Department of Biochemistry

Newsletter

All India Institute of Medical Sciences,Rajkot

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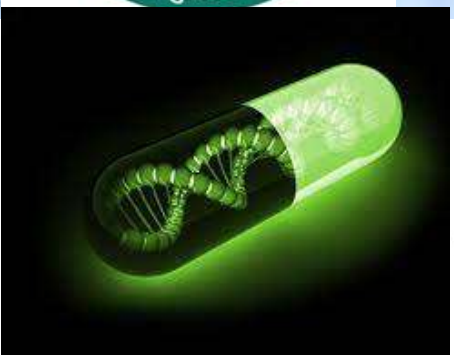


This issue is dedicated to

CRISPR TECHNIQUE

What is CRISPR?

CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats.

Repetitive DNA sequences, called CRISPR, were observed in bacteria with "spacer" DNA sequences (non-repetitive nucleotides) in between the repeats that exactly match viral sequences. It was subsequently discovered that bacteria transcribe these DNA elements to RNA upon viral infection. The RNA guides a nuclease (a protein that cleaves DNA) to the viral DNA to cut it, providing protection against the virus. The nucleases are named "Cas," for "CRISPR-associated."

While the size of repeats typically ranges from 24 to 47 base pairs, the number of repeats varies from 2 to 249. In a single CRISPR array, the size and sequence of repetitions are consistent.

When bacteria are exposed to a novel virus, they often pick up a new spacer that gives them protection from that virus. Therefore, the presence of spacers in a given bacterium often reveals the phage exposure history of that organism.

About 45 Cas proteins have been found in close proximity to CRISPR arrays, and CRISPR/Cas systems can be further divided into three groups based on the ratio of Cas proteins to crRNA. Cas3, a protein found in the Type I CRISPR/Cas system, has been revealed to have helicase and DNase activity by Makarova et al. In addition, the presence of Cas9 protein, which generates crRNA and cleaves the target DNA, is a defining feature of type II CRISPR/Cas systems. Type III CRISPR/Cas systems, like the Cascade complex,

are characterized by the presence of polymerase and repeat-associated mystery protein (RAMP) modules, which are thought to contribute to the production of spacer-repeat transcripts.The CRISPR/Cas systems are crucial for bacterial defense against bacteriophages and exogenous plasmids. CRISPR/Cas systems, in particular CRISPR/Cas9 and CRISPR/Cas13, have been used to engineer flexible gene-editing tools that can target and alter specific sequences of DNA. These two techniques are also used to create rapid and very sensitive isothermal diagnostic tools for spotting cancer, bacterial infections, and viral mutations.

CRISPR/Cas systems can be introduced into cells by means of viral vectors, lipid vesicles, gold nanoparticles, carbon nanotubes, microinjection, electroporation,acoustoporation, magnetotransfection, and laser optoporation.

Biomedical applications of CRISPR/Cas systems

1. Identification of viral and bacterial diseases

CRISPR/Cas13a system can identify many different kinds of viruses and bacteria. For example, Specific High-Sensitivity Enzymatic Reporter UnLOCKing (SHERLOCK) was developed as a molecular detection system based on the CRISPR/Cas13a system that allows for the detection of both Zika and Dengue viruses. New possibilities for the rapid and sensitive detection of viral genetic materials have been shown by this study, with the detection limit of SHERLOCK for Zika virus as low as 3.2 aM and the cost of per test as low as $0.61.

As an added bonus, an amplification free CRISPR/Cas13a test has been developed that enables direct detection of the respiratory virus, SARS-CoV-2, from nasal swab RNA that can be viewed using a mobile phone microscope. This CRISPR/Cas13a-based approach has been clinically validated and has a remarkable sensitivity of 100 copies/L for measuring SARS-CoV-2 RNA.

