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Genome editing with CRISPR-Cas9

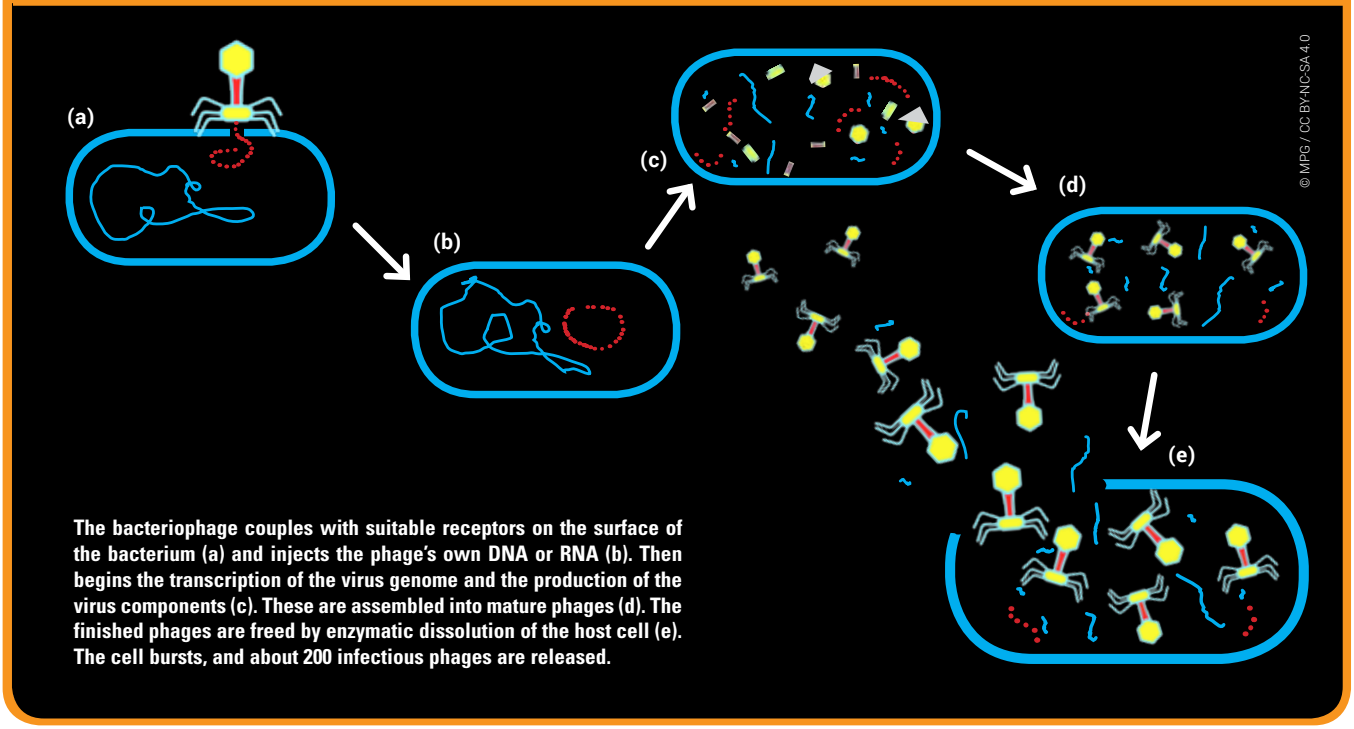
– what is now possible?

Who wouldn't want to see a real-life dragon? In their book *"How to Build a Dragon or Die Trying"*, US biologist and writer Paul Knoepfler and his 17-year-old daughter Julie explain how they would build their own dragon – thanks to one of the greatest technological innovations of recent times, genome editing. The authors write: *"While we marvelled at the cool science that already exists, we also realized – in the middle of our dragon-building plans […] – that things could go catastrophically wrong for us".* The book provides a satirical view of the most groundbreaking science. But we need to ask ourselves: what is satire and what is reality?

Advances in genomic biotechnology may offer the possibility of bringing back long extinct species – or at least "replacement" species with characteristics and ecological functions similar to those of the originals. A team led by George Church at Harvard University is trying to bring mammoths back to life by transcribing – nucleotide by nucleotide – the genome of its evolutionary relative, the Asian elephant, which is still alive today. This has been possible since researchers at Pennsylvania State University in 2008 succeeded in sequencing the genetic material of an extinct woolly mammoth for the first time, thus theoretically providing access to the information for its characteristics. Over four billion DNA bases have been decoded **(see Biomax 33)**.

