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Volume 2

GULMOHAR – BOTANY NEWSLETTER

FROM THE EDITOR'S DESK

A very warm welcome to our 2nd edition. In this edition we have tried to publish some amazing articles and photographs

In this edition you will get to know about Crispr - Cas 9 which is a simple yet powerful tool for editing genomes. You will also get information about the oldest mushroom fossil discovered, will help better explain evolution of organisms and also about Coronavirus which is spreading in different countries.

Last but not the least, don't forget to check out the photo gallery section which shows amazing photography skills of the students. Happy Reading!

- Sneha Nair
TYBSc

CRISPR-Cas9 & Targeted Genome Editing:New Era in Molecular Biology

What is CRISPR - Cas9?

The functions of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and CRISPR-associated (Cas) genes are essential in adaptive immunity in select bacteria and archaea, enabling the organisms to respond to and eliminate invading genetic material. These repeats were initially discovered in the 1980s in E. coli,but their function wasn't confirmed until 2007 by Barrangou and colleagues,who demonstrated that S. thermophilus can acquire resistance against a bacteriophage by integrating a genome fragment of an infectious virus into its CRISPR locus.Three types of CRISPR mechanisms have been identified, of which type II is the most studied. In this case, invading DNA from viruses or plasmids is cut into small fragments and incorporated into a CRISPR locus amidst a series of short repeats (around 20 bps). The loci are transcribed, and transcripts are then processed to generate small RNAs (crRNA – CRISPR RNA), which are used to guide effector endonucleases that target invading DNA based on sequence complementarity.One Cas protein, Cas9,has been shown, through knockdown and rescue experiments to be a key player in certain CRISPR mechanisms (specifically type II CRISPR systems). The type II CRISPR mechanism is unique compared to other CRISPR systems, as only one Cas protein (Cas9) is required for gene silencing. In type II systems, Cas9 participates in the processing of crRNAs, and is responsible for the destruction of the target DNA. Cas9's function in both of these steps relies on the presence of two nuclease domains, a RuvC-like nuclease domain located at the amino terminus and a HNH-like nuclease domain that resides in the mid-region of the protein. To achieve site-specific DNA recognition and cleavage, Cas9 must be complexed with both a crRNA and a separate trans-activating crRNA (tracrRNA or trRNA), that is partially complementary to the crRNA. The tracrRNA is required for crRNA maturation from a primary transcript encoding multiple pre-crRNAs. This occurs in the presence of RNase III and Cas9. During the destruction of target DNA, the HNH and RuvC-like nuclease domains cut both DNA strands, generating double-stranded breaks (DSBs) at sites defined by a 20-nucleotide target sequence within an associated crRNA transcript. The HNH domain cleaves the complementary strand, while the RuvC domain cleaves the noncomplementary strand. The double-stranded endonuclease activity of Cas9 also requires that a short conserved sequence, (2–5 nts) known as protospacer-associated motif (PAM), follows immediately 3´- of the crRNA complementary sequence . In fact, even fully complementary sequences are ignored by Cas9-RNA in the absence of a PAM sequence.

Cas9 and CRISPR as a new tool in molecular biology

The simplicity of the type II CRISPR nuclease, with only three required components (Cas9 along with the crRNA and trRNA) makes this system amenable to adaptation for genome editing. This potential was realized in 2012 by the Doudna and Charpentier labs. Based on the type II CRISPR system described previously, the authors developed a simplified two-component system by combining trRNA and crRNA into a single synthetic single guide RNA (sgRNA). sgRNAprogrammed Cas9 was shown to be as effective as Cas9 programmed with separate trRNA and crRNA in guiding targeted gene alterations . To date, three different variants of the Cas9 nuclease have been adopted in genome-editing protocols. The first is wild-type Cas9, which can site-specifically cleave double-stranded DNA, resulting in the activation of the doublestrand break repair machinery. DSBs can be repaired by the cellular Non-Homologous End Joining (NHEJ) pathway, resulting in insertions and/or deletions (indels) which disrupt the targeted locus. Alternatively, if a donor template with homology to the targeted locus is supplied, the DSB may be repaired by the homology-directed repair (HDR) pathway allowing for precise replacement mutations to be made. Cong and colleagues took the Cas9 system a step further towards increased precision by developing a mutant form, known as Cas9D10A, with only nickase activity. This means it cleaves only one DNA strand, and does not activate NHEJ. Instead, when provided with a homologous repair template, DNA repairs are conducted via the high-fidelity HDR pathway only, resulting in reduced indel mutations. Cas9D10A is even more appealing in terms of target specificity when loci are targeted by paired Cas9 complexes designed to generate adjacent DNA nicks . The third variant is a nuclease-deficient Cas9 . Mutations H840A in the HNH domain and D10A in the RuvC domain inactivate cleavage activity, but do not prevent DNA binding. Therefore, this variant can be used to sequence-specifically target any region of the genome without cleavage. Instead, by fusing with various effector domains, dCas9 can be used either as a gene silencing or activation tool. Furthermore, it can be used as a visualization tool.

