THE NOVOZYMES PRIZE

PROFESSOR EMMANUELLE CHARPENTIER
PROFESSOR VIRGINIJUS SIKSNYS

2017
The 2017 Novozymes Prize is being awarded to Emmanuelle Charpentier and Virginijus Siksnys for their groundbreaking research that forms the basis of CRISPR-Cas9 technology, which provides revolutionary ability to edit genomes for biotechnological science.

Two key advances in the ability to manipulate cellular DNA and genomes have arisen from the study of bacterial antiviral defence systems. The first came with the discovery and harnessing of bacterial restriction-modification systems in the 1970s for DNA cloning and genetic engineering. The second has arisen over the past five years from studies of the CRISPR-Cas bacterial adaptive immune system, which has given rise to innovative technologies for genome engineering in a broad spectrum of organisms.

For years, scientists have searched for easy ways to manipulate the genomes of living cells with high efficiency and precision, but this has generally been possible in only a few selected model organisms. With the discovery that the virus protection system known as CRISPR-Cas9 can be recruited for genome editing in virtually any organism, biotechnological developments involving metabolic engineering and other types of biosynthetic optimization can now progress much faster, and previous bottlenecks in choosing organisms for the biotechnological processes have been largely eliminated.

The CRISPR-Cas9 system is recognized for its wide applicability in genome editing approaches. The advantage of this system is that a single effector protein, Cas9, carries a duplex of RNA molecules, the dual-tracrRNA-CRISPR RNA (crRNA), which guides the DNA cleavage activity of Cas9, with the sequence of the crRNA determining the DNA target sequence that will be cleaved. Cas9 has been hailed as a programmable DNA scissor, which has proven to be extremely useful for genome engineering approaches.

Emmanuelle Charpentier and Virginijus Siksnys made key discoveries that paved the way for developing these novel tools for genome editing applications. These have truly transformed the prospects for genome engineering, bringing vast potential benefits for fundamental science, biotechnology, food production and the treatment of human disease.

Virginijus Siksnys studied chemistry at Vilnius University and obtained his PhD degree from Lomonosov Moscow State University before returning to Vilnius University, where he has held the position of Professor since 2002. Currently he is chief scientist and department head at the Institute of Biotechnology of Vilnius University and serves as a Chairman of the Institute's Board. Virginijus Siksnys is a member of the Lithuania Academy of Sciences, and his work has been recognized by several awards, including...
the Lithuanian Science Award. In 2016, he was awarded the highly prestigious Warren Alpert Foundation Prize (with four other scientists), recognizing his seminal contribution to the discovery and development of the CRISPR-Cas9 system for genome engineering. Also in 2016, Siksnys was elected as a member of the European Molecular Biology Organization – the first from Lithuania to receive this honour.

From his earlier research on restriction endonucleases, Virginijus Siksnys significantly contributed to understanding the mechanism of these enzymes, but his work on CRISPR-Cas nucleases has had even greater scientific impact. The activities of two *Streptococcus thermophilus* CRISPR-Cas systems, Type I Cas3 and Type II Cas9, were studied first. The laboratory of Virginijus Siksnys was able to elucidate the key activity mechanisms of both Cas3 and Cas9. More recently, his group investigated a third type of CRISPR-Cas systems, Type III-A. In all these cases, the findings were based on solid biochemical research involving recombinant, purified proteins and activity assays.

In 2011, Virginijus Siksnys and colleagues showed that CRISPR effector systems could be introduced by genetic engineering into unrelated bacterial species. The resulting strains showed enhanced immunity against invasive nucleic acids such as phages or plasmids. This opened the door to engineering bacterial strains with biotechnological potential for improved resistance to viral attack. Subsequently, Virginijus Siksnys capitalized on his wealth of expertise in studying nucleic acid enzymes to carry out experiments showing that the Type II CRISPR enzyme Cas9-crRNA complex could be reprogrammed using designed crRNA species to cleave double-stranded DNA targets specifically.

The professional life of Virginijus Siksnys has been devoted to studying enzymes that manipulate DNA – enzymes that have had profoundly affected biotechnology over decades. In the past 6 years, Virginijus Siksnys has been a leader in the explosive development of the CRISPR-Cas field. His articles are characterized by stringency, high-quality data and insightful analysis. Based on this, Virginijus Siksnys is clearly a worthy candidate to receive the Novozymes Prize.

