will there be an obligation to 'design'?

These risks and ethical concerns are why most scientists are not (yet) using CRISPR to edit human DNA in the embryo or germline. However, in November 2018 a scientist in China announced the birth of a pair of twin girls whose genes he had edited with CRISPR-Cas. The modification was supposed to make them resistant to HIV. The girls had been born in secrecy in October 2018. This experiment has been widely condemned as immoral and irresponsible. The scientist, He Jiankui, was sentenced to three years in prison on 30 December 2019.

Conclusion

Tiny as they are and not even properly alive, phages have a huge influence on our world. By infecting bacteria in natural environments, they contribute to the shaping the ecosystems of the Earth. By infecting human pathogens, they can be harmful or beneficial to human health. Thanks to their small sizes and their fast replication, bacteria and phages are great study systems in the lab, in addition to their roles in the environment. In this way, they have contributed to fundamental scientific discoveries and the development of new technologies. We don't know what new breakthroughs the future will bring, but they will certainly be exciting!

Other useful resources

- Phages killing bacteria: `https://twitter.com/MarraffiniLab/status/1211864511918956545`
- A video about phages: `www.youtube.com/watch?v=YI3tsmFsrOg`
- CRISPR-Cas9 technology explained by Jennifer Doudna, one of its inventers: `https://www.youtube.com/watch?v=TdBAHexVYzc`

Instructions for the competition

The deadline for this series is **midnight on February 24th, 2020.**
Questions: Answer the questions from the Questions section in the online form here: `https://forms.gle/tNGsKztqXUFnshXz9`. A question may have multiple correct answers.
The project: This time, you will write an assay. Follow the instructions in the project section. Send the essay as a pdf, named `Lastname_Firstname_ProjectB3.pdf` to **EvoBioSeminar@gmail.com**.

Project: Your own title

This time, you won't need any colorful paper, scissors or dice. Instead, you will need to google, think, think even more, and write! Write a short essay (about 700 words) on the CRISPR technology. Follow the instruction below to write a good essay.

1. Give an example of a specific hereditary disease that could potentially be treated with this technology. Briefly describe the disease.
2. Describe in detail one possibility how CRISPR could be used to treat this disease: which part of the genome would need to be edited, which cells would be treated, at what age or life stage should the patient be treated, etc.
3. Describe how the CRISPR editing procedure could change the condition caused by the deficient genes or what in the human body would work differently following the procedure.
4. Describe the possible drawbacks and ethical considerations that would arise if the CRISPR method of gene editing became widely used.
5. Suggest some government regulations and guidelines that should be followed by scientists and medical workers using CRISPR to edit the human genome.
6. Cite your references and provide a bibliography.

Express yourself clearly and concisely. We are not counting your words, we're interested in your ideas! **Your essay will be graded based on the quality of the content and the clarity of your writing.** Specifically, we will judge your work based on the following questions:

1. Did you argue well why the chosen disease would be suitable to be treated by CRISPR?
2. Did you explain clearly how your proposed procedure would work?
3. Do your statements and reasoning make sense?
4. Are your ideas based on clearly stated facts?
5. Is it easy to understand your thoughts?
6. Do sentences follow a logical order and make sense together?

CRISPR is a hot, important topic that is not only exciting for biologists – it could change mankind and nature forever, and should be of great interest to every human. We're looking forward to reading your thoughts!

Questions:

Answer the questions from the Questions section in the online form here: `https://forms.gle/tNGsKztqXUFnshXz9`. A question may have multiple correct answers. You can get up to 30 points for this part.

1. **Which of the following statements is/are correct?**
   (a) All bacteria cause diseases in humans.
   (b) All viruses cause diseases in humans.
   (c) All viruses need bacterial cells to reproduce.
   (d) All viruses infect bacteria.
   (e) Most viral infections can be treated with antibiotics.
   (f) Suitable viruses can be used to treat bacterial infections.
2. **Viruses are capable of which of the following?**
   (a) They can metabolize organic compounds.
   (b) They can use the host cell to make copies of themselves.
   (c) They can have their proteins made by the host cell.
   (d) They can make copies of their DNA within the host cell and then make their proteins after leaving the cell.
   (e) They can mutate and evolve.
   (f) Some viruses are able to reproduce on their own, given the right humidity and temperature and the availability of suitable nutrients.
3. **Diphtheria is a bacterial infection of the upper respiratory tract. If it remains untreated, the mortality rate is high. The bacterium that causes Diphtheria is called *Corynebacterium diphtheriae*. The deleterious effect on our health is caused by a toxin – the Diphtheria toxin – that kills our human cells. The *tox* gene encoding this toxin is located on a prophage. Which of the following statements is/are true?**
   (a) Strains of *Corynebacterium diphtheriae* that do not carry the prophage cause Diphtheria as well.
   (b) Since the toxin is produced by a virus, Diphtheria is a viral disease. It thus cannot be treated with antibiotics.
   (c) The *tox* gene cannot be transferred to uninfected cells of *Corynebacterium diphtheriae*.
   (d) Vaccination protects against Diphtheria.
   (e) Antitoxins can be used for treatment.
   (f) Mutations in the *tox* gene can lead to the production of an altered non-toxic protein.
4. **Consider a population of bacteria that do not possess any CRISPR-Cas system. Which of the following statements is/are correct?**
   (a) When phages are added to the bacterial culture, beneficial mutations are induced that allow the bacteria to survive.
   (b) When phages are added to the bacterial culture, some 'lucky' bacteria may contain beneficial mutations that allow them to survive.
   (c) In a bacterial population originating from a single bacterial cell, all cells are identical.
   (d) If bacterial mutations were induced by the addition of phages, we would expect all bacteria to survive.
   (e) If bacterial mutations were induced by the addition of phages, we would expect more or less the same number of bacteria to survive and produce a colony every time we repeat the experiment.

(f) If bacterial mutations were independent of the environment, we would expect more or less the same number of bacteria to survive and produce a colony every time we repeat the experiment.

5. **Which of the following statements about the CRISPR-Cas9 technology are correct? CRISPR-Cas9...**

(a) is a new tool for DNA-editing.
(b) is based on a defence mechanism in bacteria.
(c) is based on the viral immune system against bacteria.
(d) is based on a system with which viruses modify the bacterial genome.
(e) does not work in cells of the germline.
(f) does not work in eukaryotic cells.