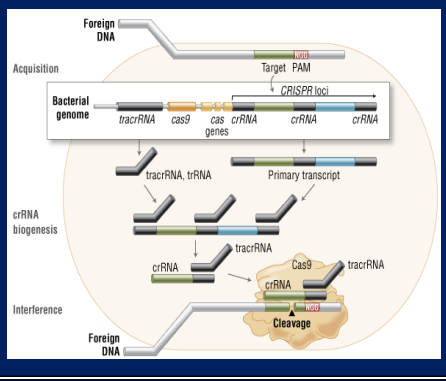
Targeting efficiency and off-target mutations

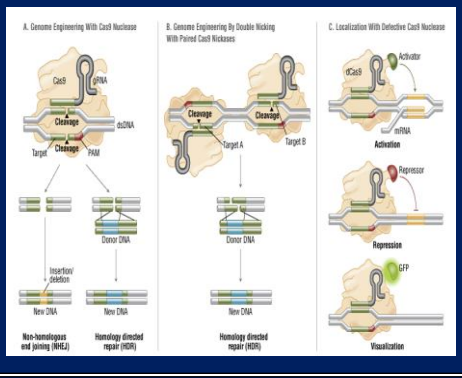
Targeting efficiency, or the percentage of desired mutation achieved, is one of the most important parameters by which to assess a genome-editing tool. The targeting efficiency of Cas9 compares favorably with more established methods, such as TALENs or ZFNs. For example, in human cells, custom-designed ZFNs and TALENs could only achieve efficiencies ranging from 1% to 50%. In contrast, the Cas9 system has been reported to have efficiencies up to >70% in zebrafish and plants,and ranging from 2–5% in induced pluripotent stem cells. In addition, Zhou and colleagues were able to improve genome targeting up to 78% in one-cell mouse embryos, and achieved effective germline transmission through the use of dual sgRNAs to simultaneously target an individual gene. A widely used method to identify mutations is the T7 Endonuclease I mutation detection assay. This assay detects heteroduplex DNA that results from the annealing of a DNA strand, including desired mutations, with a wildtype DNA strand. Another important parameter is the incidence of off-target mutations. Such mutations are likely to appear in sites that have differences of only a few nucleotides compared to the original sequence, as long as they are adjacent to a PAM sequence. This occurs as Cas9 can tolerate up to 5 base mismatches within the protospacer region or a single base difference in the PAM sequence.

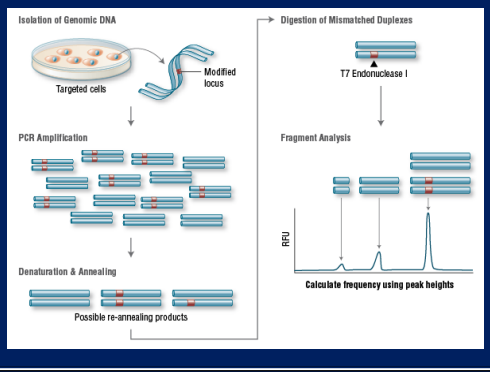
Off-target mutations are generally more difficult to detect, requiring whole-genome sequencing to rule them out completely. Recent improvements to the CRISPR system for reducing off-target mutations have been made through the use of truncated gRNA (truncated within the crRNA-derived sequence) or by adding two extra guanine nucleotides to the 5´ end. Another way researchers have attempted to minimize off-target effects is with the use of “paired nickases”. This strategy uses D10A Cas9 and two sgRNAs complementary to the adjacent area on opposite strands of the target site. While this induces DSBs in the target DNA, it is expected to create only single nicks in off-target locations and, therefore, result in minimal off-target mutations. By leveraging computation to reduce off-target mutations, several groups have developed webbased tools to facilitate the identification of potential CRISPR target sites and assess their potential for off-target cleavage. Examples include the CRISPR Design Tool and the ZiFiT Targeter.