Emmanuelle Charpentier studied Biology, Microbiology, Biochemistry and Genetics at the University Pierre and Marie Curie in Paris and obtained a PhD degree in Microbiology from the Pasteur Institute in Paris in 1995. After her PhD, she went to the Rockefeller University for postdoctoral research (1 year), and following this she held positions as research associate at various institutions in the United States. She obtained an independent position (Lab Head) as Assistant Professor at the Department of Microbiology and Immunology at the University of Vienna in 2002, where she was later tenured as Associate Professor in 2006. After 4 years (2009–2013) as head of the Laboratory for Molecular Infection Medicine at the Department of Molecular Biology at the University of Umeå in Sweden, she moved to the Helmholtz Centre for Infection Research and Hannover Medical School, where she took a position as Head of the Department of Regulation in Infection Biology. In 2015, she became Director of the Department of Regulation in Infection Biology of the Max Planck Institute for Infection Biology in Berlin, a position she currently holds, together with a visiting professor position at the University of Umeå and an honorary professorship at the Humboldt University of Berlin. Emmanuelle Charpentier has received a long list of awards, including the Breakthrough Prize in Life Sciences. She has been elected a member of the European Molecular Biology Organization, the German National Academy of Sciences, Leopoldina and the Royal Swedish Academy of Sciences.

Emmanuelle Charpentier’s main research focus has been on bacterial infections and antibiotic resistance. She has made several contributions to understanding the mechanisms by which pathogenic bacteria acquire resistance to antibiotics, such as by modifying transporters and signal transduction. This led her to study the role of small RNAs in bacteria, and investigation of CRISPR RNAs was a natural consequence. The studies of Emmanuelle Charpentier and co-workers identified the key tracr-RNA of the CRISPR-Cas9 system and demonstrated that tracrRNA forms a duplex of RNAs by base-pairing with the CRISPR RNA of the CRISPR system (crRNA), deciphering further the mechanisms of co-maturation of the RNAs. It was further shown that this chimeric mature two-RNA structure directs the CRISPR-associated protein Cas9 to introduce double-stranded breaks in target DNA. They also demonstrated that the dual RNA, when engineered as a single RNA chimera, also directs sequence-specific Cas9 double-stranded DNA cleavage and that changing the sequence of the dual RNA could reprogramme the Cas9 enzyme to a target site of choice. This was the origin of an efficient genome editing strategy using CRISPR-Cas9.

Emmanuelle Charpentier has developed a world-class research programme in the CRISPR-Cas field and is regularly invited as a keynote speaker at international scientific meetings. Based on her pioneering research, it is therefore well founded to award Emmanuelle Charpentier the 2017 Novozymes Prize.

CONCLUDING REMARKS
The 2017 Novozymes Prize is being awarded for the first time to honour research leading to an exceptional scientific discovery, which has already had striking positive impact in biotechnology. The rapid progression of green, sustainable solutions towards next-generation bioproduction of chemicals and medicine largely depends on technological breakthrough developments, which will enable cell factories to be designed and optimized more easily and rapidly. The CRISPR-Cas technology for rapid and precise engineering of a broad spectrum of relevant production organisms clearly has the potential to create a revolution in future biotechnological innovations. The technology was pioneered by Virginijus Siksnys’ characterization of the properties of the Cas9 nuclease and demonstration that Cas9 specificity is directed by CRISPR RNA and by Emmanuelle Charpentier’s demonstration of the dual-tracrRNA-CRISPR RNA structure required for guiding the Cas9 enzyme to its target on the genome. For these groundbreaking complementary discoveries, Virginijus Siksnys and Emmanuelle Charpentier share the 2017 Novozymes Prize.
A HUNT FOR BACTERIAL TREASURES

BY MORTEN BUSCH

Emmanuelle Charpentier has been one of the driving forces behind harnessing a bacterial immune system into the transformative CRISPR-Cas technology that has the potential to cure genetic diseases. Nevertheless, she asserts that she still has not achieved her goal, since bacteria comprise an inexhaustible resource of knowledge to be exploited for further biotechnological and biomedical applications.

Bacteria can be compared to the characters of Dr. Jekyll and Mr. Hyde. Just when we think we know them, they surprise us completely. At their worst, bacteria can kill people. Conversely, at their best, bacterial populations such as those in our gut microbiome can improve our health. Emmanuelle Charpentier has dedicated her life to answering fundamental questions about physiological and regulatory processes in bacteria.

This mission led her to investigate a bacterial immune system called CRISPR-Cas9 and to develop this system into a genome engineering technology.

“Bacteria have always fascinated me. The incredible diversity of the bacterial world testifies to how evolution works in practice. There are countless types – those that make us sick and those that help us. Research on bacteria is invaluable because it gives us not only strategies to combat multidrug-resistant bacteria but also tools such as CRISPR-Cas9 for biotechnological and medical applications,” explains Emmanuelle Charpentier, Director of the Department of Regulation in Infection Biology at the Max Planck Institute for Infection Biology in Berlin, Germany.

