Editorial

Biotech Rainbow Magazine

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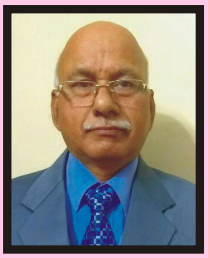

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Electron Microscope Emerging a Powerful Tool for Research in Biotechnology

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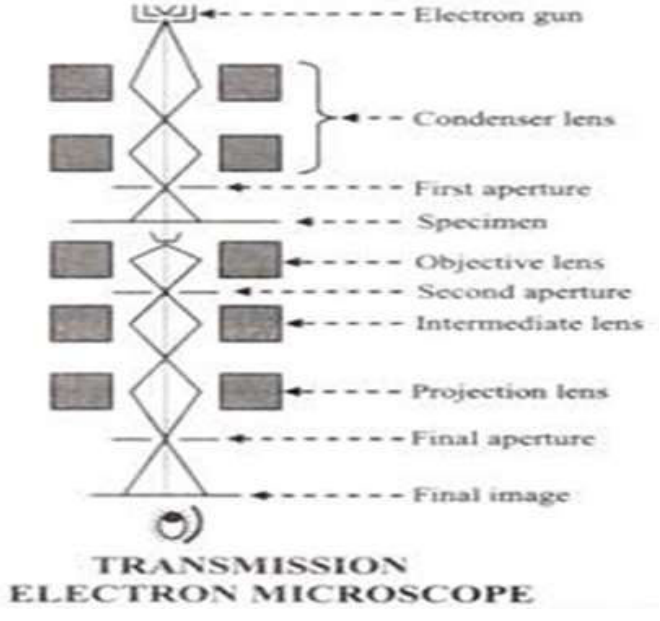
Infectious diseases of animals are important especially when they are capable of infecting humans. Such diseases are called zoonoses. Based on their aetiologies, zoonoses may be bacterial, viral, mycotic or parasitic. Zoonotic infections occur worldwide and often spread to humans through their companion domestic animals as well as through wild animals. As a result of our interconnectedness, infectious diseases emerge more frequently, spread greater distances, pass more easily between humans and animals, and change rapidly into new and more virulent strains. Therefore, electron microscope is an important tool for biomedical investigations.

The electron microscope a high energy beam of electrons is shone through a very thin sample, and the interactions between the electrons and the atoms can be used to observe features such as the crystal structure and features in the structure like dislocations and grain boundaries. It has already been known that, the smaller is the wavelength of light, the greater is its resolving power. The wavelength of green light (=0.55μ) is 1, 10,000 times longer than that of electron beam (=0.000005μ or 0.05 Å; 1μ = 10,000 Å). That is why, despite its smaller numerical aperture, an electron microscope can resolve objects as small as 0.001μ (=10 Å), as compared to 0.2μ by a light microscope. Thus, the resolving power of an electron microscope is 200 times greater than that of a light microscope. It produces useful magnification up to X 400,000, as compared to X 2000 in a light microscope. Thus, the useful magnification is 200 times greater in an electron microscope than that in a light microscope.

There are two types of electron microscopes as described below :

(1) Transmission Electron Microscope (TEM) :

In this microscope, an electron beam from an electron gun is transmitted through an ultra-thin section of the microscopic object and the image is magnified by the electromagnetic fields. It is used to observe finer details of internal structures of microscopic objects like bacteria and other cells. The specimen to be examined is prepared as an extremely thin dry film or as an ultra-thin section on a small screen and is introduced into the microscope at a point between the magnetic condenser and the magnetic objective.



TRANSMISSION ELECTRON MICROSCOPE

The point is comparable to the stage of a light microscope. The magnified image may be viewed on a fluorescent screen through an airtight window or recorded on a photographic plate by an in-built camera. Modern variants have facility to record the photograph by digital camera.

(2) Scanning Electron Microscope (SEM) :

In a scanning electron microscope, the specimen is exposed to a narrow electron beam from an electron gun, which rapidly moves over or scans the surface of the specimen. This causes the release of a shower of secondary electrons and other types of radiations from the specimen surface. The intensity of these secondary electrons depends upon the shape and the chemical composition of the irradiated object. These electrons are collected by a detector, which generates electronic signals. These signals are scanned in the manner of a television system to produce an image on a cathode ray tube (CRT). The image is recorded by capturing it from the CRT. Modern variants have facility to record the photograph by digital camera. This microscope is used to observe the surface structure of microscopic objects.

It has both transmission and scanning electron microscope functions.

Limitations of Electron Microscopes :

The limitations of electron microscopes are as follows :

(a) Live specimen cannot be observed.

(b) As the penetration power of electron beam is very low, the object should be ultra-thin. For this, the specimen is dried and cut into ultra-thin sections before observation.

Principle of Electron Microscope :

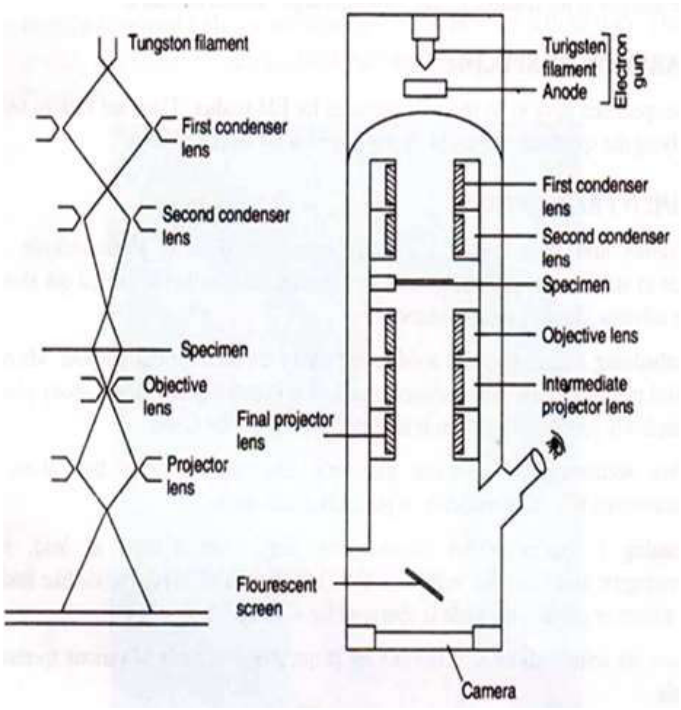
Electrons are subatomic particles, which orbit around the atomic nucleus. When atoms of a metal are excited by heat energy, electrons fly off from the atom. In electron microscope, tungston is heated by applying a high voltage current, electrons form a continuous stream, which is used like a light beam.The lenses used in EM are magnetic coils capable of focusing the electron beam on the specimen and illuminating it. The strength of the magnetic lens depends upon the current that flows through it. Greater the flow of the current, greater will be strength of the magnetic field. The electron beam cannot pass through the glass lens.

Components of Transmission Electron Microscope :

EM is in the form of a tall column which is vertically mounted.

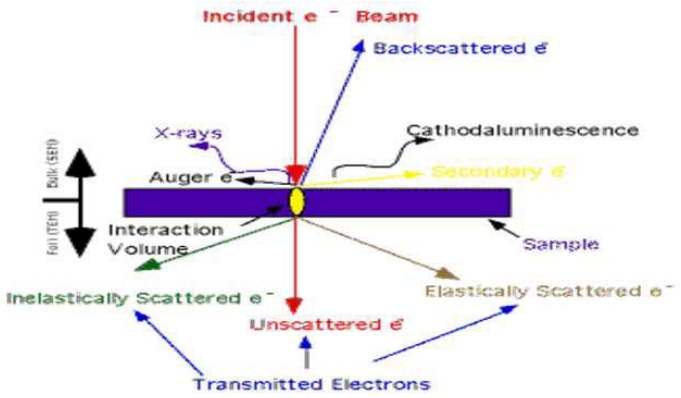
It consists of the following main components :

1. Electron gun
2. Electromagnetic lenses—three sets.
3. Image viewing and recording system.



Electron gun is a heated tungsten filament, which generates electrons. Condenser lens focuses the electron beam on the specimen. A second condenser lens forms the electrons into a thin tight beam.To move electrons down the column, an accelerating voltage is applied between tungsten filament and anode. Now most EMs use accelerating voltages between 100 kV-1000 kV. Electrons also function as a source of illumination for the specimen. High velocity electrons pass into the system of condenser lenses, which focus them on the specimen. The specimen to be examined must be extremely thin, at least 200 times thinner than those used in optical microscope. Ultra thin sections of 20-100 nm are cut. The specimen holder is an extremely thin film of carbon or collodion held by a metal grid.

Scattering of Electrons



The electronic beam passes through the specimen and electrons are scattered depending upon the thickness or refractive index of different parts of the specimen. The denser regions in the specimen scatter more electrons and therefore appear darker

in the image since fewer electrons strike that area of the screen. In contrast, transparent regions are brighter. The electron beam coming out of the specimen passes down the second of magnetic coils called objective lens, which has high power and forms the intermediate magnified image. Finally, a third set of magnetic lenses called projector (ocular) lenses produce the final further magnified image. Each of these lenses acts as image magnifier all the while maintaining an incredible level of details and resolution. His whole image remains in focus. This image is projected on a fluorescent screen. Below the fluorescent screen is a camera for recording the image. These lenses provide immense magnification and resolution.

As the EM works in vacuum, the specimen should be completely dry. Air molecules present in the column of EM scatter the electrons causing flicker in the electron beam. Vacuum is created in two steps. Firstly, a mechanical vacuum pump is used to create vacuum. Secondly, a diffusion pump uses a fast downward moving liquid, either oil or mercury which traps air and gas in the column. In this way, ultra high vacuum is created, It consists of a tungsten filament or cathode that emits electrons on receiving high voltage electric current (50,000-100,000 volts). Near the top of the tube is an anode which attracts electrons.

