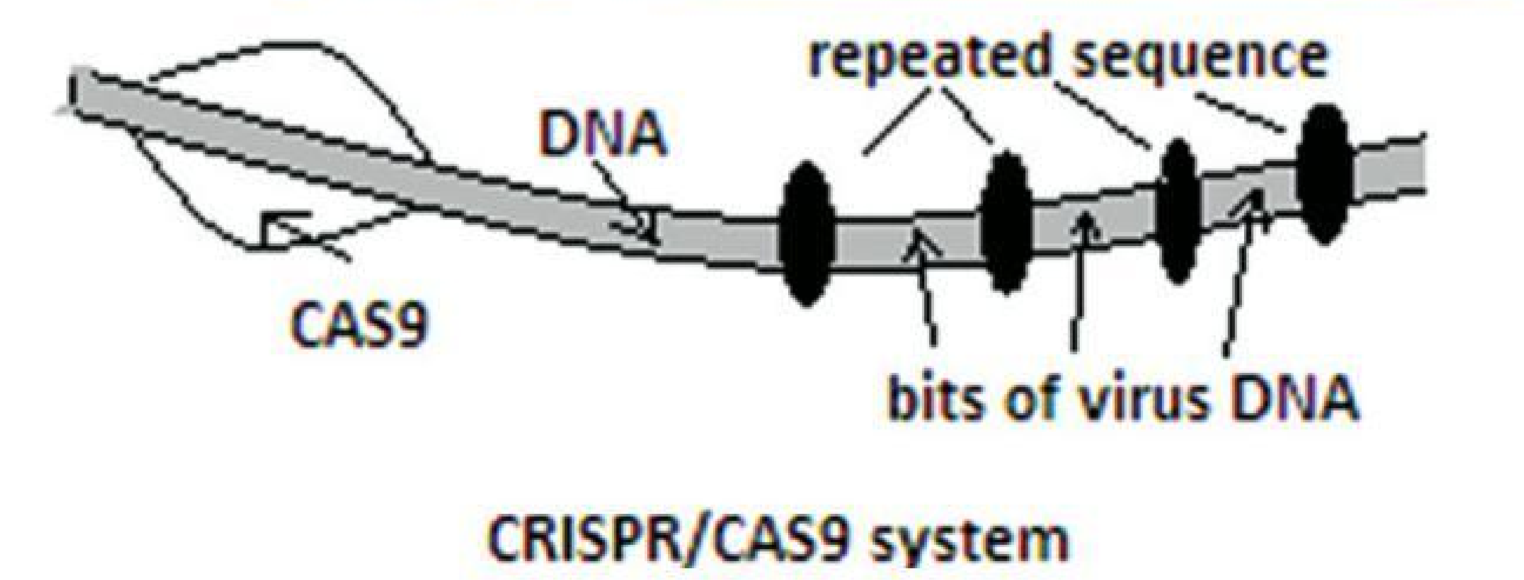
Cut & paste in genetics

New tools have made routine work of DNA manipulation

S ANANTHANARAYANAN



In 2001, Stephen Hawking said we had entered a century of biotechnology. The last two years, if the pandemic should be thanked for anything, have raised public understanding of the internals of genetics, the virus, and DNA. And the field of genetic engineering, with methods of manipulating the DNA itself, has become the central technology of our times.

This was not the case at the start, however. The start was in 1953, with the discovery of the DNA's structure, by Francis Crick, James Watson and Maurice Wilkins, based on the seminal work of Rosalind Franklin. And this explained how the complementary pair of strands, that DNA, or deoxyribonucleic acid, consists of, split apart at the time of cell division, so that the two halves could recreate the whole in daughter cells.

Just over a decade later, Robert Holley, Marshall Nirenberg and Har Gobind Khorana uncovered the mechanism by which the millions-of-units-long DNA encoded and passed on the templates for the 20 amino acids that all proteins consist of.

It was a start, but the nature of DNA was still far from understood. It was known that the DNA molecule was a series of units that came from only four chemical groups, called bases. And it was known that the four bases formed base pairs that appeared together. The pattern, or the order of the appearance, over the length of the DNA molecule, however, was out of reach.

The task of mapping out the entire structure of the human DNA, or the genome, was undertaken in 1990, with 2,800 researchers working, worldwide, and was completed in 2003. The structure worked out is of three billion base pairs, in about 20,500 groups, called genes, which code for proteins. This was the progress made in the first 50 years since 1953.

But the progress since 2003 has been rapid and we are now able to identify the structure, down to base pairs, of the genes responsible for diseases, and then, in cases, repair errors that have appeared. The last two years have shown us the capability to build, in the laboratory, versions of segments of the DNA, to create vaccines that can train the immune system to recognise the Sars-CoV2 virus.