Emmanuelle Charpentier has led a diverse and highly mobile research career, and has experienced both the positive and negative aspects of such a varied path. She has strived for 20 years to be recognized by a research field that didn’t place much value on moves between universities and research environments.

“I have always considered it important to follow my intuition. From the beginning, I believed that I – like many other professors – would eventually find a new niche and open a new field of research for myself and others. However, it slowly dawned on me that I was not looking for a specific theme, and that I enjoyed the freedom of exploring different fields. Throughout my career, the red thread has been working at the crossroads between basic, clinical and applied research.”
Bacteria use CRISPR-Cas systems to provide adaptive immunity against foreign invaders such as viruses. Upon infection, new foreign DNA sequences are captured and integrated into the bacterial CRISPR locus as spacers. The CRISPR locus is transcribed to generate CRISPR RNAs, each with one unique spacer sequence. Each CRISPR RNA associates with the Cas enzyme and guides the enzyme to DNA sequences of attacking viruses that match the CRISPR RNA sequence. Finally, the Cas enzyme cuts the DNA to destroy the virus. In some systems – such as the CRISPR-Cas9 system – a trans-activating CRISPR RNA is required.

“My research should be novel, and it should make a difference.”

The discovery of CRISPR-Cas9 genome editing, and the recognition that came with it, have encouraged Emmanuelle Charpentier to further explore the world of bacteria for additional potential breakthroughs.

FACE-TO-FACE WITH ANTIBIOTIC RESISTANCE

The hunt to reveal the secrets of bacteria began at the Pasteur Institute in Paris. Emmanuelle Charpentier, a young PhD student working in the laboratory of Patrice Courvalin, started to work on antibiotic resistance, which was emerging as a major worldwide health threat. At that time most antibiotics were still effective, so the discovery of multidrug-resistant Listeria bacteria came as a huge shock. These bacteria can cause meningitis and blood poisoning among infants and older people.

“It certainly makes you want to make a difference when, as a young person, you find yourself looking at the test results from an 84-year-old man with meningoencephalitis whose life was being threatened by multidrug-resistant bacteria. This was the moment that piqued my interest in the physiology of bacteria and clinical research. How did the resistance occur, and how could we develop treatment?”

Charpentier and Courvalin showed that the transfer of genes that confer resistance to antibiotics occurs not only between bacteria of the same species but also between bacteria of different species. For instance, Listeria could inherit resistance genes from bacteria in the human gut. In this way, bacteria can rapidly pick up each other’s tricks for combating specific types of antibiotics.

“Although I had already become interested in bacteria, clinical research and developing treatments, I understood that I had to go out into the world and forge my own research field. I figured that if I went to the United States and became an expert in streptococci, I would be able to return and start my own research group within this field.”

THE FIRST MAJOR BREAKTHROUGH

In 1996, Charpentier relocated to the laboratory of Elaine Tuomanen at the Rockefeller University in New York City and then to St. Jude Children’s Research Hospital in Memphis, Tennessee with the purpose of studying streptococci, which are an even greater health threat than Listeria. But when she arrived, stark differences in the research environment in the United States gave Charpentier cause for thought.

“Witnessing the massive biotechnology efforts that gripped the United States from the mid-1990s onwards was incredibly inspiring. As a medical microbiologist coming from a more conservative European research tradition, it was incredible to see how the research environment in the United States built bridges between the academic world and the pharmaceutical industry.”

Charpentier’s research also scaled new heights under Elaine Tuomanen, some of whose research focused on life-threatening streptococcal diseases among children. The antibiotic vancomycin had been the last bastion of defence against these bacteria, and researchers knew that a catastrophe would be inevitable when this antibiotic ceased to work. Their critical discovery of the molecular mechanism in the streptococci that caused the vancomycin resistance was published in Nature, one of the world’s most prominent journals.

“I sought out and was fortunate to find the best mentors. Each one has greatly influenced my career. The combination of basic and applied research has also driven me forward. Finally, it has been important for me to constantly re-evaluate my research, to be open for changes in direction and hopefully make a difference in my field of research.”

Before her next move, Charpentier and her fellow researchers, especially Rodger Novak, succeeded in revealing important details about the mechanisms of antibiotics. What they discovered was that antibiotics such as penicillin do not directly kill cells but first initiate a process of apoptosis in the bacteria that ultimately leads to the bacteria dissolving their own cell membranes and dying.