(b) Ray tube (Microscope Column) :

It is a high vacuum metal tube (2mt. high) through which electrons travel.

(c) Condense lens :

It is the electromagnetic coil which focuses the electron beam in the plane of the specimen.

(d) Objective lens :

It is the electromagnetic coil which produces the first magnified image formed by the objective lens and produces the final image.

(e) Projector lens :

It is also an electromagnetic coil which further magnifies the first image formed by the objective lens and produces the final image. Each electromagnetic coil has a coil of wire encased by a soft iron casing.

(f) Fluorescent Screen or Photographic Film :

Since unaided human eye cannot observe electrons, therefore, a fluorescent screen is used for viewing the final image of the specimen. The final image can be captured on photographic film and die photograph obtained is called an electron micrograph.

Sample preparation for Transmission electron microscopy (TEM)

In the TEM electrons is transmitted through an ultrathin sections, of the specimen The image is formed from the electrons transmitted through the specimen, magnified and focused by an objective lens and appears on a fluorescent screen, plus a monitor, or to be detected by a sensor such as a CCD camera. Biological materials contain large quantities of water. To be able to view it in the electron microscopy, the first stage in preparing is the fixation, one of the most important and most critical stages. We need to fixed it in a way that the ultrastructure of the cells or tissues remain as close to the living material as possible. The sample is then dehydrated through an acetone or ethanol series, passed through a “transition solvent” such as propylene oxide and then infiltrated and embedded in a liquid resin such as epoxy and LR White resin. After embedding in the resin, block were made and then thin sectioned by a process known as ultramicrotomy, sections of 50-70 nm thickness are collected on metal mesh ‘grids’ and stained with electron dense stains before observation in the TEM. Sectioning the sample allows us to look at cross-sections through samples to view internal (ultra)structure. Many modifications to the basic protocol can be applied to achieve many different goals, immunogold labeling for example; the in situ localization of specific tissue constituents using antibody and colloidal gold marker systems. Every sample is different. Please consult with the EM Staff before starting a project. Support film on TEM grids Formvar film is useful for the support of ultrathin sections on the finer mesh grids. Using of support film are ideal for those applications requiring large viewing areas without grid bar interference. These films must be strong, clean and remain attached to the specimen grids during specimen preparation. A Formvar film covered with a “light” layer of carbon will help to stabilize the film when the film is exposed to the electron beam. Sectioning with ultramicrotome Materials for TEM must be specially prepared to thicknesses which allow electrons to transmit through the sample, much like light is transmitted through materials in conventional optical microscopy. Because the wavelength of electrons is much smaller than that of light, the optimal resolution attainable for TEM images is many orders of magnitude better than that from a light microscope. The block is cut into semithin sections (1 µm) with a glass knife, using an ultramicrotome. The sections are then stained with Toluidine Blue for LM for orientation, and for selecting of a small area for ultrathin sectioning. Ultrathin sections are made at 50 70 nm using a diamond knife and placed/collected on a grid of metal. Positive staining Side 2 Details in light microscope samples

can be enhanced by stains that absorb light; similarly TEM samples of biological tissues can utilize high atomic number stains to enhance contrast. The stain absorbs electrons or scatters part of the electron beam which otherwise is projected onto the imaging system. Uses heavy metals such as lead and uranium to scatter imaging electrons and thus give contrast between different structures, since many (especially biological) materials are nearly "transparent" to electrons (weak phase objects). Heavy metal salts attach to various organelles or macromolecules within the sections to increase their electron density and they appear dark against a lighter background. Uranyl ions react strongly with phosphate and amino groups so that nucleic acids and certain proteins are highly stained. Lead ions bind to negatively charged components and osmium reacted areas (membranes). Grids are stained with heavy metals, such as uranyl acetate and lead citrate. The grids, with the specimen side down, remain in 4% uranyl acetate for 25 minutes and are then rinsed in a series of four beakers of pure water. After rinsing, the grids are then stained with 1% lead citrate for 5 minutes, rinsed again in pure water, and stored in a grid box.

The specimen have to be specially prepared for EM studies. There are various techniques for studying the specimen under EM. Some of which are discussed here.

Fixation and Dehydration :

The specimens are fixed in glutaraldehyde, osmium tetroxide to stabilize the cell structure. After fixation, dehydration is carried out slowly with organic solvents like acetone and ethanol.

Embedding :

Resins such as araldite and epoxy are used for this purpose. Microbes are embedded in plastic resin. The specimen is soaked in un-polymerized, liquid epoxy plastic until it is completely permeated and then is hardened to form a solid block.

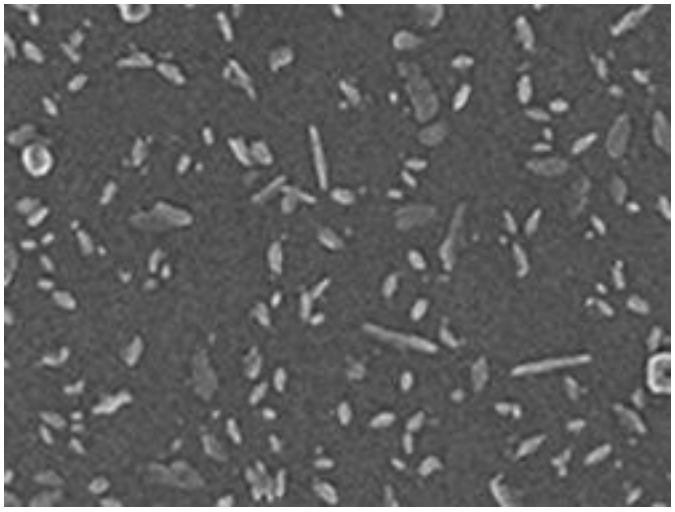
Ultra sectioning :

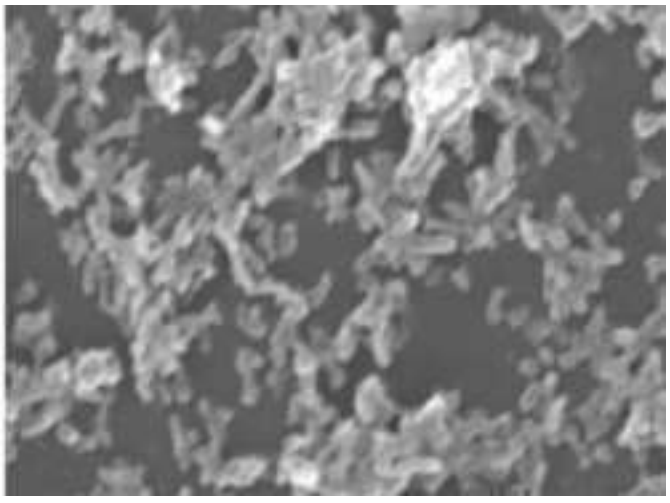
To obtain extremely thin sections from this plastic block, Ultra-microtomes with diamond knife or glass knives are used.

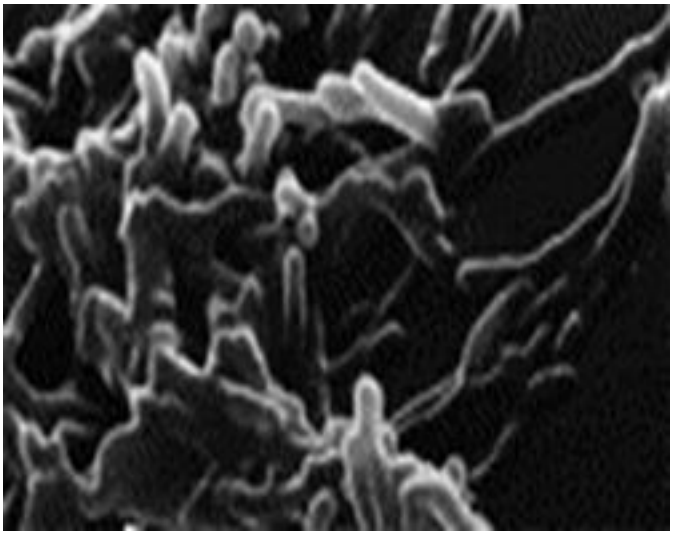
Staining :

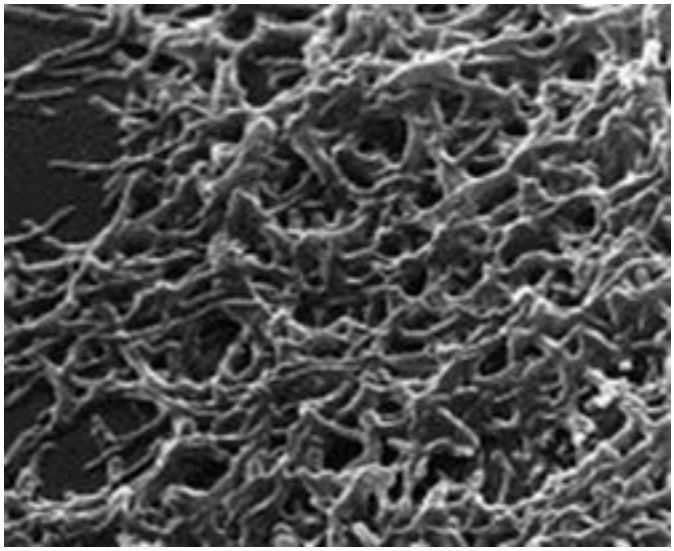
Specimens are stained with heavy metals such as lead, uranium, phosphotungstic acid. The thin sections soaked in solutions of heavy metals like lead citrate, uranyl acetate or osmium tetroxide is also used for staining.