This capacity is due, to a large extent, to a procedure called CRISPR-Cas9, developed by Jennifer Doudna and Emmanuelle Carpentier in 2016. While genetic engineering is set to modify natural processes for improved health, environment control and industrial processes, CRISPR-Cas9 was developed by mimicking a natural process, where bacteria make use of genetic engineering in defense against viruses.

The discovery started with the observation that the DNA of many bacteria contains a series of sequences which are repeated, interspersed with sequences that are derived from viruses. And it was noticed that the repeats usually occurred in the vicinity of another set of genes, of a kind that seemed to be involved in the process of DNA repair. It was then thought that the repeats were perhaps some kind of template acquired from encounters with viruses and later used for resisting the same viruses. The repeats were named CRISPR, a short form for "clusters of regularly interspaced short palindromic repeats", and the associated genes were called CAS genes, for CRISPR associated genes.

While the DNA is the construction formula of all the proteins that the organism would need, the whole DNA is never active at any one time. Instead, it is portions of the DNA, which represent the code for just a set of proteins, that are extracted and utilised for creating different proteins that define the nature of a cell.

The medium to transmit the partial code to the cells' engines that build the proteins is the RNA, or ribonucleic acid, a variation of the DNA molecule structure. Now, when a virus attacks a bacterium, the identifying sequences of the virus get copied on to scraps of RNA which form during the encounter with the virus and these get stored in the CRISPR sequences. When the virus attacks again, it is not a passive action of taking and storing copies of parts of the virus DNA that happens, but the existing patterns of the virus DNA are copied quickly on to RNA, which then combine with proteins, or enzymes, that are formed from the CAS genes portion, and an important group called CAS9.

CAS9, equipped with the template for a portion of the virus, is able to zero in to the portion of the virus DNA that CRISPR has recorded and CAS9 acts by snipping the DNA at that place. The virus, which consists mainly of its own DNA, is then destroyed and the bacterium stays healthy.

In 2012, Doudna, of the University of California at Berkeley, and her colleague Carpentier, and their team, studied the process of this action and they identified CAS9 as the enzyme which could cause a rupture in the two strands of the DNA molecule at the portion marked by the pattern of the RNA, called the guide RNA. And when Doudna and Carpentier got down to how this happened in the bacteria, they wondered if the process could be mimicked with a given RNA to serve as the guide RNA, to create a system that could sever the DNA at the place specified by the guide RNA.

The team went on to do this and then they devised a test to see if the target DNA had actually been cloven at the point they wanted. They first generated a pair of guide RNA that matched specific parts of a known DNA molecule and then formed the cleaving enzymes by letting the RNA attach to CAS9.

The target DNA was then incubated with the cleaving enzymes, and then, the DNA was examined with the help of a gel in which segments of DNA move with different speeds. The gel was able to separate two parts of the DNA and the sizes of the portions showed that the original DNA had been cut just where the cleavage was programmed by the guide RNA.

Studies had shown for some time that cells had ways of repairing DNA damage. In such cases, when a DNA was separated, the two portions rejoined, either just as they had broken or with a small section added, or there could be the addition of a whole part of DNA which matched the two broken ends. Where the DNA rejoined in the ordinary way, there was often damage to the gene involved, which had its value in studying the properties or function of that gene. Where a portion was added, the genome now had additional genes, with their value in research.

The CRISPR and CAS9 system, which allow DNA to be divided accurately, and then modified, has made many forms of DNA manipulation possible. The technique is said to be "igniting a revolution across the life sciences" and is quickly becoming a standard tool in many labs.

The writer can be contacted at response@simplescience.in

More uses

The use of CRISPR has gone beyond the traditional "gene knockouts" and "knock-ins".

New technologies include CRISPRi, or CRISPR interference, where the DNA is not cleaved, but the functioning of a gene is suppressed. Doing this with genes in succession enables identifying gene function. Another is CRISPRa, or CRISPR activation, where genes are made more active. An application is where a gene is switched on by blue light and repressed when the light is turned off.

Yet another is the "anti-CRISPR protein", which enables a gene to evade CRISPER action. On the other hand, "CRISPR screens" allow inhibition of a group of genes, "gene tagging" helps attach a marker to specific genes, and "prime and base editing" allow changes to be made within individual DNA components.

Preparing for a collision

An asteroid impact could wipe out an entire city. A space security expert explains Nasa's plans to prevent a potential catastrophe

SVETLA BEN-ITZHAK


Nasa's Dart mission will crash a small spacecraft into the double asteroid Didymos to see if it will change the asteroid's orbit

The Earth exists in a dangerous environment. Cosmic bodies, like asteroids and comets, are constantly zooming through space and often crash into our planet. Most of them are too small to pose a threat, but some can be cause for concern.