A DECISION TO STAY IN ACADEMIA

Although Emmanuelle Charpentier had actually planned to return to Europe after her stay in the United States, she extended her visit with positions at the New York University Langone Medical Center and the Skirball Institute of Biomolecular Medicine in New York City. However, no long-term positions were waiting for her when she returned to Europe in 2002.

“It was as if they considered it a fault that my research career had not focused on a specific theme. I instead had found a way of carrying out research that integrated basic and medical research and spanned studies of bacteria and eukaryotes. I had become used to the research freedom in the United States. In those days, the research environment in Europe was much more old-fashioned.”
Although at one point Charpentier considered applying for a job in biotechnology, she ended up being recruited as a junior group leader at the University of Vienna, where she had the freedom to develop her own research group. For a variety of reasons, she became interested in the emerging regulatory role of RNA molecules in bacteria and set out to discover and characterize novel RNAs in streptococci.

“The research clearly showed that RNA molecules have important regulatory functions in bacteria. Researchers had already discovered multiple mechanisms of regulation of gene expression by RNA molecules. For example, it had been shown that RNA can act at the level of transcription and translation by using anti-sense mechanisms or by binding to protein effectors. Very little was known about RNA biology in the human pathogen Streptococcus pyogenes, and I thought there was a good chance that novel mechanisms might be found. It certainly seemed to be an exciting field around which to build a laboratory.”

DETECT AND DESTROY
Charpentier’s group subsequently discovered that an RNA molecule plays an important role in the ability of streptococci to invade tissue. During this work on RNA molecules, Charpentier came across several studies on a bacterial immune system called CRISPR, where bacteria cut small fragments of DNA from invading viruses and then incorporate them into their genome so they can remember the DNA sequence again when they are attacked. You could call it a sort of genetic vaccination card.

What really captured Charpentier’s attention, however, was that bacteria make small RNA copies of the incorporated viral DNA sequences. If these RNA molecules recognize and bind to the corresponding viral DNA sequence upon subsequent infection, the cell knows that this is an invasive virus that must be destroyed.

During this time, Charpentier had started a new laboratory at the Laboratory for Molecular Infection Medicine Sweden in Umeå, Sweden. In 2006, she and her team performed a bioinformatics screen that resulted in the identification of numerous novel small RNA species in Streptococcus pyogenes, and this gave the researchers a surprise.

“We also found the small CRISPR RNA (crRNA) molecules with sequences that were identical to the viruses the bacteria had encountered along the way. However, what astonished us was the finding of an abundant RNA species containing a stretch of sequence with remarkable similarity to the repeats of crRNA.”

LIKE A SMARTPHONE
Charpentier and her colleagues had discovered the last piece of the puzzle in the CRISPR-Cas9 system – the transactivating RNA, or tracrRNA. As reported in a study published with Jennifer Doudna and other colleagues, the tool that resulted when crRNA and Cas9 protein were mixed with the new RNA fragments in a test tube was a fully functional DNA cleavage system.

Not surprisingly, Charpentier’s discovery of tracrRNA triggered an explosion of research in the CRISPR field.

“We discovered with Jennifer Doudna and our teams that by simply changing the sequence of the crRNA we could reprogramme the Cas9 enzyme to cut in another location. This is an incredibly beautiful design and nearly as elegant as a smartphone – simple and user-friendly, but nevertheless both versatile and sophisticated. When we saw this, we knew that we had discovered a gene technology tool that matched anything we had previously seen.”

CRISPR-Cas became the revolution that Charpentier had envisioned. After these groundbreaking studies, several groups showed that the system not only functioned in bacteria and in the test tube but also in diverse cell types in plants, animals and humans. The system could thereby be used to repair the human genome by getting CRISPR-Cas9 to precisely cut out incorrect gene sequences and replace them with new sequences.

TALK OF THE TOWN
Overnight, CRISPR-Cas9 grew from a specialist field of scientific research to a topic in the mainstream media. Excitement grew around the potential application of CRISPR-Cas9 for curing genetic diseases. Charpentier and
her colleagues have received numerous scientific accolades, and they were listed in Time Magazine’s Top 100 most influential people in the world.

“As a young group leader, I focused on building my research programme with the hope of discovering new mechanisms that could be relevant for the scientific community. I knew when I received the first scientific prize for CRISPR-Cas9 in 2014 that it would not be the last one. One of my friends said to me: ‘You should accept, because if you don’t, there will almost certainly be someone else in the wings ready to claim the honour’.”