However, electron microscopy emerging a power tool to investigate the detailed structure and configuration of the mycobacteria which may lead to an understanding the role of infections and in transmitting the diseases. Bacterial biofilms are often associated with infections specially with medical implants such as catheters and other medical devices. In the natural world more than 99% of bacteria exist as biofilms and according to NIH report more then 75% of all human infections are associated with biofilms formation. Biofilms are slimy, glue-like substance that excreted by bacteria and aggregate on living surface. Biofilms are formed to protect the bacteria from host defences, antibiotics and from harsh environmental conditions. Biofilms are found almost everywhere in nature, including soil, water pipes, and even inside the human body. Attachment of mycobacteria involved in biofilm formation in the liquid air interface is a complex process, with many variables such as pH, nutrient levels, iron, oxygen, ionic strength and temperature, affecting the outcome.





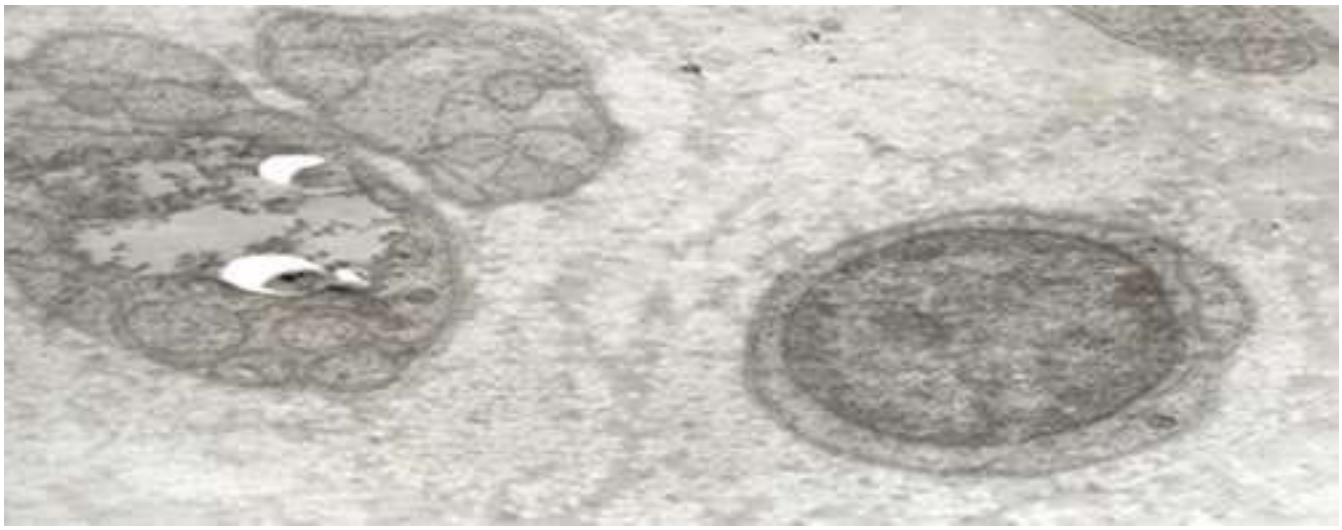




Figures : *Showing various forms of Biofilm developed by Mycobacteria*

However, many mycobacterial species are known to form biofilms, little is known about either the genetic requirements, patterns of gene expression. In micro array hybridisation we have found that six genes were expressed in *M.avium.* In *M. tuberculosis* MDR isolates seven genes were expressed and two genes Rv0359 and Rv3526 were homologus as earlier reported in *P. areuginosa* and *M. avium* which might be responsible for biofilm formation.

On the other hand electron microscopy used to investigate the detailed structure and configuration of the mycobacteria in the Schwann cells (SC) and endothelial cells in leprosy patients. In multibacillary patients, the ultrastructural study showed significant changes in peripheral nerves and endoneurial blood vessels. It was revealed that besides the SC the endothelial cells of endoneurial blood vessels also frequently harbor bacilli. In possibacillary patients showed the degenerative changes of SC and hypertrophy of endothelial cells leading to narrowing or complete occlusion of lumen of endoneurial blood vessels. The endothelial cells of endoneurial blood vessels were found to be loaded with bacilli and this bacillary loaded endothelial cell was found to release bacilli into the lumen through its ruptured membrane. Therefore, we have concluded that SC is not only targeted cells of *M.leprae* infection but endothelial cells are equally responsible for harboring bacilli and transmission of *M.leprae* in various parts of the body.



Figures : *Showing various forms of Biofilm developed by Mycobacteria*

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Emergence of Zika Virus (ZiV)

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Introduction :

Zika virus (ZiV) is a mosquito-borne flavivirus (family Flaviviridae); Vector: aedes (*ae. Aegypti, ae. Albopictus*) also causes dengue, chikungunya, yellow fever. Bit by female mosquitoes mostly in daylight hour, associated with a neurological birth complication and also the rapid spread of this virus across the globe. First analyses in rhesus monkey of zika forest Uganda in April 1947, during the research on the yellow fever, Rockefeller foundation initiative. WHO declared the Zika outbreak to a 'Public health emergency of international concern' on January 2016. In 2007, first large outbreak of disease caused by Zika infection was reported from the island of YAP (Federated States of Micronesia). In July 2015, Brazil described an association between Zika and Guillain-Barre disorder. In Oct 2015, Brazil described an association between Zika and Microcephaly. Sexual transmission of Zika is also possible and blood transfusion can also cause Zika.

Current scenario of Zika Virus(ZiV) outbreaks :

It has been spread over more than 25 countries till now. First indigenous case of America was found in February 2015, in Chile. Since April 2015, a large outbreak of ZiV has spread across much of central and south America. Then it began in Brazil, in May 2015, 16 cases investigated with Zika infection. In June 2015, first case of ZiV was presented in Dominican Republic. In Jan 2016, a travel alert was issued by CDC for traveler towards ZiV affected countries. Researchers from Brazil identify a new vector of ZiV is *Culex quinquefasciatus*.

Research done so far

OX513A, is a genetically modified male Aedes aegypti, creation of british company Oxitec. OX513A stop the spread of ZiV by passing along gene that makes his offspring die. Each OX513A carries a fluorescent marker so he can be tracked by the scientist. Field trials in Brazil in 2011 were hugely successful. A new release of males in Brazil in 2014 was 92% successful.

Bharat biotech claimed to have attained a breakthrough in developing a ZiV vaccine to fight the dreaded mosquito born disease. Bharat biotech claimed that it had started working on vaccine using live ZiV but nobody knows that from where /when they got this virus because normally nobody could import any exotic virus into the country, it requires government authority by investigate all the aspects.

Sanofi (French pharma company) geared up to launch a project to develop vaccine at the end of this year. Researchers from the University of Canada announce first vaccine ready for human testing and also approved by food and drug administration (FDA) and Health, Canada.

Researchers from the University of Southern California (USC) have recently analyze the key proteins associated with this disease, may helps to correct Zika-related malformations.

Tanaka Kikinzoku Kogyo, Tokyo has developed the world's first kit able to direct analyze the Zika virus in blood. The kit is capable of rapid ZiV detection in just 10 to 15 minutes.

Scientists from the University of Massachusetts Medical School (UMMS) in the US, advised that boosting the activity of the interferon induced protein 3 (IFITM3) may be useful for inhibiting ZiV and other emerging viral infections.

It was also found that of the ten proteins that make up the ZiV, two proteins (NS4A and NS4B) are playing key role in the development of small brains in infected babies, i.e. Microcephaly.

NIV (ICMR) Pune, scientist reveals the similarity between E protein of ZiV and dengue viruses. Envelope glycoprotein (E protein) of ZiV may be necessary as E Protein is the main target for host antibodies. ZiV E protein amino acid sequence has

been compared (using bioinformatics tools) with from other flavivirus like dengue type 2 (DEN2), Japanese encephalitis (JEV), West nile (WNL), Kyasanur forest disease (KFDV), Yellow fever (YFV), Tick-borne encephalitis (TBEV) and St. Louis encephalitis (StLU). The following observations came out:

Maximum homolog (DEN2): 54.2% of identity, 81% of similarity

Least homolog (KFDV): 36.3% identity, 67.2% similarity

Structure of Zika virus :

The virion is about to 40 nm in diameter. Nucleocapsid is about to 25-30 nm in diameter surrounded by a host membrane derived lipid bilayer. Enveloped, Spherical Icosahedral symmetry (of surface protein), Contains envelope proteins E and M, Single stranded Positive sense RNA genome containing 10794 nucleotide, Encoding 3419 amino acids.

Diagnosis of Zika Virus :

About one in five people infected with ZiV become ill (develop Zika). Symptoms could be seen within 2-7 days after the Zika infection, are same as dengue and chikungunya for example fever, rash, joint pain, conjunctivitis, muscle pain, headache.

Acute phase (3-5 days): detection of viral genome by RT-PCR in maternal serum and amniotic fluid Ambulatory phase (>5 days):

Serology by testing IgM antibodies in blood through ELISA

Plaque reduction neutralization test (PRNT): it may give cross reactive results in case of secondary flavivirus infection.

MAC ELISA: IgM antibody capture ELISA is most commonly used format in diagnostic laboratories and commercial available diagnostic kits. It is based on capturing human IgM antibodies on a micro titer plate by using anti human IgM antibody followed by the addition of ZiV specific antigen (DENV1-4). The antigens used for this methods, are derived from the virus envelope protein. One of the disadvantage of this testing is the cross reactivity between other flaviviruses.