2. Gene Therapy

There is growing evidence that CRISPR/Cas systems can be used to effectively treat a wide range of inherited diseases, including Duchenne muscular dystrophy (DMD), sickle cell disease (SCD), beta-thalassemia, hemophilia A and B, and recessive dystrophic epidermolysis bullosa (RDEB). The CRISPR/Cas9-based approach has been used to correct the exon 44 deletion mutation in the dystrophin gene, a mutation that is responsible for Duchenne muscular dystrophy. In addition, he CRISPR/Cas9 system has been used as gene-editing tools to target the enhancer region of the B-cell lymphoma/leukemia 11 A (BCL11A) gene in hematopoietic stem and progenitor cells (HSPCs) in one recent study to treat SCD. Mutations in the HBB gene, which are a leading cause of -thalassemia, can also be corrected using the CRISPR/Cas9 system. Hemophilia A is a monogenic disorder caused by mutations in the factor VIII (F8) gene; mice with this disorder have shown improvement after receiving CRISPR-based therapeutic treatment.

3. For Cancer Research

a. For treatment of cancer: A recent study shows that increasing macrophage phagocytic capabilities by knocking out signal regulatory protein- (SIRP-) with the CRISPR/Cas9 system improves their ability to engulf and digest cancer cells. Human epidermal growth factor receptor 2 (HER2) is a well-studied oncogene whose proliferation can be suppressed by targeting its exons with the CRISPR/Cas9 system.Clinical trials for the use of CRISPR/Cas systems to treat various forms of cancer have been conducted in several countries, including the United States, China, the United Kingdom, and Australia, all because of the promising potential of CRISPR biotechnology in cancer therapy.

b. For cancer modelling: Breast cancer, colorectal cancer, pancreatic cancer, lung cancer, and liver cancer models are just some of the cancer types for which CRISPR/Cas systems have been shown to play important roles in recent years. Loss-of-function mutations in tumor suppressor genes and gain-of-function mutations in a proto-oncogene were both created by using a Cre-dependent Cas9 knockin animal. This cutting-edge biotechnology enables the simulation of multi-mutational processes in cancer development.

4. Miscellaneous Uses

CRISPR/Cas systems have important applications beyond cancer research, particularly in the search for new drug-resistant genes. A CRISPR-pooled library can be used to

identify novel genes that, when silenced by knockdown, contribute to resistance to the acute myeloid leukemia treatment medication quizartinib (AC200).

Additionally, CRISPR screens with the indigenous cancer model in mice allow for the quick mapping of variations of tumor suppressor genes.

Limitations and future perspectives

Off-target effects, limited editing effectiveness, a lack of adequate delivery mechanisms in vivo, and ethical difficulties of editing the human genome directly have all been raised as potential roadblocks to the widespread adoption of CRISPR/Cas systems as therapeutic tools.

Thus, substantial work has been done to overcome these obstacles by enhancing viral CRISPR delivery and non-viral delivery methods and refining sgRNA design. Increasing the selectivity and efficiency of this novel gene-editing system will require more biochemical and structural studies to decipher the process by which Cas proteins connect with and cleave target DNA/RNA.

Tid-Bits From History

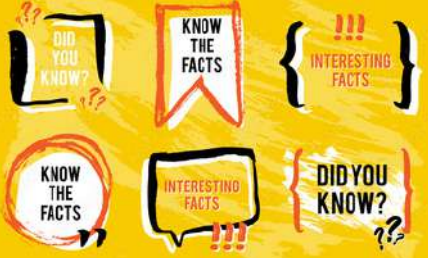
Nobel Prize Awarded to Jennifer Doudna And Emmanuelle Charpentier For CRISPR Discovery

Emmanuelle Charpentier and Jennifer Doudna won the Nobel Prize in Chemistry for their groundbreaking work on CRISPR technology, in year 2020.

In the year 2011, Emmanuelle Charpentier discovered the tracrRNA while studying the bacteria Streptococcus pyogenes. Through her work, she demonstrated that tracrRNA is part of bacteria's ancient immune system, CRISPR/Cas, that can disarm viruses by cleaving their DNA. She then initiated a collaboration with Jennifer A. Doudna, who had a lot of experience working with RNA. Their collaboration resulted in the successful recreation of the bacteria's genetic scissors in a test tube.