Applications as a genome-editing and genome targeting tool

Following its initial demonstration in 2012, the CRISPR/Cas9 system has been widely adopted. This has already been successfully used to target important genes in many cell lines and organisms, including human, bacteria, zebrafish, *C. elegans*, plants, *Xenopus tropicalis*, yeast, Drosophila, monkeys, rabbits, pigs, rats and mice. Several groups have now taken advantage of this method to introduce single point mutations (deletions or insertions) in a particular target gene, via a single gRNA. Using a pair of gRNA-directed Cas9 nucleases instead, it is also possible to induce large deletions or genomic rearrangements, such as inversions or translocations. A recent exciting development is the use of the dCas9 version of the CRISPR/Cas9 system to target protein domains for transcriptional regulation, epigenetic modification, and microscopic visualization of specific genome loci.



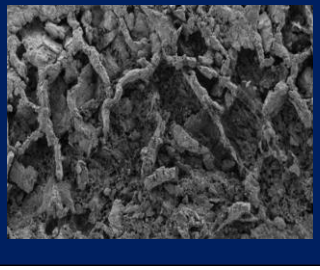


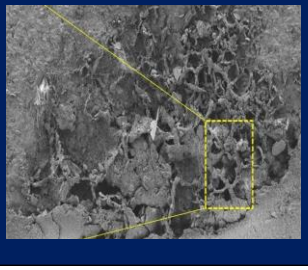


Oldest mushroom fossil discovered, will help better explain evolution of organisms

The oldest mushroom fossil was discovered in rocks, whose age is between 715 and 810 million years, found in the Democratic Republic of the Congo and likely formed in a lagoon or coastal lake environment. According to the scientists, including those from Universite Libre de Bruxelles in France, the oldest confirmed mushroom fossil until now was dated to 460 million years ago. In the current study, published in the journal Science Advances, researchers found fossilised remains of microscopic mushroom parts called mycelium in rocks whose age is between 715 and 810 million years. The scientists said these rocks were found in the Democratic Republic of the Congo and likely formed in a lagoon or coastal lake environment. Putting the discovery in perspective, the researchers said, this was a time in the Earth's history when life on the continents' surface was in its very infancy. "The presence of fungi in this transitional area between water and land leads us to believe that these microscopic mushrooms were important partners of the first plants that colonised the Earth's surface around 500 million years ago," explained Steeve Bonneville, one of the researchers part of the study from the Universite libre de Bruxelles."This is a major discovery, and one that prompts us to reconsider our timeline of the evolution of organisms on Earth. The next step will be to look further back in time, in even more ancient rocks, for evidence of those microorganisms that are truly at the origins of the animal kingdom,"Bonneville said.

While previously discovered mushroom fossils were identified based on the morphology of organic remains found in rocks using corrosive acid compounds,Bonneville said this method damaged the chemistry of the fossils, and only allowed analysis of the microscopic structures. He added that this may lead to incorrect interpretations as certain morphological traits are common to different branches of living organisms. In the current study, the scientists used multiple molecular analysis techniques at a microscopic scale with which they could study the chemistry of organic remains in the site, without corrosive chemical treatment. The technique enabled the researchers to detect traces of the complex chemical chitin - a very tough compound found in the cell walls of fungi. On further analysis, they also demonstrated that the fossil mushroom cells had a prominent nucleus. "Only by cross-correlating chemical and micro-spectroscopic analyses could we demonstrate that the structures found in the old rock are indeed about 800-million-year-old fungal remains," said study co-author Liane G Benning from GFZ German Research Centre for Geosciences.





CORONAVIRUS

What is coronavirus ?

Coronaviruses are a large group of viruses that are common among animals. In rare cases, they can be transmitted from animals to humans. The spikes protruding from the virus's membrane look like the sun's corona. It is from this that the virus gets get the name 'coronavirus'. It causes illnesses of the respiratory tract, ranging from the common cold to severe conditions like SARS.The ability of the virus to pass from one person to others is sufficient to cause sustained community transmission. It is primarily passed between people via respiratory droplets produced when an infected person coughs or sneezes. The virus can also be spread by people who do not show symptoms.

Virology

SARS-CoV-2 is closely related to SARS-CoV-1 (75% to 80% identical). It is thought to have a zoonotic origin based on probable epidemiological links to the Huanan Seafood Market. Genetic analysis has revealed that the coronavirus genetically clusters with the genus Betacoronavirus, in lineage B of the subgenus Sarbecovirus together with two bat-derived strains. It is 96% identical at the whole genome level to other bat coronavirus samples. In February 2020, researchers from South China Agricultural University announced that there is a 99% similarity in genome sequences between the viruses found in pangolins and those from human patients, suggesting that the animal may be an intermediary host for the virus, but did not release evidence. At least five genomes of the novel coronavirus have been isolated and reported.The coronavirus enters human cells through a receptor called angiotensin-converting enzyme 2 (ACE 2), a membrane exopeptidase. Bayesian analysis by Benvenuto et al. of the genome sequences of SARS-CoV-2 and related coronaviruses, shows that the nucleocapsid and the spike glycoprotein have some sites under positive selective pressure. Homology modelling indicated certain molecular and structural differences among the viruses. The phylogenetic tree showed that SARS-CoV-2 significantly clustered with a bat SARS-like coronavirus sequence, whereas structural analysis revealed mutations in spike glycoprotein and nucleocapsid protein. The authors conclude SARS-CoV-2 is a coronavirus distinct from SARS virus that probably was transmitted from bats or another host that provided the ability to infect humans.