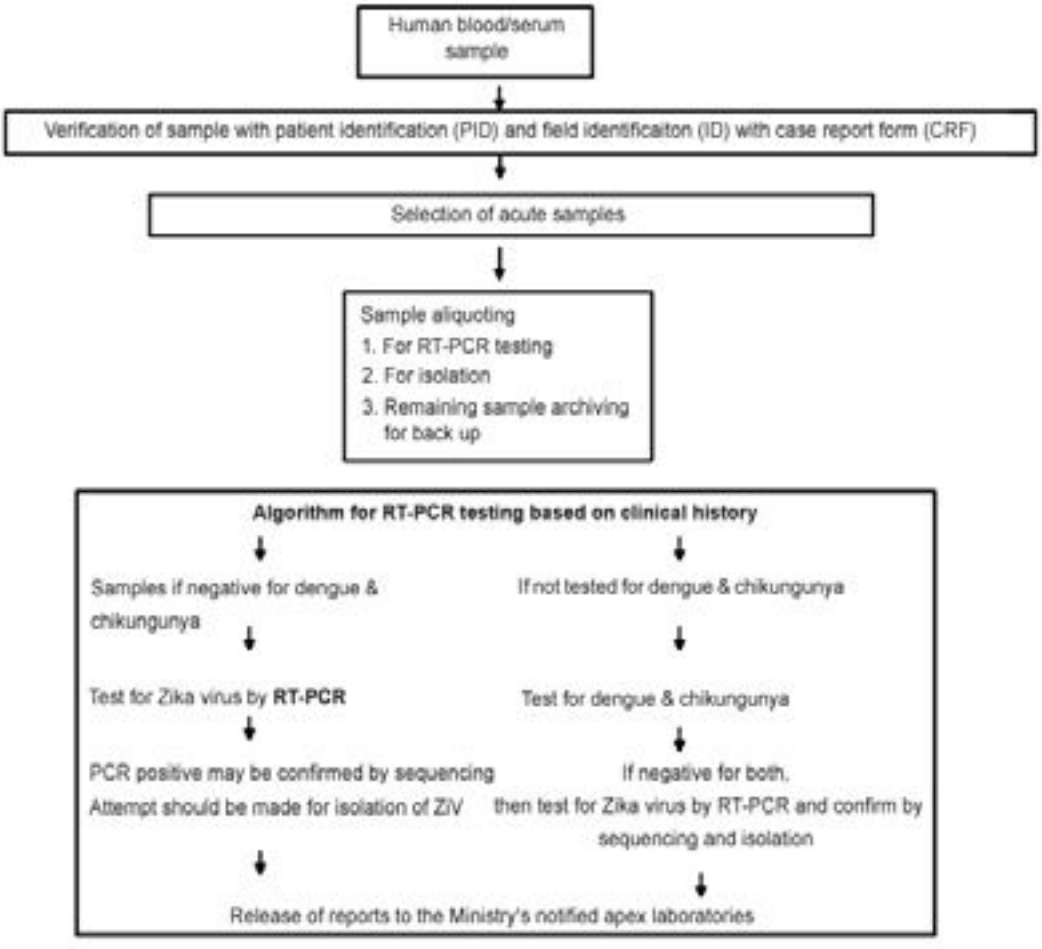

Fig : Flowchart for Zika diagnostic protocol

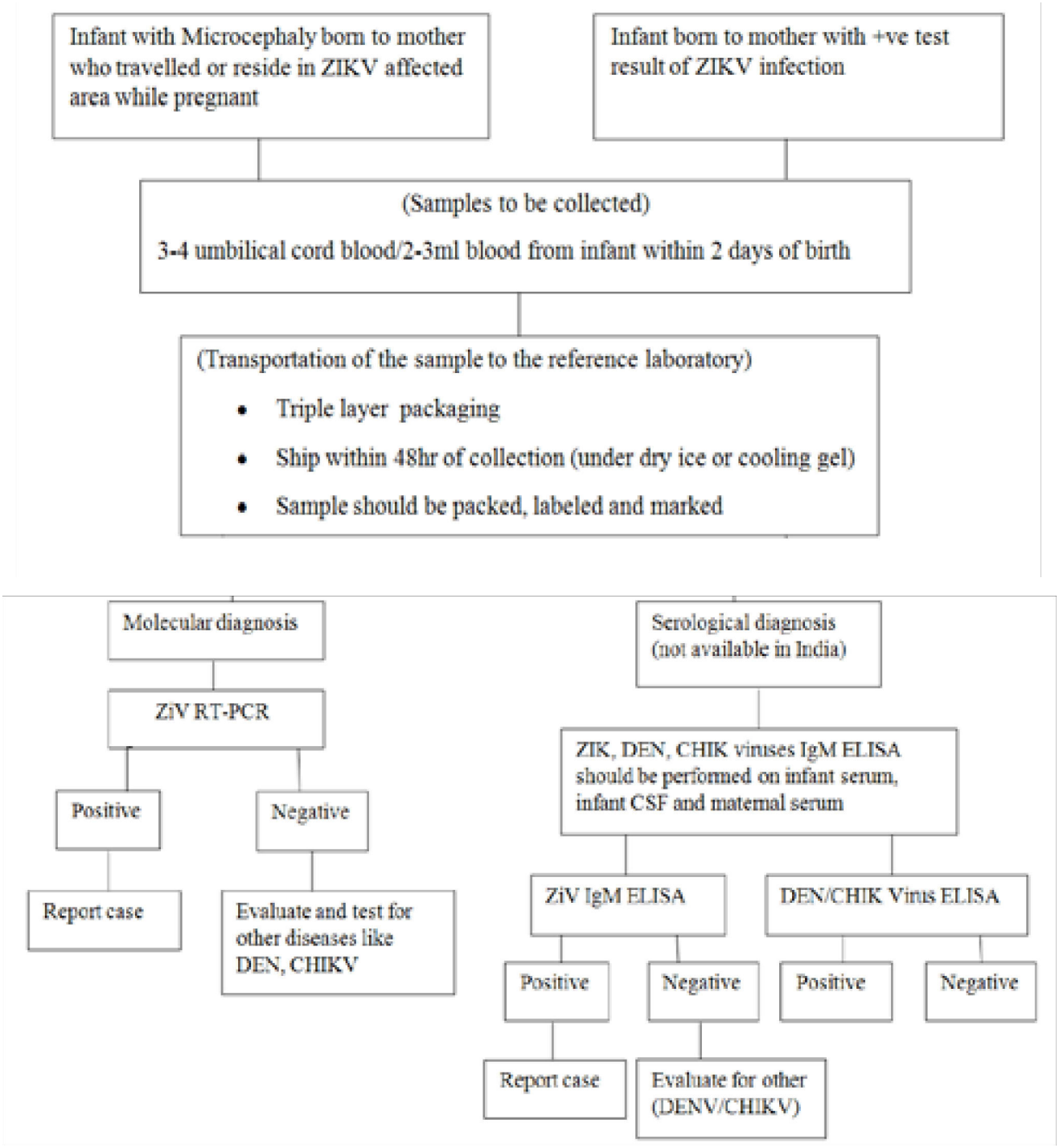

Fig : Flowchart for infants with possible congenital ZiV infection

Treatment and control :

There is no vaccine available in the world for ZiV so precaution is only way to cure in case of Zika infection.

Reducing mosquito through source deduction (elimination and modification of breeding sites).

Reducing contacts between mosquitoes and peoples

Wearing full body cloths

Close door and windows, use screen in windows

Use mosquito net for sleeping

Clean/cover the bucket, flower pots, tyers etc

Use insecticides/insect repellent like DEET, IR3535 OR Icaridin, KBR3023 or Picaridin, Bayrepel

Drink enough fluids, take vitamin C rich fruits

Treat pain and fever with common medicine like paracetamol

Use integrated vector management (chemical, biological, environmental)

Ayurveda treatment :

5 basil leaves + 4 black pepper beads + 3 clove buds + 1 teaspoon of fresh ginger. Boil the entire mixture until

the quantity of the water is halved. Filter it and add a teaspoon of honey. Take this twice daily

Tinospora cordifolia: take two leaves of this in the morning and evening as well

Add one teaspoon of turmeric powder in a glass of warm milk during it every night.

Zika through the years :

1947 (discovered in Zika forest of Uganda)

1950-1980 (Human infection across Africa and Asia)

2007 (Large outbreak from Island of Yap)

Oct, 2013 (Reported in French Polynesia, 66% population get affected till Apr 14th)

Apr, 2015 (Identified in Brazil, 1.5 million people get affected at the end of 2015)

Feb, 2016 (WHO declared health emergency for ZIKV)

Apr, 2016 (CDC confirm the link between ZIKV and Microcephaly)

Today (Spread rapidly through more than 25 countries, Americas and 30+ of its territories)

Conclusion :

Though the presence of ZiV has not been detected yet in India but the possible association of this virus infection with Microcephaly, Guillain-Barre and other neurological symptoms is exposed. Thus, the preparedness for the ZiV has to be there in our country. This epidemic has been notified recently internationally and requires very strict surveillance programme to spread awareness among the peoples.

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CRISPR – Theory and Technology

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Advent of recombinant DNA technology paved way towards genome editing. Since then, genome editing technology evolved steadily with consistent improvisation of the former technology. One such improvised technology was CRISPR-Cas9 which exploited the microbial adaptive immune system – CRISPR - found in bacteria and archaea. It provides acquired immunity against foreign viruses and plasmids. It was initially found in the E.coli K12 bacterial genome as repeated motifs of <50bp that were neatly and consistently ordered. At that time, it was considered to be "exotic junk of DNA". Later, in the year 2002, this junk of DNA was named as Clustered Regularly Interspaced Short Palindromic Repeat' – CRISPR.

Image courtesy : Crispr-Cas9 plasmid www.systembio.com.

CRISPR Locus

The presence of CRISPR on a bacterial genome is called CRISPR locus. The figure 1 shows cmponent of a CRISPR locus :

Components of a CRISPR locus

• CRISPR Array

o Spacers - Small variable sequences in the bacterial genome that are acquired from foreign nucleic acids flanked by repeats

o Leader – Includes a promoter and contains long A-T rich region

• Cas nuclease/ Cas proteins

o Contains DNA endonuclease and two RNA components

o crRNA and tracrRNA

o Required for multistep defense against invasive element

• Protospacer adjacent motifs (PAM)

o Differentiates between self and non-self DNA

Types of CRISPR

The CRISPR-Cas system is generally divided into three types, depending on the Cas protein sequence and structure :

Type	Function	Cas proteins used
I	Cleaves and degrades DNA	Several Cas proteins
II	Cleaves DNA only	Only a single Cas protein - Cas 9
III	Cleaves DNA or RNA	Several Cas proteins

The CRISPR-Cas9 system is widely used for genome editing. It belongs to type II CRISPR-Cas system adapted from Streptococcus pyogenes.