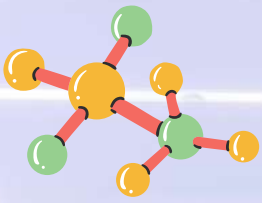
In their natural form, the scissors recognise DNA from viruses, but Charpentier and Doudna proved that they could be controlled so that they can cut any DNA molecule at a predetermined site. Where the DNA is cut it is then easy to rewrite the code of life. The programmable gene-editing system developed by the duo has paved way for countless applications in basic science, medicine and agriculture.

FUNFACT

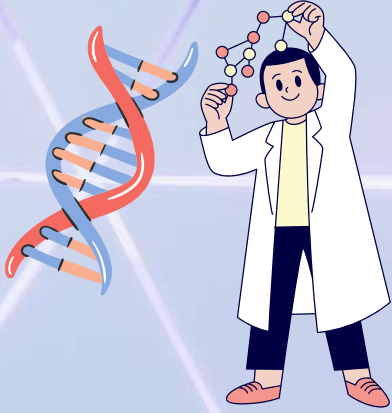


Why Do I Get Sleepy After Eating?

- **Postprandial sleepiness, also called postprandial somnolence, refers to the feeling of sleepiness after consuming food. It is sometimes known as the post-lunch dip or referred to more informally as a "food coma."**
- **The composition of a meal can affect whether a person feels sleepy after eating. A big meal may be more likely to cause sleepiness, and certain foods and nutrients can have an impact as well. Research has found that meals that are high in fat, carbohydrates, or calories may increase sleepiness.**
- **Foods That Can Make You Sleepy**
- **<u>High-fat foods, High-carbohydrate foods, Foods containing tryptophan:</u> Tryptophan is an amino acid that is linked to a number of sleep-promoting processes in the brain. Turkey contains high levels of tryptophan, so it is famously blamed for sleepiness after Thanksgiving dinner. Other foods with tryptophan include milk, bananas, oats, and chocolate.**
- **<u>Nuts</u>: Walnuts, pistachios, and other nuts have some of the highest melatonin levels. Given the effects of melatonin on sleep, eating a significant amount of nuts may induce a feeling of drowsiness.**



Laboratory Touchup



Heavy Metal Analysis (Heavy metal toxicity) – Metal Toxicity

Human body need small amount of metals/heavy metals (e.g., zinc, copper, chromium, iron, and manganese) to function normally. However, beyond a certain limit, either in free or bound form, metals are harmful, resulting in toxic effects (heavy metal toxicity/poisoning).

- Sources of Heavy metal poisoning: It occurs due to exposure through food, water, industrial chemicals, medicines, improperly coated food containers, plates, and cookware, ingestion of lead-based paints, Ingestion of insecticides, herbicides, and pesticides
- Symptoms of heavy metal poisoning depend on the type of metal causing toxicity, and the duration of exposure. By and large, acute symptoms include Confusion, Numbness, Nausea, Vomiting, falling into a coma and Long-term or chronic exposure to lower levels of heavy metals may include – Headache, Weakness, Tiredness, Muscle pain, Joint pain, dyspnea

Analytical methods: Common analytical methods used for trace metal analysis are

1. **Atomic absorption spectroscopy (AAS),**
2. **Inductively coupled plasma optical emission spectroscopy (ICP-OES) and**
3. **Inductively coupled plasma mass spectrometry (ICP-MS).**

Atomic absorption spectrometry (AAS) is an easy, high-throughput, and inexpensive technology, whereas analysis of trace metals by ICP is a highly sensitive procedure.

Atomic absorption spectrometry (AAS) is an analytical method of elemental analysis, allowing the determination of metals in a variety of samples at the picogram level.