Since 2014, Charpentier has received more than 40 major scientific prizes and distinctions, including the prestigious Warren Alpert Foundation Prize, the Gruber Foundation International Prize and the Breakthrough Prize in Life Sciences. It would not be surprising if she would be considered for a Nobel Prize at some point.

“An award such as the Novozymes Prize really means a great deal to me because it is people from another research field – biotechnology – that have examined my work and said that it is very important for their work. I see the Prize primarily as a prize for my field – microbiology – and as recognition of the importance of this research.”

BEYOND CRISPR
The significance of microbiology and the search for new ways to apply knowledge of bacterial systems to benefit people has filled much of Charpentier’s daily activities in recent years. CRISPR has dominated the attention the last 6 years, and the worry that this would swallow up everything has caused her to pause for thought. With receiving up to 20 prizes and distinctions annually, and the associated interviews and travel, staying focused is important.

“There are many distractions that can make me lose focus on what I really believe to be important – science – and I am concerned that I will be snared in my own net and will forever be known solely as the woman who discovered CRISPR-Cas9. However, I feel that there is much more that I want to accomplish, which is why I am doing my best to integrate my responsibilities and duties associated with CRISPR-Cas9 into my scientific life.”

Since 2015, Emmanuelle Charpentier has been the Director of the Department of Regulation in Infection Biology at the Max Planck Institute for Infection Biology in Berlin. Here she has expanded her research programme, which currently comprises five research fields.

“We are still working on the biology of CRISPR-Cas. The greatest progress we made was the work published in Nature last year, when we reported our discovery of an even simpler system from the bacterium Francisella novicida. In this system, the Cpf1 enzyme not only activates crRNAs but also targets the viral DNA. This means that we end up with an even simpler and more elegant system.”

A FREE ELECTRON
Emmanuelle Charpentier feels liberated at this stage of her career, or as she puts it, “like a free electron” that can move freely through the bacterial treasure trove in places she believes are important.

“We see bacteria as toolmakers. Bacteria encode a large variety of enzymes that have revolutionized biology. These enzymes have enabled us to read whole genomes, including the human genome. They also produce the enzymes that can cleave and ligate DNA molecules, which have enabled cloning. With the CRISPR-Cas9 system, they have supplied us with a powerful tool to perform gene surgery to repair genetic mutations.”

The next major challenge is to deliver the CRISPR-Cas9 system into the right tissues so the repair can take place in exactly the right places, for example, the brain, the heart or any other tissue in the body. For Emmanuelle Charpentier, however, there is much interesting work to be done beyond CRISPR-Cas9.

“Apart from our continuing studies of CRISPR, we also study, for example, the small regulatory RNAs that interfere with bacterial pathogenicity and the mechanisms of bacterial recognition by immune cells. Continuing basic science on bacteria will reveal new types of enzymes that may also be very useful for genome editing. And with the growing antibiotic resistance, there is certainly also a need to better understand infectious diseases caused by bacteria.”

Most important for Charpentier, however, is her continuing work to train young scientists to ensure the future of science. Major donations from the Kempe Foundation and the Wallenberg Foundations have given her the opportunity to train more young researchers.

“Working with young students is the most inspiring part of the job and extremely important for the scientific community. I try to teach what has been most important for my career and my research: to follow your gut feeling and to believe in your ideas but to keep an open mind to new ideas. Without this open mind, I would never have found what we discovered with CRISPR-Cas9.”
A tiny bacterium has upended the world as we thought we knew it just a few years ago. It has taken 25 years for researchers to understand what the bacterium was hiding. Virginijus Siksnys, a biochemist in Lithuania, cracked the code. He showed how tiny scissors in the core of the bacterium can be used to cut and paste genes together in a brand new way. This technology has such incredible potential that it may be able to heal sick people and even help save the environment if we learn to use it correctly.

THE MAN WITH THE GOLDEN DNA SCISSORS

The ability to cut or break down large molecules correctly is an essential process in humans and most other organisms. Sugar has to be cleaved into smaller units before we can use it. Proteins from invading viruses or bacteria need to be cut into smaller fragments so that the immune system can recognize them as being alien in the future. Faulty DNA fragments need to be excised and replaced with the right ones to protect against genetic diseases, including cancer.

The different molecular scissors that carry out these reactions cut small units precisely and rapidly and on a scale that makes all scissors created by humans pale by comparison.

The dream of discovering and controlling nature’s amazing scissors has fascinated the world of research for at least 50 years since the first examples were discovered. Virginijus Siksnys has been involved most of the way. It was therefore fitting that he was the first researcher to understand how to use the most golden of all scissors currently known: the CRISPR-Cas system.