Types of Cas proteins

There are 45 major Cas proteins identified so far. Among them, Cas 1-10 is of major importance. There are three types of Cas proteins based on the process it is involved with :

• Universal – Spacer acquisition – Cas 1 and 2

• Signature – Target interference – Cas 3, 9, 10

• Type specific – crRNA expression – Cas 4, 5, 6, 7 and 8

Mechanism of CRISPR mediated immunity in bacteria

CRISPR-Cas systems in bacteria target viruses, plasmids, chromosomal sequences (transposons, prophages) and produce immunity against the same.

The mechanism of immunity includes 2 phases :

• Immunization phase – Includes spacer acquisition

• Immunity phase – Expression and interference

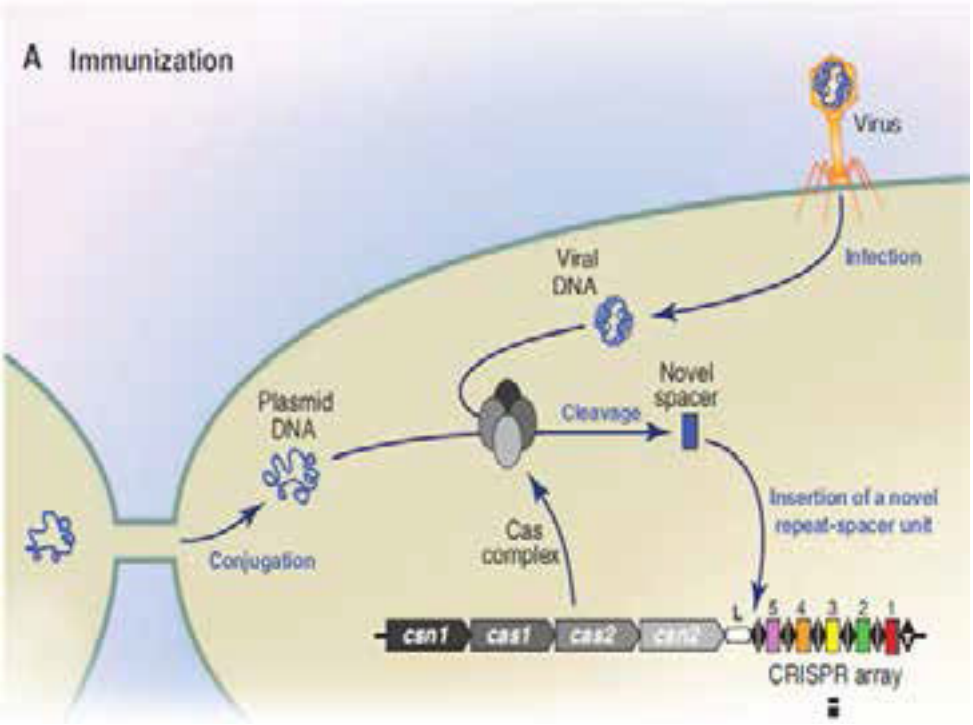


Fig : Immunization phase

Upon introduction of a foreign DNA, it is cleaved by DNA endonuclease in the Cas proteins and is inserted as spacers into the CRISPR locus.

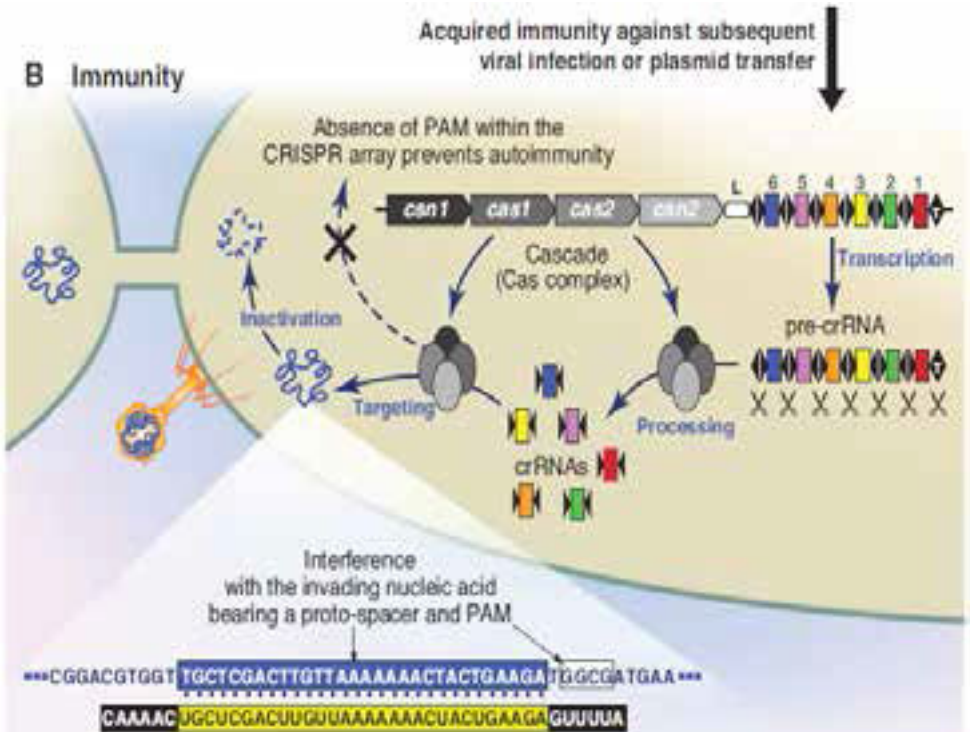


Fig : Immunity phase

Reinfection - Repeat spacer units transcribed - Pre-CRISPR RNA (pre-crRNA) - Cas9 binds to pre-crRNA - crRNA-tracrRNA-Cas9 complex - Foreign DNA destroyed.

CRISPR-Cas9 — Technology

The ability to edit genomes precisely has been fulfilled by CRISPR-Cas9 technology. With the advent of this technology, any gene of interest can be inserted or deleted effortlessly and efficiently. It is an easy and versatile platform for genome editing when compared to the strenuous zinc finger and TALEN approaches.

Components of CRISPR-Cas9 system

Genome editing using CRISPR Cas9 technology has two components :

• an endonuclease – Cas9

• a short guide RNA (sgRNA)

Endonuclease – Cas9

The endonuclease is the bacterial Cas9 nuclease protein from Streptococcus pyogenes. The Cas9 nuclease possesses two DNA cleavage domains (the RuvC1 and HNH-like nuclease domains) that cleave double-stranded DNA, making double strand breaks (DSB).

Short guide RNA

• crRNA – 20 nucleotide guide RNA + 14 nucleotide repeat region

• tracrRNA – 14 nucleotide anti-repeat region + 3 stem loops (Loop1,2,3)

o Stem loop 1 – formation of functional sgRNA : Cas9 complex

o Stem loop 2 and 3 – stability and activity of CRISPR-Cas9 system

CRISPR-Cas9 regulation

• Last 20bp at the 5' end of the sgRNA acts as a homing device that,

• Recruits Cas9 to cleave a specific dsDNAdirectly upstream of a protospacer adjacent motif (PAM)

o The target sequences which are immediately followed by the PAM sequence will be targeted for genome editing. PAM recognition sequence differs depending on the species and the type of bacteria from which the Cas9 nuclease is derived.