The mammoth is more suitable than almost any other extinct vertebrate for the analysis of prehistoric genetic material. This is because the fossils of the ice-age elephants originate mainly from the permafrost of Siberia, where they are relatively well preserved. The closest living evolutionary relative of the woolly mammoth is the Asian elephant. According to genetic analyses by Svante Pääbo and his team at the Max Planck Institute for Evolutionary Anthropology, the Asian elephant and the woolly mammoth split into different species around 440,000 years ago. The genomes of the woolly mammoth and the Asian elephant therefore differ "only" by about 1.4 million mutations. An Asian elephant is therefore practically already 99.96% woolly mammoth.

FIG. A: LIFE CYCLE OF A BACTERIOPHAGE



The bacteriophage couples with suitable receptors on the surface of the bacterium (a) and injects the phage's own DNA or RNA (b). Then begins the transcription of the virus genome and the production of the virus components (c). These are assembled into mature phages (d). The finished phages are freed by enzymatic dissolution of the host cell (e). The cell bursts, and about 200 infectious phages are released.

JURASSIC PARK – FROM FICTION TO REALITY?

In 2015, the Harvard Woolly Mammoth Revival team first analyzed the genome of a woolly mammoth and then artificially produced exact copies of certain mammoth genes. These were successfully incorporated into **fibroblast cell lines** of the Asian elephant. "Most of the genes we took have something to do with cold resistance – genes for long fur, smaller ears, the storage of subcutaneous fat and, above all, for mammoth haemoglobin", George Church told the media. Although it is an initial success, it is only relative. Even if researchers limited themselves to the bare minimum, in order to obtain a genome very similar to that of the mammoth they would, according to a conservative estimate, have to replace a few hundred thousand genome segments. What's more: they do not yet know all sequences relevant to the mammoth characteristics.

Nevertheless, the US scientists also want to study the expression of mammoth mutations in living elephant cells in order to test predictions about gene function. How does evolution shape the same gene to adapt to tropical habitats in one version or cold habitats in an alternate version? This research not only forms the basis for the **"de-extinction"** of the mammoth but also provides potentially valuable insight into evolution under different climatic conditions. The findings could reveal new approaches for genetic biotechnology that would facilitate adaptation for wildlife threatened by climate change. But at this point in time, this is all still a long way off.

A WEAPON AGAINST BACTERIOPHAGES

Which technology has so fundamentally expanded the possibilities of molecular biology in recent years and sparks the imagination of scientists? Let's go back to the year 1987: When studying *E. coli* bacteria, Japanese microbiologists came across unusual, repetitive DNA sequences in the genetic material of a bacterium. "The biological significance of these sequences is completely unknown", they wrote. Shortly after this, microbiologist Francisco Mojica from the University of Alicante took a closer look at these sequences. They can be read forwards and backwards like the **palindromic** words "madam" or "racecar" in human language. But while these words have a meaning, palindromes make no sense in the vocabulary of genetics; they cannot be translated into functional proteins.

Mojica called these sequences *Clustered Regularly Interspaced Short Palindromic Repeats* – or CRISPR for short. In 2005, he discovered that they matched segments of the genome of a **bacteriophage**, a virus harmful to bacteria. He suspected that CRISPR had the function of an **adaptive immune system** in bacteria. Two years later, a French scientist from Danisco, the world's largest producer of food additives, actually succeeded in providing experimental evidence when investigating the streptococci used in the production of yoghurt. Philippe Horvath and his colleagues integrated segments of phage DNA into the CRISPR fragment and were thus able to combat subsequent phage attacks.

Bacteria are constantly under attack by bacteriophages. This is because they are unable to reproduce independently. They must "hijack" another organism into which they can introduce their genetic material. The foreign genes introduced by the phage reprogram the genome of the host. The bacterium no longer produces proteins for itself but instead becomes a small "phage factory." It works at full capacity until the bacterial cell is full of phages and then bursts so that the phages are released (Fig. A).

However, bacteria have developed defence mechanisms to defend themselves against such infections. If the enzymes of a bacterium succeed in cutting the injected viral DNA into small pieces, other enzymes incorporate these fragments into the CRISPR fragment in the bacteria's own genome. The strangely constructed sequences thus represent a "memory" of past viral infections. In this respect, it is a library of all pathogens the bacterium has been exposed to. This library can even be passed on to its offspring.