- **The atomic absorption phenomenon involves a measurement of the reduction of the intensity of optical radiation subsequent to its passage through a cell containing gaseous atoms.**
- **AAS typically consists of:**

A. a light source called a hollow cathode lamp (HCL), which emits specific wavelengths of light that are ideally only absorbable by the analyte;

B. an 'atom cell', which serves to convert the samples into gaseous atoms that can absorb light from the HCL;

C. a 'detection system' that serves to isolate and quantify the wavelengths of interest;
D. and a computer system to control instrument operation and collect and process data.

The most common atom cells employed for AAS are flames and electrothermal atomizers (ETAs). ·

- Flames, employ a carefully controlled combustion environment to produce atoms, have the advantages of speed, ease of use, and continuous operation. However, atoms are dispersed across a relatively large volume, causing the sensitivity of flame AAS to be relatively poor (typically parts per million level).
- ETAs (Graphite furnaces), employ a small graphite tube whose temperature can be accurately controlled by a power supply. Argon gas flows around the tube to prevent its combustion at elevated temperatures. ETA-AAS provides much higher sensitivity (typically parts per billion level, or picograms on a mass basis) compared to flame AAS because the atoms are concentrated in a relatively small volume and its high-(essentially 100%) atomization efficiency.

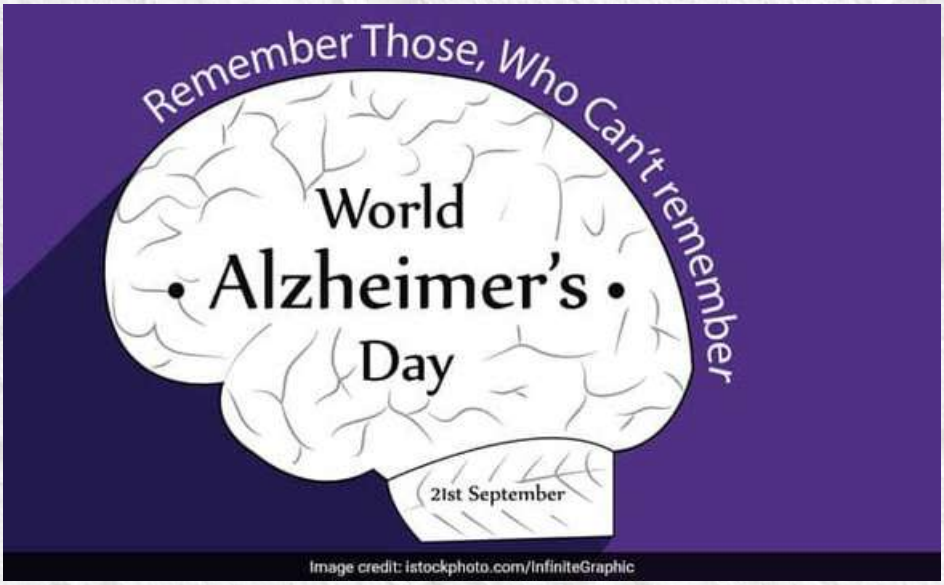
o In addition, ETA-AAS is capable of analysing small volumes (typically 20 µl per measurement).

Compared to AAS, ICP-MS has greater speed, precision, and sensitivity.

1. ***Inductively coupled plasma atomic emission spectroscopy (ICP-AES) OR inductively coupled plasma optical emission spectrometry (ICP-OES):*** **It is a type of emission spectroscopy that uses the inductively coupled plasma to produce excited atoms and ions that emit electromagnetic radiation at wavelengths characteristic of a particular element.**
2. ***Inductively coupled plasma mass spectrometry (ICP-MS):*** **It uses an inductively coupled plasma to ionize the sample. It atomizes the sample and creates atomic and small polyatomic ions, which are then detected.**

Key difference between ICP-AES and ICP-MS: ICP-AES provides a higher detection limit down to ppm or ppb, whereas ICP-MS provides a lower detection limit down to ppt (part per trillion).

Upcoming Events



Quote of the day