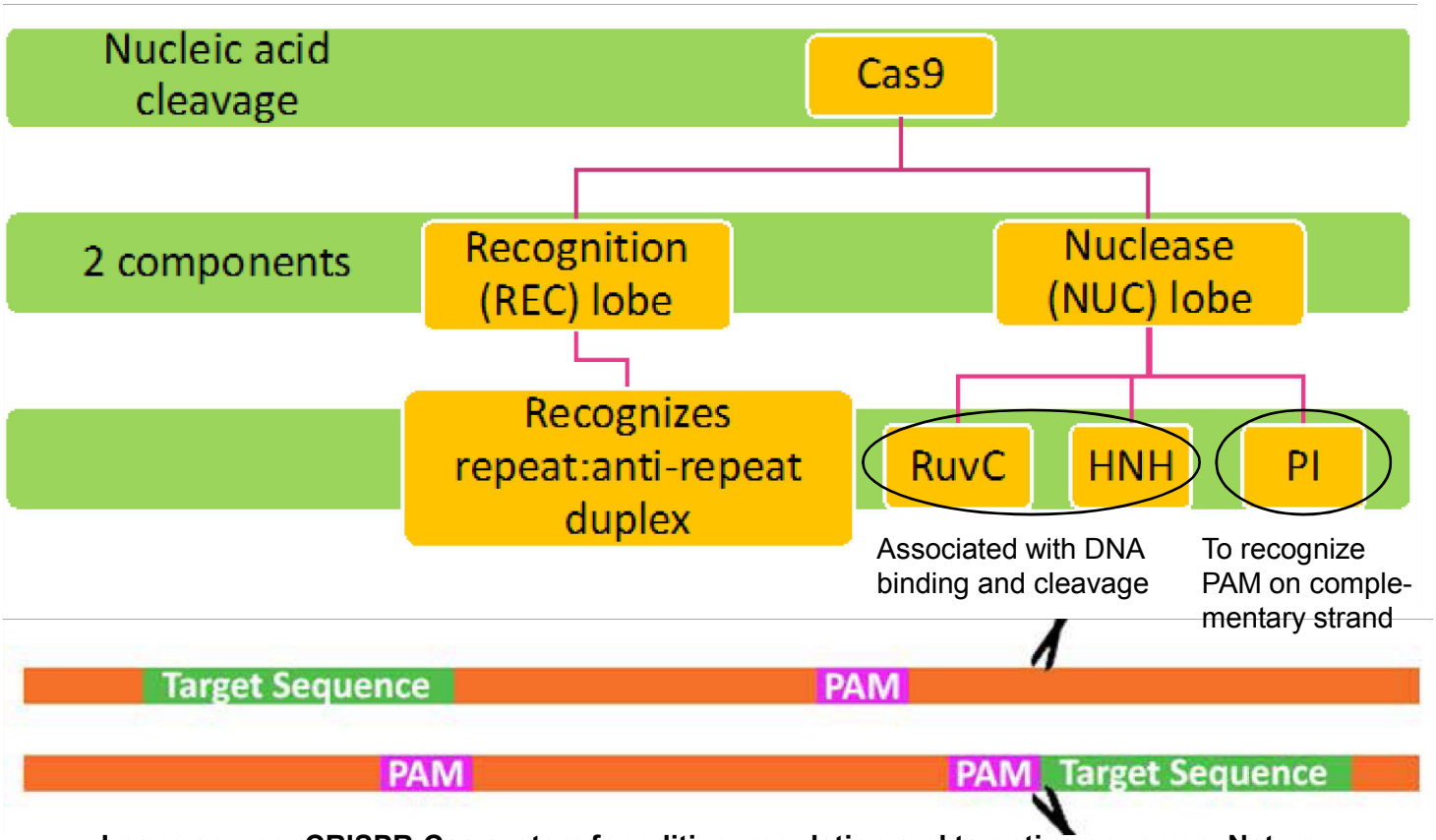


Image source: CRISPR-Cas system for editing, regulation and targeting genomes. Nature Biotechnology, 2014, Vol. 32.

• sgRNA serves as a bridge between Cas9 and target sequence

• It can be designed to recognize a particular sequence

• Successful genome editing using CRISPR-Cas9 depends on the sgRNA sequence as well as the PAM Sequence

CRISPR-Cas9 – Double strand break

Double strand break can be repaired by - Non-homologous end joining (NHEJ) or Homology directed repair (HDR)

• NHEJ: Does not use template

More error prone

Occurs with high frequency

• HR: Requires template

More accurate

Workflow of CRISPR

• Gene sequence analysis

o Essential to sequence the gene of interest of the target genome

• Design of guide RNA for Cas9

o Selection criteria:

Should highly match target sequence – To minimize off target activity

Should not have more than 3 mismatches

20nt sgRNA is often used

17 or 18nt sgRNA – More specific

Designing tools for guide RNA

• Optimized CRISPR Design

• sgRNA Scorer

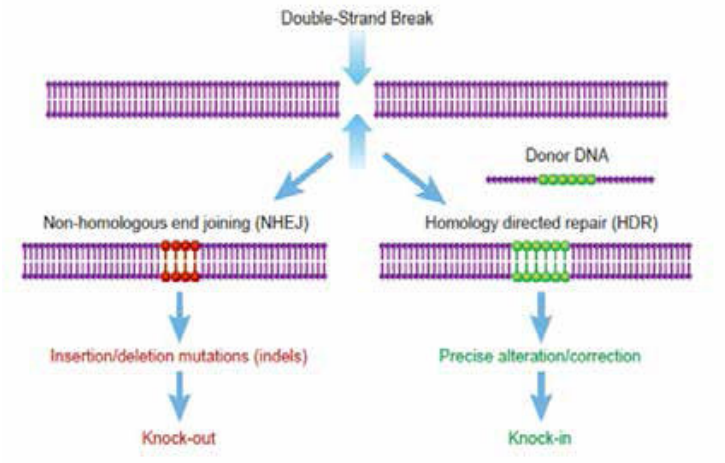
• sgRNA Designer

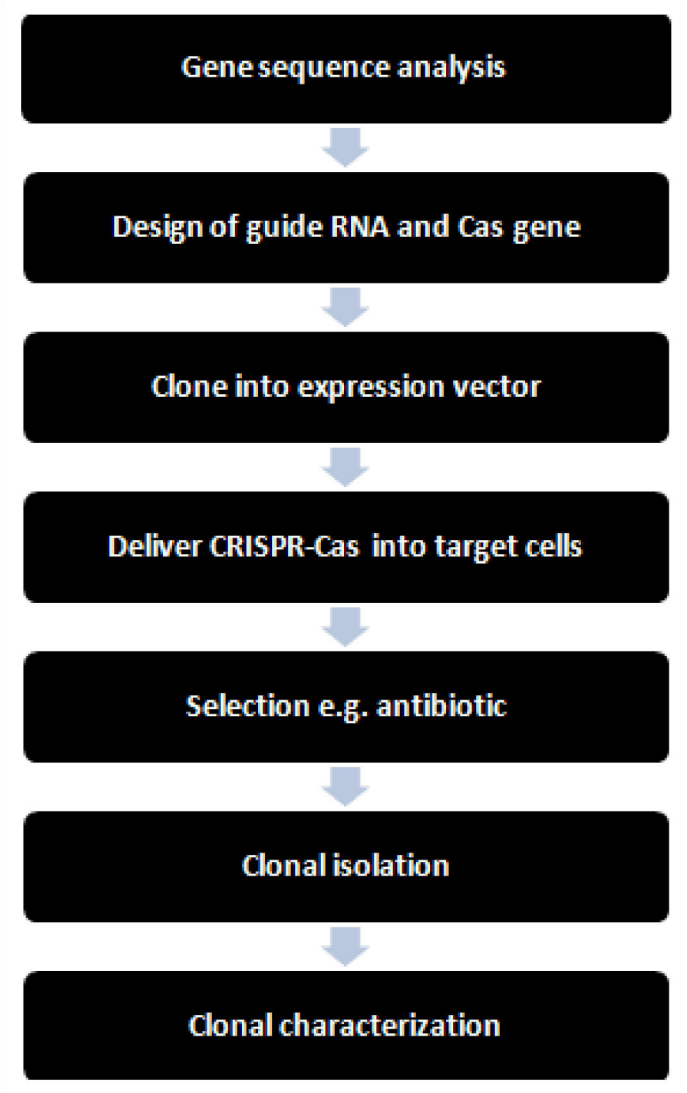
• ChopChop web tool

• E-CRISP

• CRISPR Finder

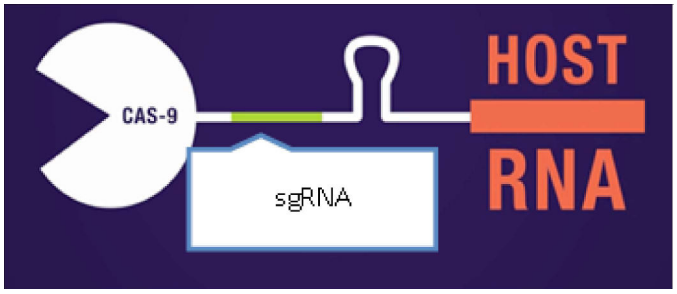
• RepeatMasker - to double check and avoid selecting target sites with repeated sequences Image source : Bhaya et al., 2011. CRISPR-Cas Systems in Bacteria and Archaea : Versatile Small RNAs for Adaptive Defense and Regulation.





So, the complex of CRISPR should contain :

o sgRNA + Cas9 + Host genome sequence (identical to the host genome)



• Design of single stranded oligonucleotide DNA – Repair template

• Choice of Cas protein

o Cas9 – Efficient in homologous recombination

o Cas9 nickase – Double nicking with two separate gRNAs

o DeadCas9 – Transcriptional pertubation of target genes without modifying the DNA

o Light-activated Cas9 – Activation of gene transcription with light stimulation

• Alteration of PAM

o PAM interacting amino acids may be replaced with different a.a to improve its specificity with Cas

• Expression vector

o Lentiviral vectors – to transfect cells

o Recombinant adeno viral vectors – in vivo gene delivery

o All in one vector – guide RNA and Cas9 genes

• Delivery of CRISPR into target cell

Target cell	Method of delivery
Mammalian cells	- Lipofection-based transfection of DNA plasmids - Electroporation of DNA plasmids or RNP - Lentiviral transduction of DNA plasmids
Microbial organisms	Transformation of plasmids into competent cells
Yeast	Electroporation of plasmids and galactose induction of Cas9
Plants	Agrobacterium mediated transformation of sgRNA and Cas9 vector
Mouse	- Direct injection of AAV into the tissue - Electroporation in zygotes

CRISPR-Cas9 — Variations to the System

There are two variations to the system introduced above that are also commonly available today : the Cas9 Nicakse and the Cas9 Double Mutant. Each of these variants has their own benefits and applications.

Applications of CRISPR-Cas9 system

• Gene disruption (without donor template DNA)

• Gene knock-out (with a reporter knock-in)

• Non-protein coding gene disruption

• Specific mutations

o Desired SNP introduction or correction

o Desired insertions/deletions

• Promoter Study

o Luciferase replaced the 5' exon

• Conditional knockout

o For essential genes or tissue-specific study inserting LoxP sites

o Around the exon to be knocked-out

• Large chromosomal deletions

o Using two signature Cas9 RNAs to induce double stranded breaks at sites that flank the region of interest

• Exogenous gene Insertion

o Adeno-associated virus integration site 1 (AAVS1) in human genome is a safe harbor for transgene integration

o A controlled Gene Knock-in e.g. controlled copy number and location

• CRISPR interference and activation of transcription

CRISPR in various disciplines

• Neuroscience

o Novel rat model for muscular dystrophy reveals new treatment targets

• Cancer biology

o Novel tumor suppressor genes and new animal models for brain tumors

• Vaccinology

o T cell engineering with CRISPR-Cas reveals a new therapeutic strategy for HIV

• Immunology

• Plant biology

o Successful adaptation of the CRISPR-Cas editing system in rice

• Therapeutics – Cancer, HIV, cardiovascular disease, SCID

• Epigenetic modifications and Stem cell differentiation

Importance of CRISPR

• High potency and specificity

• Broad applicability in vitro and in vivo

• Potential one-time curative treatment

• Ability to edit out diseases

• Ability to address any site in the genome or foreign genome

• Ability to target multiple DNA sites simultaneously

• Multi-functional programmability – Delete, insert, repair

Any enthusiastic scientist would have the curiosity and excitement to explore CRISPR technology that lead to various CRISPR based genome editing. But, considering the ethical and biosafety concerns it could raise, strict laws and regulations have been formed to exempt misuse or improper use of this technology in scientific research.