As the uses of this gene technology tool have enormous potential, there are competing claims about its discovery. However, financial rewards and fame have never motivated Siksnys, and his discoveries in this field were very close to not happening.

“After so many years in this field, I had actually become tired of trying to find or engineer the perfect DNA cutting tool that would cut at any desired sequence. I was therefore well on the way to switching my scientific focus when I happened to read an article on the immune system of bacteria. That really made me think – mostly because the bacteria seem similar to humans in their ability to remember the organisms that previously tried to attack them, so they can counterattack effectively the next time they are attacked,” explains Virginijus Siksnys.
The story of the golden CRISPR-Cas scissors is yet another research saga of how seeking to understand nature often results in discovering the most valuable treasures – and often when least expected.

**BACTERIA'S ANTIVIRAL DEFENCE**

Virginijus Siksnys started his golden scissors odyssey in a completely different place. He studied chemistry at Vilnius University and received his PhD from Lomonosov Moscow State University, where he carried out research on enzymes that cut proteins. Returning to the Institute of Biotechnology in Vilnius, he decided to switch the focus and aim at a very fundamental question on how bacteria protect themselves against invasive external viruses known as bacteriophages.

> "Unlike humans, who have many cells, bacteria are unicellular. If a virus gets control of a bacterium, it quickly multiplies and spreads across the whole bacterium population. Bacteria thus have only one try to eliminate an invader, and this requires very effective defence to protect themselves."

One of the first lines of defence of bacteria are restriction enzymes that can cut DNA into fragments, thereby destroying the DNA of the invading virus. Whether a DNA molecule can be cut depends on the DNA sequence. For example, *Escherichia coli* has a restriction enzyme, EcoRI, that cuts at the GAATTC DNA sequence. Bacteria protect their own DNA by attaching methyl group tags to the sequence recognized by a restriction enzyme that is unable to cut a methylated target sequence.

> "When I started my research career in the 1980s, very few of these enzymes had been identified. However, the enzymes rapidly spread as indispensable tools for DNA manipulation and genetic engineering in bacteria. What interested me and continues to interest me today is understanding how these enzymes achieve their functioning."

**INCREASING NEED FOR GENETIC SCISSORS**

From 1982 and for two decades, Virginijus Siksnys built up a key centre within this field at the Institute of Biotechnology of Vilnius University. It focused on the structural and molecular mechanisms of restriction enzymes, addressing questions such as how restriction enzymes recognize particular DNA sequences, which common structures are shared by the enzymes and how enzyme structure can be linked to function.

> "We hoped that finding answers to these questions would enable us to understand the enzymes' structures and mechanisms so well that we could engineer them and thereby change their functioning. Ultimately, this could mean that we would be able to design and engineer tailor-made restriction enzymes that would cut precisely where we wanted."

The need for specific types of genetic scissors grew in the 1990s as gene technology began to gather momentum. The more knowledge obtained about specific genes from the genome of various organisms, the greater researchers desired and needed enzymes to remove genes from one organism and paste them into another.

> "Today, we have identified more than 4000 different restriction enzymes that can cut at nearly 300 different sequences, so the diversity is incredibly large. However, although we had improved our understanding of the biochemical mechanisms, and although we could determine the structure by using X-ray crystallography, we could only make limited changes to the enzymes using the technology available at that time."

**TIRED OF SCISSORS**

In those days, the methods for the rational design of enzymes required a lot of protein engineering and were very time-consuming. An enzyme such as EcoRI comprises a chain of 277 amino acids in a specific order. To change the enzyme, researchers had to replace individual amino acids at the protein–DNA interface, test whether the enzyme functioned differently, make another change and test again.

> "Although our structural studies implied which amino acids we should change, it was like trying to find a needle in a haystack. In about 2005, I thought I had reached a crossroads since the restriction enzymes seemed to be a dead end."

Virginijus Siksnys therefore decided to switch tracks with his research, but some unexpected and intriguing research studies occurring around that convinced him to change his mind.

Francisco Mojica, a young researcher in Spain, discovered some mysterious structures in the *Haloferax mediterranei* bacterium. Mojica found many repeated DNA sequences that each comprised precisely 30 base pairs – separated by precisely 36 base pairs. Mojica became almost obsessed by the structures and spent the next 10 years seeking an explanation. The structures were dubbed clustered regularly interspaced short palindromic repeats or CRISPR.
The CRISPR phenomenon did not create much attention in the next couple of years, but research often accelerates when pioneering scientific discoveries are made, and the CRISPR research suddenly began to take off in 2005. The next significant step convinced Virginijus Siksnys to return to the path he had previously left. The discovery came from a rather unusual source: sauerkraut.