As a scholar who studies space and international security, it is my job to ask what the likelihood of an object crashing into the planet really is -- and whether governments are spending enough money to prevent such an event.

To find the answers to these questions, one has to know what near-Earth objects are out there. To date, the National Aeronautics and Space Administration, or Nasa, has tracked only an estimated 40 per cent of the bigger ones. Surprise asteroids have visited Earth in the past and will undoubtedly do so in the future. When they do appear, how prepared will humanity be?

The threat from asteroids and comets

Millions of objects of various sizes orbit the Sun. Near-Earth objects include asteroids and comets whose orbits will bring them within 193 million kilometres of the Sun.

Astronomers consider a near-Earth object a threat if it comes within 7.4 million km of the planet and is at least 140 metres in diameter. If a celestial body of this size crashed into Earth, it could destroy an entire city and cause extreme regional devastation. Larger objects -- one km or more -- could have global effects and even cause mass extinctions.

The most famous and destructive impact took place 65 million years ago when a 10-km-diameter asteroid crashed into what is now the Yucatán Peninsula. It wiped out most plant and animal species on Earth, including the dinosaurs.

But smaller objects can also cause significant damage. In 1908, an approximately 50m celestial body exploded over the Tunguska river in Siberia. It levelled more than 80 million trees over 2,100 square km. In 2013, an asteroid only 20m across burst in the atmosphere 32 km above Chelyabinsk, Russia. It released the equivalent of 30 Hiroshima bombs worth of energy, injured over 1,100 people and caused $ (United States) 33 million in damage.

The next asteroid of substantial size to potentially hit Earth is asteroid 2005 ED224. When the 50m asteroid passes by on 11 March 2023, there is roughly a one in 500,000 chance of impact.

Watching the skies

While the chances of a larger cosmic body impacting Earth are small, the devastation would be enormous.

The U S Congress recognised this threat, and in the 1998 Spaceguard Survey, it tasked Nasa to find and track 90 per cent of near-Earth objects one km across or bigger within 10 years. Nasa surpassed the 90 per cent goal in 2011.

In 2005, the Congress passed another bill requiring Nasa to expand its search and track at least 90 per cent of all near-Earth objects 140m or larger by the end of 2020. That year has come and gone and, mostly due to a lack of financial resources, only 40 per cent of those objects have been mapped.

As of 14 February this year, astronomers have located 28,266 near-Earth asteroids, of which 10,033 are 140m or larger in diameter and 888 at least one km across. About 30 new objects are added each week.

A new mission, funded by the Congress in 2018, is scheduled to launch in 2026. It is an infrared, space-based telescope -- NEO Surveyor -- dedicated to searching for potentially dangerous asteroids.

Cosmic surprises

We can only prevent a disaster if we know it is coming, and asteroids have sneaked up on Earth before.

An asteroid the size of a football field -- dubbed the "City-killer" -- passed less than 73,000 km from Earth in 2019. An asteroid the size of a 747 jet came close in 2021 as did a one-km-wide asteroid in 2012. Each of these was discovered only about a day before they passed Earth.

Research suggests that one reason may be that Earth's rotation creates a blind spot whereby some asteroids remain undetected or appear stationary. This may be a problem, as some surprise asteroids do not miss us. In 2008, astronomers spotted a small asteroid only 19 hours before it crashed into rural Sudan. And the recent discovery of an asteroid two km in diameter suggests that there are still big objects lurking.

What can be done?

To protect the planet from cosmic dangers, early detection is key. At the 2021 Planetary Defence Conference, scientists recommended a minimum of five to 10 years' preparation time to mount a successful defence against hazardous asteroids.

If astronomers find a dangerous object, there are four ways to mitigate a disaster. The first involves regional first-aid and evacuation measures. A second approach would involve sending a spacecraft to fly near a small- or medium-sized asteroid; the gravity of the craft would slowly change the object's orbit. To change a bigger asteroid's path, we can either crash something into it at high speeds or detonate a nuclear warhead nearby.

These may seem like far-fetched ideas, but in November 2021, Nasa launched the world's first full-scale planetary defence mission as a proof of concept -- the Double Asteroid Redirection Test, or Dart. The large asteroid Didymos and its small moon currently pose no threat to Earth. In September this year, however, Nasa plans to change the asteroid's orbit by crashing a 610 kg probe into Didymos' moon at a speed of approximately 22,500 km an hour.