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News in Focus

Borlaug Global Rust Initiative Honors Women In Wheat Research

April 25, 2018, BGRI The Borlaug Global Rust Initiative (BGRI) awarded a group of women who have made significant contributions in wheat research and development. The winners of the 2018 Women in Triticum Award were honored at the 2018 BGRI Technical Workshop in Marrakech, Morocco.

Image : Dr Urmi Bansal

The Early Career Awards were given to the following researchers :

Meriem Aoun, who applied both conventional and molecular breeding techniques toward the release of resistant durum varieties to rusts and Fusarium head blight;

Radhika Bartaula, a plant geneticist working to unravel the genetic mechanism of resistance to wheat stem rust pathogen in barberry;

Sreya Gosh, work focuses on understanding and exploiting genes controlling resistance to leaf rust in wheat;

Raheela Rehman, who conducts studies to better understand and characterize the differences in root absorption and translocation of zinc in wheat and maize plants, as well as in various wheat genotypes with high grain zinc concentrations; and

Hannah Robinson, who engages with researchers throughout Australia and across the globe to develop research projects aimed at improving wheat and barley production.

Dr. Urmil Bansal, a molecular geneticist at the University of Sydney Plant Breeding Institute, was hailed with the Mentor Award. Aside from developing and validating closely linked markers for more than 20 rust resistance genes to facilitate marker-assisted pyramiding to control of rust diseases in wheat, she has mentored 29 M.Sc. and Ph.D. students mostly from developing countries including South Asia and Africa.

FDA Approves Application for AquaBounty Salmon Facility in Indiana

April 26, 2018

The U.S. Food and Drug Administration today approved a supplemental New Animal Drug Application (NADA) submitted by AquaBounty Technologies, Inc. The supplemental NADA requested FDA approval to raise AquAdvantage Salmon – a product under an application previously approved in 2015 – at a land-based contained facility near Albany, Indiana. While the Indiana facility is approved for production, the company is prohibited from importing the eggs necessary for producing genetically engineered (GE) salmon at the facility because of a requirement in FDA's current appropriations law.

Plant Scientists Boost Malaria Drug Yield In Plant

May 2, 2018, BBC

Scientists from Shanghai Jiao Tong University and other research institutions in China modified the genetic sequence of the plant Artemisia annua to make it produce high levels of a key drug for malaria. Their research study is published in *Molecular Plant*.

According to the World Health Organization (WHO), malaria has affected about 216 million people in 91 countries in 2016, and caused around 445,000 deaths all over the globe in the same year only. A. annua is the main source of artemisinin, the only WHO recommended treatment for the devastating disease. Thus, the researchers identified the genes involved in making artemisinin and modified the plant to make it produce three times more drug than the usual amount. They did this by simultaneously increasing the activity of three genes, HMGR, FPS, and DBR2.

ICRISAT's top Honour for Two Women Scientists

APRIL 17, 2018 , The Hindu

Both bag Doreen Margaret Mashler Award for their work in plant pathology and biotechnology

Women scientists Mamta Sharma and Pooja Bhatnagar-Mathur are the joint recipients of Doreen Margaret Mashler Award for 2018, for significant work

in plant pathology and biotechnology respectively at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)

In 2017, she led a team that developed the Loop-Mediated Isothermal Amplification (LAMP) method to identify a pathogen affecting chickpea and over 500 crops globally. She also established the Centre of Excellence on Climate Change Research for Plant Protection to address the effects of climate change on insect-pests and diseases.

Dr. Bhatnagar-Mathur, led an international, multi-institutional effort, for innovative biotechnology solutions to combat aflatoxin in groundnut using a 'double-defence' approach. This breakthrough resulted in resistance to fungal infection as well as remarkably low levels of aflatoxin contamination.

Janssen Strikes Deal to Acquire BeneVir and its Oncolytic Virus Platform in Deal Worth Up to $1 Billion

Published : May 02, 2018 By Alex Keown

Janssen Biotech, a subsidiary of Johnson & Johnson's Janssen Pharmaceuticals, cut the deal in order to acquire BeneVir's proprietary T-Stealth Oncolytic Virus Platform that can be used to develop oncolytic viruses used to infect and destroy cancer cells. The deal was facilitated by Johnson & Johnson Innovation LLC.

Oncolytic viruses are a growing field in immuno-oncology. Oncolytics have been shown to make a difference in treating various cancers. In 2015 the U.S. Food and Drug Administration approved the first oncolytic virus therapy for melanoma, Amgen's Imlygic. Multiple companies have jumped into the field, including Merck, which in February acquired Australia-based Viralytics Limited and its oncolytic immunotherapy treatments for **$394 million**.

While Janssen was mum on the financial details of the transaction, HC2 said the deal could be **worth up to $1.04 billion** when milestones are included. Under the terms of the agreement, Janssen will make an upfront cash payment of $140 million and additional contingent payments of up to $900 million based on achievement of certain predetermined milestones, HC2 announced. The deal is expected to close in the second quarter of 2018.

DuPont & ADM Open Biobased FDME Pilot production Facility in Illinois

April 30, 2018, http://www.dow-dupont.com.

DuPont Industrial Biosciences (DuPont) and Archer Daniels Midland Company (ADM) announced the opening of the world's first biobased furan dicarboxylic methyl ester (FDME) pilot production facility in Decatur, Illinois.

Nearly one-tenth of the world's oil is used to make the plastic products we use every day. From shampoo bottles to frozen food containers, fossil-fuel-based plastics are virtually impossible to avoid because of a lack of commercially available alternatives — a significant gap in the marketplace that DuPont and ADM's new biobased FDME will help address. FDME is a molecule derived from fructose that can be used to create a variety of biobased chemicals and materials, including plastics, that are ultimately more cost-effective, efficient and sustainable than their fossil fuel-based counterparts.

Sterling Biotech suspended on BSE

Earlier Sterling Biotech was warned by BSE and last date was given to comply with the norms of stock exchange. Last date was 12th April 2018, but it seems that Sterling Biotech could not comply and thus BSE suspended account of company from Bombay Stock Exchange.

New Medical Hub proposed in Ghaziabad Uttar Pradesh

A new pharma industry cluster will come soon in the industrial area of Madhuban Bapudham, near NH-58. The GDA has started the process already, we will roll out bid call in May, said Ritu Maheshwari, GDA vice-chairperson.

The space allotted to develop industries here is nearly 1 lakh 14 thousand sqm. Along the line, a multispeciality hospital hub has also been proposed to develop in 3 hectare of area in this region. For this, single or group of bidders can bid for the space. Earlier it was proposed to be develop on PPP model.

Kymriah® first-in-class CAR-T therapy from Novartis, receives second FDA approval to treat large B-cell lymphomas

May 01, 2018

Kymriah is an innovative immunocellular therapy that is a one-time treatment manufactured individually for each patient using the patient's own T cells. Kymriah uses the 4-1BB costimulatory domain in its chimeric antigen receptor to enhance cellular expansion and persistence. In 2012, Novartis and Penn entered into a global collaboration to further research, develop and commercialize CAR-T cell therapies, including Kymriah, for the investigational treatment of cancers.

Novartis announced the US Food and Drug Administration (FDA) has approved Kymriah® (tisagenlecleucel) suspension for intravenous infusion for its second indication - the treatment of adult patients with relapsed or refractory (r/r) large B-cell lymphoma after two or more lines of systemic therapy including diffuse large B-cell lymphoma (DLBCL), high grade B-cell lymphoma and DLBCL arising from follicular lymphoma. Kymriah is not indicated for the treatment of patients with primary central nervous system lymphoma. Kymriah, developed in collaboration with the University of Pennsylvania, became the first chimeric antigen receptor T cell (CAR-T) therapy to receive regulatory approval in August 2017 for the treatment of patients up to 25 years of age with B-cell precursor acute lymphoblastic leukemia (ALL) that is refractory or in second or later relapse. Kymriah is now the only CAR-T cell therapy to receive FDA approval for two distinct indications in non-Hodgkin lymphoma (NHL) and B-cell ALL.

"Today's FDA approval of Kymriah provides another opportunity for Novartis to build on its leadership in CAR-T development, delivering a potentially transformative therapy with durable and sustained response rates and a well-characterized safety profile to help patients in dire need of new treatment options," said Liz Barrett, CEO, Novartis Oncology. "We look forward to leveraging all of our learnings and new capabilities from the initial launch of Kymriah in pediatric and young adult B-cell ALL for this larger group of patients."

Kymriah is manufactured for each individual patient using their own cells at the Novartis Morris Plains, New Jersey facility. In the US, the target turnaround time for manufacturing Kymriah is 22 days. The reliable and integrated manufacturing and supply chain platform for Kymriah allows for an individualized treatment approach on a global scale. The process includes cryopreservation of a patient's harvested (or leukapheresed) cells, giving treating physicians and centers the flexibility to initiate therapy with Kymriah based on the individual patient's condition. Novartis has significant CAR-T manufacturing experience and has demonstrated a reproducible product. Novartis has manufactured CAR-T cells for more than 300 patients from 11 countries. Novartis continues to advance its CAR-T manufacturing expertise in Morris Plains where we have been supplying CAR-T cells for global clinical trials and where we continue to invest in support of the anticipated demand to meet the needs of patients.