THE LAST PIECE OF THE PUZZLE IN THE CRISPR-CAS SYSTEM

In 2011, French microbiologist Emmanuelle Charpentier at the University of Umeå in Sweden investigated how the underlying mechanism of immune defence works. Charpentier found the final piece of the puzzle in the CRISPR-Cas system by performing RNA sequencing on a *Streptococcus* bacterium and discovering two short fragments of RNA. The bacterium transcribes the foreign DNA in the CRISPR fragment into an RNA molecule called CRISPR-RNA (crRNA). This CRISPR RNA is essentially a molecular profile. It provides the recognition sequence with which the enzyme Cas9, a nuclease, detects the corresponding DNA sequence of the invading virus. However, in order for Cas9 to become active, a second small RNA, trans-activating CRISPR RNA (tracrRNA) is required. Only the crRNA and tracrRNA complex lead the Cas enzyme to its target. By cutting both strands of viral DNA, Cas9 prevents successful infection by the bacteriophage (Fig. B).

Together with Jennifer Doudna from the University of California at Berkeley, Emmanuelle Charpentier, who currently heads the Max Planck Unit for the Science of Pathogens in Berlin, achieved the decisive technological breakthrough one year later. In the laboratory, they fused the crRNA and tracrRNA into a single molecule, a **single guide RNA**. To use the CRISPR/Cas method, only one RNA must be cloned. The two female scientists thus radically simplified the functional principle of CRISPR-Cas9 commonly referred to as **gene scissors**.

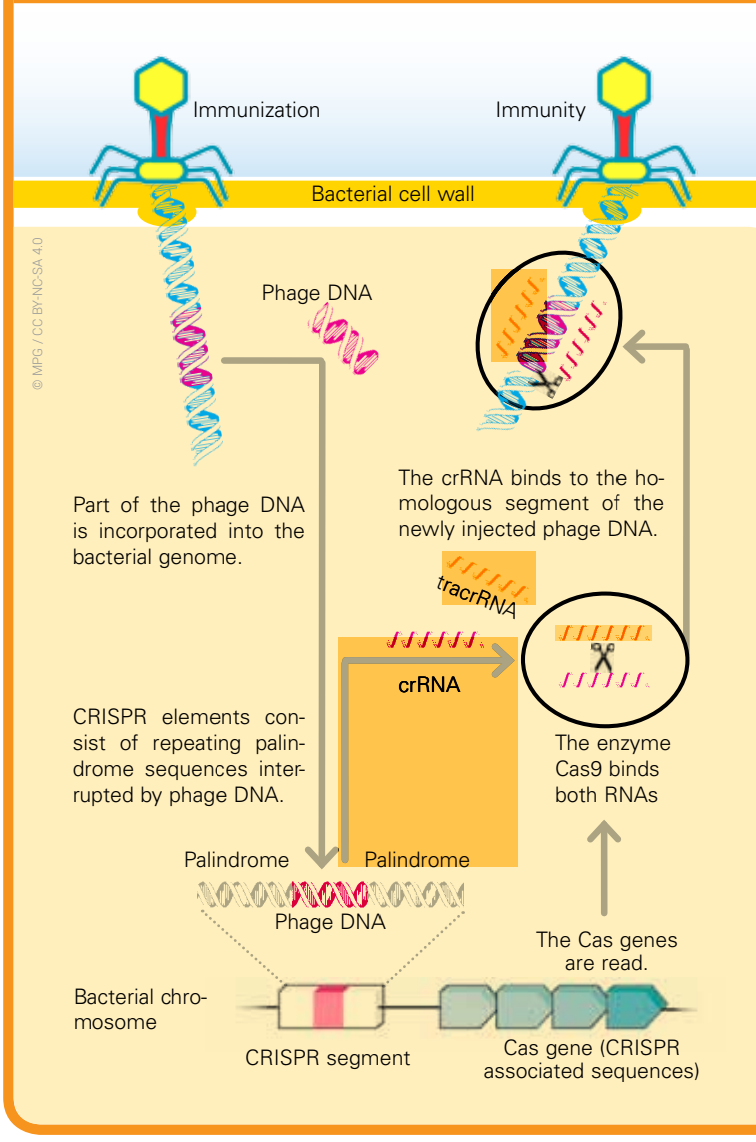
In 2013, biochemist Feng Zhang, a researcher at the Broad Institute of MIT and Harvard University, successfully adapted CRISPR-Cas9 for **genome editing** in eukaryotic cells. Zhang and his team succeeded in targeted genome editing in cultured mouse and human cells. They showed that the **CRISPR-Cas system** can be programmed to modify different genomic segments. George Church, who wants to revive the woolly mammoth, reported similar results in the same issue of Science.

Genome editing is not new; various techniques have been around for years. What makes CRISPR so revolutionary is its precision. It is also incredibly cheap and easy. Researchers used to have to spend thousands of dollars and weeks or months in the laboratory to modify a gene. Now it costs only about 75 dollars and takes only a few hours. This technique has also worked in every organism it has been tried on – from nematodes and plants to humans.