**YOGURT AND SAUERKRAUT**

In France, PhD student Philippe Horvath dedicated his studies to investigating something as exotic as the genetics of lactic acid bacteria used to ferment sauerkraut. Rhodia Food hired Horvath to examine the lactic acid bacteria the company used. Bacteriophages often attacked the bacteria used for producing yogurt, ruining an entire batch.

Horvath had already heard about CRISPR in 2002 and was therefore aware of its possible importance when he discovered the very same genetic system in the lactic acid bacteria he was examining. He decided to look more closely at their genes together with another young PhD student, Rodolphe Barrangou from Danisco USA, Inc. and bacteriophage expert Sylvain Moineau from Quebec, Canada.

Horvath discovered several bacteria that he knew had become resistant to specific bacteriophages. He examined their genome more closely and found that the bacteria had cut out DNA fragments from the bacteriophages and inserted them in their own genome – in precisely the intervals discovered in CRISPR’s repeat DNA sequences.

Virginijus Siksnys explains: “The tiny DNA fragments from the bacteriophages stored by bacteria thus enable the bacteria to recognize the bacteriophages the next time they attack. However, what really startled me was that the cas9 gene in the vicinity of the CRISPR array encoded the Cas9 protein and carried signature sequences seen before in restriction enzymes we had studied previously.”

The bacteria thus apparently place CRISPR – their memory of invading enemies – next to and under something that presumably can cut – the Cas protein. Much more was still needed to be understood about CRISPR-Cas and especially its potential, but enzyme expert Siksnys rekindled his interest in bacteria’s immune scissors.

“All my original interest in enzymes arose from the immune system of bacteria. So clearly it was a real eye-opener to suddenly read that bacteria not only had the primitive immune system we had investigated but also have a more advanced immune system.”

**A DECISIVE MOMENT**

While Virginijus Siksnys set to work studying the possible restriction enzyme in the CRISPR-Cas antiviral defence system, the pieces of the rest of the CRISPR-Cas puzzle slowly began to fall into place.

Researchers from all over the world accounted for individual pieces. John Van de Oost from the Netherlands discovered that the short DNA gene sequences that CRISPR stores to enable it to remember the invading bacteriophages were translated into short RNA molecules (crRNA) that could move around the cell and guide the enzyme cutter to the bacteriophage that needed to be attacked. Emmanuelle Charpentier from Umeå University in Sweden later discovered that crRNA required another RNA guide molecule (transactivating RNA) to enable it to find the invading bacteriophages.

In 2011, this knowledge enabled Virginijus Siksnys to become the first person to successfully compile and transfer a complete CRISPR-Cas locus from a bacterium, *Streptococcus thermophilus*, which is resistant to bacteriophages, into a non-resistant strain of *E. coli* and showed that this locus enables the *E. coli* to fight the unwelcome enemy.

“This was a decisive moment for us. We could easily have been missing some components or we might not have been able to transfer the system from one organism to another. But our results clearly showed that we had collected the whole locus and were able to move it around freely. However, we still did not understand how it worked at the molecular level.”
Researchers in the United States, Luciano Marraffini and Erik Sontheimer, had shown that CRISPR-Cas eliminates bacteriophages by cleaving their DNA, and Sylvain Moineau had shown that Cas9 was probably the enzyme scissors that did the cutting. Researchers thus had all the pieces of the puzzle in front of them. They just needed to assemble them.

HARDLY BELIEVING YOUR OWN EYES

The race to be the first to assemble the pieces seriously intensified, and deciding who actually crossed the finish line first is still difficult today, but the image that emerged was more magnificent than anyone had expected. After initially cloning the whole CRISPR-Cas system to make it function in another bacterium, Siksnys was ready to focus on his field of expertise: enzymes.

The time had come to determine the mechanism of Cas9, the enzyme bacteria seemed to use to attack the bacteriophages. Siksnys therefore carried out a thorough study with only Cas9-RNA complex and DNA present in a test tube that was the first to confirm that CRISPR-Cas is the enzyme scissors that cuts up the DNA of the bacteriophages. Siksnys’ next discovery was even more surprising and decisive for the breakthrough that CRISPR-Cas subsequently created.

“We strongly suspected that the Cas9 enzyme only cut the DNA fragments if these were identical to the DNA sequences the bacteria had stored from previous attacks. So we thought: What if we change them? Can we make the enzyme cut the sequence we want it to cut?“

Researchers could scarcely believe their eyes. What they had sought for several decades – to reprogramme a restriction enzyme – could now be achieved rapidly. When a 20-base-pair sequence of the crRNA was edited, Cas9 now cut in another location – exactly where the researchers had requested.