Replication

After entry into the host cell, the virus particle is uncoated, and its genome enters the cell cytoplasm. The coronavirus RNA genome has a 5′ methylated cap and a 3′ polyadenylated tail, which allows the RNA to attach to the host cell's ribosome for translation. Coronavirus genomes also encode a protein called RNA-dependent RNA polymerase (RdRp), which allows the viral genome to be transcribed into new RNA copies using the host cell's machinery. The RdRp is the first protein to be made; once the gene encoding the RdRp is translated, translation is stopped by a stop codon. This is known as a nested transcript. When the mRNA transcript only encodes one gene, it is monocistronic. Coronavirus non-structural proteins provide extra fidelity to replication, because they confer a proofreading function, which is lacking in RNA-dependent RNA polymerase enzymes alone. The genome is replicated and a long polyprotein is formed, where all of the proteins are attached. Coronaviruses have a non-structural protein – a protease – which is able to cleave the polyprotein. This process is a form of genetic economy, allowing the virus to encode the greatest number of genes in a small number of nucleotides.

Genomic cis acting elements

In common with the genomes of all other RNA viruses, coronavirus genomes contain cis-acting RNA elements that ensure the specific replication of viral RNA by a virally encoded RNA-dependent RNA polymerase. The embedded cis-acting elements devoted to coronavirus replication constitute a small fraction of the total genome, but this is, it is presumed, a reflection of the fact that coronaviruses have the largest genomes of all RNA viruses. The boundaries of cis-acting elements essential to replication are fairly well-defined, and an increasingly well-resolved picture of the RNA secondary structures of these regions is emerging. However, we are in only the early stages of understanding how these cis-acting structures and sequences interact with the viral replicase and host cell components, and much remains to be done before we understand the precise mechanistic roles of such elements in RNA synthesis.

Genome packaging

The assembly of infectious coronavirus particles requires the selection of viral genomic RNA from a cellular pool that contains an abundant excess of non-viral and viral RNAs. Among the seven to ten specific viral mRNAs synthesized in virus-infected cells, only the full-length genomic RNA is packaged efficiently into coronavirus particles. Studies have revealed cis-acting elements and trans-acting viral factors involved in coronavirus genome encapsidation and packaging.

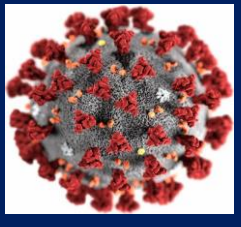


PHOTO GALLERY

Chrysanthemum

Gazania

Centaurea cyanus

Dahlia sp.

- Ruchika Dani, TYBSc

FACT 'O' MANIA

- The *Psychotria elata*, more commonly known as Hooker's Lips can be found in tropical rain forests of Central and South America. It looks like big red lips and it puckers up to attract Hummingbirds and butterflies.
- The *Senecio peregrinus* plant is a succulent that has leaves that look like tiny little dolphins jumping out of the waves.
- Some species of the *Trachyandra* genus of plants produce extensively twisty and curly foliage ; an adaptation in response to hot climates.
- Lots of plants are used as dyes. You can colour cloth with stewed onion skin, tea bags or walnut juice. One of the oldest blue dyes comes from a plant called 'woad' that has been used since Neolithic times – more than 6000 years ago.
- The Poison Garden at England's Alnwick Garden is filled with plants that can kill you.
- There is a plant in Australia known as the "Suicide Plant" because the effect of its sting can last for years, and its pain is so unbearable that people have killed themselves after touching it.

TEACHER IN-CHARGE – Dr.Mahavir Gosavi.
LAYOUT DESIGNER – Ms. Shweta Vaidande.
EDITOR – Ms. Sneha Nair
WEB MANAGERS – Mr. Shubham Patkar, Ms. Ruchika Dani and Ms. Shrawani kharkhande

Email address : gulmohar.botanynewsletter@gmail.com
Instagram : gulmohar_botany_newsletter
Facebook : Gulmohar – Botany Newsletter