CRISPR is now one of the hottest areas of research. In 2011, there were fewer than 100 publications on CRISPR; in 2018, there were more than 17,000. The number will continue to increase with new techniques for manipulating genes and improvements in precision as well as other types of CRISPR proteins that also work as gene editors. For example, Cas13 can edit RNA instead of DNA. "We have reached the point where the efficiency of gene processing is at a level that will clearly be useful both therapeutically and for various other applications", said Jennifer Doudna in an interview.

This is precisely why, for some years now, there has been an intense legal battle over who should be granted the potentially lucrative patent rights for the CRISPR technology. In September 2018, a US Federal Appeals Court rejected the objections of the University of California at Berkeley and upheld the patents of the Broad Institute for some CRISPR applications. In turn, the European regulatory authorities have granted the university basic patents in Europe. These cover the single guide RNA for CRISPR-Cas9 in all areas, including eukaryotic cells. In 2019, the University of California presented new documents challenging the decision of the US authorities. The patent battle wages on.

FIG. B: IMMUNE DEFENCE AGAINST BACTERIA



NO INTERFERENCE IN THE HUMAN GERM LINE

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In November 2018, Chinese researcher Jiankui He reported the birth of twins in whom he had specifically switched off a gene at the embryonic stage, thus triggering a worldwide ethical debate. In an October 2019 position paper on genome editing, the Max-Planck-Gesellschaft declared that because of the consequences of artificial hereditary mutations (e.g. those caused by genome editing of **germ line** cells), it will not carry out any research on the genetic modification of germ line cells until further notice. The German Ethics Council also considers germ line interference to be too risky at present but does not want to rule them out ethically.

GENOME EDITING – OPPORTUNITIES AND RISKS

Many doctors are convinced that by editing genes they can, for example, treat hereditary diseases in which one or more genes do not function properly. They want to use it for mutations that trigger Huntington's disease or cystic fibrosis, among others. Experiments on mice have shown that faulty gene segments (as they also occur in human hereditary diseases) can be removed by editing genes and in this way the various symptoms can be treated.

One of the biggest problems in trying to change human DNA is the **"off-target"** effects. These occur when Cas9 cuts a piece of DNA for which it has not been programmed. This is like programming the navigation device in your car with the address "restaurant". In each city, this search leads to numerous individual locations. But which location is the correct one? In the same way, Cas9 is guided by the guide RNA to its DNA target. If the address given by the guide RNA is not unique – which is easily possible with only 20 base pairs – Cas9 is guided to several sites, where it cuts the DNA. This could lead to unwanted and serious side effects, including cancer. For any therapeutic application in humans using CRISPR, minimizing these off-target effects is therefore of utmost importance.

However, there are already promising initial approaches. Two patients with **beta-thalassaemia** and **sickle cell anaemia** were treated with the CRISPR technique. In both diseases, the production of the blood pigment haemoglobin is disturbed. Until now, they were treatable only with frequent blood transfusions. However, these have life-shortening side effects. Patients have now been able to do without blood transfusions for months as reported by the Boston on-line magazine STAT in November 2019. The gene therapies were developed by the biotech companies Vertex Pharmaceuticals and CRISPR Therapeutics. Emmanuelle Charpentier, who founded CRISPR Therapeutics, happily told the press "that CRISPR-based gene therapies have a beneficial effect on patients with beta thalassaemia and sickle cell anaemia after a single treatment". However, the study has not yet been completed; a total of 45 patients are to be treated. For many of them, the treatment has not even started. It is not yet possible to say whether the therapy will work forever and whether it will show side effects – possibly at a much later date.

Keywords

fibroblast cell lines, de-extinction, bacteriophages, palindrome, adaptive immune system, CRISPR-Cas system, gene scissors, single guide RNA, genome editing, off-target effects, beta thalassaemia, sickle cell anaemia, germ line

Additional reading

- Doudna, J. A. & Sternberg, S. H. (2017). *A Crack in Creation: Gene Editing and the Unthinkable Power to Control Evolution.* Houghton Mifflin Harcourt.
- Knoepfler, P. & Knoepfler, J. (2019). *How to Build a Dragon or Die Trying: A Satirical Look at Cutting-Edge Science.* Singapore: World Scientific.

Links

- *Genome-Editing with CRISPR*
  www.mpg.de/10729275
- *Statement on Genome Editing*
  www.mpg.de/13509625
- *Mammoth Revival*
  https://reviverestore.org/projects/woolly-mammoth

Video-Tipp

- *Genome-Editing with CRISPR (english subtitles)*
  www.t1p.de/mpg-Gen-editing > *YouTube*

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