Meanwhile, Emmanuelle Charpentier and her United States colleague Jennifer Doudna had achieved exactly the same result, albeit slightly differently, at about the same time. In April 2011, Siksnys submitted his manuscript to Cell. However, the journal rejected it, since they did not find it sufficiently important. He therefore resubmitted it to Proceedings of the National Academy of Sciences of the United States of America, which finally published it on 4 September 2012. Charpentier and Doudna submitted their article to Science 2 months after Siksnys’ submission, but it was published earlier, on 8 June 2012.

THE SCISSORS BECOME GOLDEN

The two 2012 publications are already landmarks for the life sciences.

“Taken together, these findings pave the way for the development of unique molecular tools for RNA-directed DNA surgery,” wrote Siksnys in his article.

The new results meant that the world now has a gene technology tool that can very accurately edit damaged or incorrect gene sequences and can repair defective genomes by inserting the correct sequences after the DNA is cleaved.

Within six months, CRISPR became one of the most frequently searched words on Google. In January 2013, when Boston-based researcher Feng Zhang reported in Science that he had used CRISPR-Cas to edit the human genome, interest on the Internet exploded.

The potential applications for CRISPR-Cas appear to be almost unlimited. Potentially, people with diseases can be healed by repairing the genetic abnormalities. Genetic abnormalities can be introduced into cells to enable researchers to study genetic disorders and thus more easily test new drugs. The new wonder scissors will also make producing drugs, enzymes or other chemicals in cell factories far easier.

CRISPR-Cas has also raised bioethical concerns because it can potentially be used to alter the genome of healthy foetuses. This aspect of CRISPR-Cas technology is still science fiction. Companies are battling for the patents globally, but many researchers are calling for calm and want a moratorium so that the technology cannot begin to be used prematurely.

In the meantime, while the CRISPR-Cas debate rages, one of the chief architects is back in his laboratory in Vilnius – where he started and where he plans to continue for many years to come, that is in basic research.

“In my view, the most important lesson of CRISPR-Cas is that basic research laid its foundations. The key breakthroughs often have the most unpredictable origins. My goal was to understand the antiviral defence mechanisms of bacteria – genome editing came as a second derivative of the basic research. If we had not sharply focused on the fundamental goal, we might never have achieved what we did.”
THE NOVOZYMES PRIZE COMMITTEE

The Novozymes Prize is a European research award instituted by the Novo Nordisk Foundation. The Novozymes Prize is awarded in the name and with the funds of the Foundation. The purpose of the Prize is to raise awareness of basic and applied biotechnology research.

The Novozymes Prize is awarded to recognize outstanding research or technology contributions that benefit the development of biotechnological science for innovative solutions. It consists of a funding amount for the Prize recipient's research of DKK 2.5 million and a personal award of DKK 0.5 million. An additional element of the Prize is an international symposium within the Prize recipient’s field of research.

Prize recipients must have a current position at a public or non-profit research institution in a European country. They may previously have worked anywhere and may have any nationality.

The Novozymes Prize is awarded by a prize committee that selects the successful candidate based on scientific achievements after a confidential nomination and review process.

The members of the Novozymes Prize Committee are appointed by the Novo Nordisk Foundation Board of Directors, and the Committee currently comprises six members:

- Søren Molin, professor, chair
- Henrik Callesen, professor
- Liisa Viikari, professor emeritus
- Claus Hviid Christensen, CEO
- Michael Broberg Palmgren, professor
- Birgitte Nauntofte, CEO, Novo Nordisk Foundation

The award event takes place in the spring at the Novo Nordisk Foundation Prize Celebration, at which the Novo Nordisk Prize is also awarded.
In addition, in celebration of the award, the awardee gives a lecture lasting about 1 hour at his or her workplace. Before the end of the year, the recipient and the Foundation arrange an international symposium within the scientific field of the prize winner.

Candidates for the Novozymes Prize can be nominated by the prize committee and former prize winners.

Additionally a “Call for nominations” is published in the Spring and candidates can be nominated on the basis of this call.

At the committee meetings the nominated candidates are thoroughly discussed with regard to their research contribution and impact, and a comprehensive bibliometric report is produced. A limited number of candidates are then selected for a thorough international peer review. On the basis of the international peer reviews the committee reaches a decision about the year’s prize winner.

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PREVIOUS RECIPIENT OF THE NOVOZYMES PRIZE

2015  PROFESSOR, DIRECTOR BERNARD HENRISSAT
2016  PROFESSOR, JENS NIELSEN