Learning more about what threatening asteroids are made of is also important, as their composition may affect how successful we are at deflecting them. The asteroid Bennu is 490m in diameter. Its orbit will bring it dangerously close to Earth on 24 September 2182, and there is a one in 2,700 chance of a collision. An asteroid of this size could wipe out an entire continent, so to learn more about Bennu, Nasa launched the Osiris-Rex probe in 2016. The spacecraft arrived at Bennu, took pictures, collected samples and is due to return to Earth in 2023.

Spending on planetary defence

In 2021, Nasa's planetary defence budget was $ 158 million. This is just 0.7 per cent of Nasa's total budget and just 0.02 per cent of the roughly $ 700 billion 2021 U S defence budget. It supports several missions, including the NEO Surveyor at $ 83 million, Dart at $ 324 million and Osiris-Rex at around $ one billion over several years.

Is this the right amount to invest in monitoring the skies, given the fact that some 60 per cent of all potentially dangerous asteroids remain undetected? This is an important question to ask when one considers the potential consequences.

Investing in planetary defence is akin to buying homeowners insurance. The likelihood of experiencing an event that destroys your house is very small, yet people buy insurance, nonetheless.

If even a single object larger than 140m hits the planet, the devastation and loss of life would be extreme. A bigger impact could quite literally wipe out most species on Earth. Even if no such body is expected to hit Earth in the next 100 years, the chance is not zero. In this low likelihood versus high consequences scenario, investing in protecting the planet from dangerous cosmic objects may give humanity some peace of mind and could prevent a catastrophe.

The writer is assistant professor of space and international relations, West Space Seminar, Air War College, Air University, United States. This article first appeared on www.theconversation.com

Unique fossil

Scientists have discovered a new species of stegosaur that is the most ancient of the group of armoured herbivorous dinosaurs unearthed in Asia and could be the oldest in the world.

The previously unknown species, *Bashanosaurus primitivus*, roamed the planet about 168 million years ago. Its new fossil sheds more light on the origin and evolution of stegosauruses -- one of the most easily recognisable types of dinosaurs with their huge back plates, long tail spikes and tiny head. The relatively small but "fearsome-looking" stegosaur measured about nine feet from nose to tail, but researchers, including those from Zigong Dinosaur Museum in China, said they are unsure if the remains are those of an adult or juvenile.

Remains of the dinosaur, described in the *Journal of Vertebrate Paleontology*, included bones from the back, shoulder, thigh, feet, and ribs, as well as several armour plates. Scientists dated the fossil to the Bajocian stage of the Middle Jurassic period -- much earlier than most known stegosauruses -- and suggested that this clade of dinosaurs may have originated from Asia.

"Our analysis of the family tree indicates that it is one of the earliest-diverging stegosauruses along with the Chongqing Lizard (*Chungkingosaurus*) and *Huayangosaurus*. These were all unearthed from the Middle to Late Jurassic Shaximiao Formation in China, suggesting that stegosauruses might have originated in Asia," study co-author Dai Hui, from the Chongqing Bureau of Geological and Mineral Resource Exploration and Development, said in a statement.

Researchers said this dinosaur species had a smaller and less developed shoulder blade, narrower and thicker bases to its armour plates, as well as other features that are different from all other Middle Jurassic stegosauruses discovered so far.

The independent

New discovery

Clues to the mechanism of yeast infections, which present risks to both humans and crops, have been identified.

The study has focused on a family of proteins, known as Mep-Amt-Rh, which enable them to transport ammonium, a significant compound involved in growth and differentiation of yeasts. Three proteins of the family are found in baker's yeast but only one of these, Mep2, is capable of triggering filamentation, the process of cell growth which can lead to infection by pathogenic fungi. It has been discovered that variations in Mep-Amt-Rh proteins affect the specificity and the type of mechanism for transporting ammonium. When two mechanisms co-exist within Mep2, they disrupt the signalling function which brings about filamentation and impede its progress.

The research could improve understanding of yeast infection in both humans and crop plants, enabling better defence against its effects. The collaborative research was led by Arnaud Javelle at the University of Strathclyde, professor Anna Maria Marini and professor Mélanie Boeckstaens at the Université Libre de Bruxelles and Professor Ulrich Zachariae at the University of Dundee. It has been published in the journal *mBio*.

Javelle, of Strathclyde Institute of Pharmacy and Biomedical Sciences, said, "Pathogenic yeasts represent a significant threat to human health and wellbeing. This can be direct, with yeast infection causing sickness or even death in humans, or indirect, through infection of crop plants severely limiting production and resulting in food shortages.

"According to a *Nature Microbiology* editorial published in 2017, more than 300 million people suffer from serious fungal-related diseases and fungi collectively kill over 1.6 million people annually, which is more than malaria and similar to the tuberculosis death toll. Fungi and the oomycetes organism destroy a third of all food crops each year, which would be enough to feed 600 million people."