GSK's Shingles Vaccine generated most revenue in First Quarter of 2018

Apr 25, 2018,

Despite strong sales of shingles vaccine Shingrix, GlaxoSmithKline reported its **sales and earnings fell 2 percent** in the first quarter of the year, largely due to challenges in respiratory sales as well as significant currency impact.

GSK said Shingrix saw sales of about $153 million in the first quarter. The first quarter of 2018 is

the first full quarter that Shingrix has been on the market in the United States. It has quickly replaced rival drugmaker Merck's shingles vaccine Soztavax. Since its launch about six months ago the GSK vaccine has seen significant growth in the United States and has won approximately 90 percent of the market share in this country. The company anticipates Shingrix will become its biggest vaccine of all time.

While the first quarter of the year was tough but expected for GSK, the company has been making moves to ensure future long-term growth. During the first quarter of this year, GSK took full control of its consumer health business.

According to news earlier in month of April 2018, the company announced it has **divested its rare disease gene therapy portfolio** to Orchard Therapeutics. As part of the agreement Orchard, which **launched in 2016**, received a number of gene therapy programs as part of the deal, which is expected to complement its existing pipeline of clinical and preclinical gene therapies for primary immune deficiencies and inherited metabolic disorders.

Under terms of the deal, GSK will receive a 19.9 percent equity stake in Orchard, as well as a seat on that company's board of directors. Additionally, GSK will receive undisclosed royalties and commercial milestone payments related to the portfolio. By taking over the GSK programs Orchard will assume all obligations from GSK's 2010 collaboration agreement with the Ospedale San Raffaele and Fondazione Telethon, as well as from GSK's collaboration agreement with MolMed, GSK announced.

Top Biotech Influencers to Watch on Twitter

Kiran Mazumdar-Shaw
Followers:1.44M

Dr. C. Michael Gibson, M.D., Harvard professor of medicine
Followers: 394K

Dr. Tedros Adhanom Ghebreyesus, Director-General of the World Health Organization
Followers:322K

Dr. Atul Gawande, surgeon and public health official, he's also the author of four New York Times bestselling books
Followers:241K

Dr. Eric Topol, M.D., Executive Vice President and Professor of Molecular Medicine at the world-renowned Scripps Research Institute
Followers:129K

Dr. Robert R. Redfield, M.D., head of Centers for Disease Control and Prevention
Followers:119K

Dr. Linda Girgis, M.D., family physician
Followers: 100K

Robin Y. Smith, CEO of Orig3n
Followers: 105K

Dr. Francis S. Collins, M.D., 16th Director of the National Institutes of Health
Followers: 86.2

Dr. Michael Mosley, BBC-affiliated influencer
Followers: 85.4K

Matthew Herper, Senior Editor covering Healthcare, Pharma, and Medicine for Forbes.com and Forbes magazine
Followers: 81.4K

Dr. Atanas G. Atanasov, Head of the Department of Molecular Biology at the Institute of Genetics and Animal Breeding within the Polish Academy of Sciences
Followers: 91.4K

Amgen Announces Rhode Island Will Be Location of First US Next-Generation Biomanufacturing Plant

April 10, 2018, Amgen

Amgen (NASDAQ:AMGN) today announced plans to build a new state-of-the-art next-generation biomanufacturing plant at its campus in West Greenwich, R.I. The new plant, the first of its kind in the United States (U.S.), will employ Amgen's proven next-generation biomanufacturing capabilities and manufacture products for the U.S. and global markets.

A next-generation biomanufacturing plant incorporates multiple innovative technologies into a single facility, and therefore is built in half the construction time with approximately one half of the operating cost of a traditional plant. Next-generation biomanufacturing plants require a smaller manufacturing footprint and offer greater environmental benefits, including reduced consumption of water and energy and lower levels of carbon emissions.

"Amgen has three decades of experience in biologics manufacturing, and we are proud of our track record of providing a reliable supply of high-quality medicines for patients around the world," said Esteban Santos, executive vice president of Operations at Amgen.

Amgen opened its first next-generation biomanufacturing plant in Singapore in 2014. The existing Amgen Rhode Island plant was licensed by the U.S. Food and Drug Administration in September 2005 and houses one of the world's largest mammalian protein manufacturing facilities. The facility manufactures commercial and clinical bulk drug substance. Amgen has invested more than $1.5 billion in its Rhode Island site, adding more than 500,000 square feet of manufacturing, utility, administrative and laboratory space to the campus. There are 625 full-time staff members employed at the Amgen Rhode Island campus.

Advaxis recruits Kenneth Berlin as New CEO

Apr 23, 2018

The company announced Kenneth A. Berlin will take over the helm of the company as president and CEO effective immediately. The announcement of a new CEO was not the only change to Advaxis' leadership team announced today. In addition to Berlin's appointment, Advaxis also announced Andres A. Gutierrez has been named chief medical officer.

With Berlin at the helm, Advaxis will be looking to harness his experience to drive growth. The company highlighted some of his corporate successes as a pharma executive for other companies. While at Rosetta Genomics Berlin spearheaded the effort to reposition the company with various microRNA-based oncology diagnostic products. Additionally, he raised nearly $100 million in capital to fund these efforts, Advaxis noted. Before Rosetta Genomics, Berlin was the Worldwide General Manager at cancer diagnostics developer Veridex, LLC, a subsidiary of Johnson & Johnson. At Veridex he grew the organization to more 100 employees, launched three cancer diagnostic products, led the acquisition of its cellular diagnostics partner, and delivered significant growth in sales as Veridex transitioned from an R&D entity to a commercial provider of oncology diagnostic products and services.

U.S. Supreme Court: Patent Office Allowed To Cancel Bad Patents

Source: Purch

In one of the most important patent cases in recent years, the Supreme Court ruled that the Patent Office can not only issue patents, but can also retract them. The ruling should deter aggressive patent holders from going after other companies, unless they are certain that their patents will withstand a review, which should result in less litigation across industries.

In a recent case between two oil drilling companies, Oil States Energy Services and Greene's Energy Group, the former argued that it was unconstitutional for an administrative law board of the

Patent Office to retract patents, because that should be the job of the courts. This administrative law board, called the Patent Trial and Appeal Board (PTAB), was created in 2012 as part of the America Invents Act as a way to lower the costs of litigation.

The Supreme Court disagreed because the way patents are granted has been decided by Congress all this time, not courts, and therefore Congress can also control how the bad patents are retracted.

Sanofi to Sell its Generic Division Zentiva to Advent for $2.4 Billion

April 2017, Reuters

Sanofi said the sale was expected to be completed before the end of the year, and Advent's offer was binding and fully financed. The 1.9 billion euros price is an enterprise value, including equity and debt.

"Following a comprehensive review of strategic options for our generics unit in Europe, we have determined that transferring this business to Advent is the best option to ensure its long-term success," Sanofi Chief Executive Olivier Brandicourt said in a statement.

Novartis appoints John Tsai Head of Global Drug Development and Chief Medical Officer

Source: http://www.novartis.com.

Novartis announced today the appointment of John Tsai, M.D. as Head of Global Drug Development (GDD) and Chief Medical Officer. Dr. Tsai will join Novartis on May 1, 2018, and will be based in Basel, Switzerland. He will report to Vas Narasimhan, M.D., CEO of Novartis and will become a member of the Executive Committee of Novartis (ECN). He succeeds Dr. Narasimhan who became CEO of Novartis on February 1, 2018.

Dr. Tsai has been Chief Medical Officer and Senior Vice President of Global Medical at Amgen Inc., since May 2017 and oversees all clinical and medical functions across multiple sites worldwide. At Novartis, he will be responsible for advancing the company's industry-leading pipeline of innovative medicines and biosimilars. Dr. Tsai will also lead the GDD organization's ongoing transformation embracing the power of advanced data sciences and digital technologies to create a more agile model for drug development.

Image: John Tsai, M.D.
Source: Front Line Genomics

"I am delighted to welcome John to Novartis," said Dr. Narasimhan. "As we continue to reimagine drug development, his expertise across multiple therapeutic areas, including cardiovascular, oncology and neuroscience combined with his background in electrical engineering will be a source of great strength for Novartis.

Dr. Tsai said: "I feel honored to have the opportunity to lead the Novartis Global Drug Development organization and do my part in bringing forward the company's strong pipeline of medicines that address some of humanity's biggest health challenges. I am also excited to work with my colleagues at Novartis to pioneer novel paradigms for drug development with data and digital technologies at the core."

Groundbreaking from 12th April - 12th May

RESEARCH NEWS

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Stem cells from adults function just as well as those from embryos

April 24, 2018

A review of research on induced pluripotent stem cells (iPSCs) finds that donor age does not appear to influence their functionality. This validates iPSCs as a viable alternative to embryonic stem cells in regenerative medicine, and highlights the enormous potential of iPSCs derived from elderly patients to treat their age-related diseases.

The 2006 discovery of induced pluripotent stem cells -- which can be derived directly from a patient -- offers an attractive alternative. Their use has already been proved in a young patient: a boy suffering from a rare genetic disease, in which the skin blisters and tears off, recovered completely after receiving a skin transplant grown from his own gene-corrected stem cells.

However, questions remained about the impact of donor age on iPSC functionality -- an especially relevant point given that the elderly stand to benefit the most from these stem cells. Kränkel and colleagues therefore critically analyzed the available research to date, to summarize what is known and identify future research needs.

The analysis revealed that the age of the donor may reduce the efficiency at which their body cells can be reprogrammed into iPSCs. However, once generated, the stem cells appear to be rejuvenated -- with typical signs of aging reversed.

"iPSCs show improved functionality compared to the donor's regular body cells, and can be differentiated into mature body cells with a similar efficiency to younger stem cell donors," says Kränkel. "This means that stem cells from an elderly patient can be developed into other cells and returned to the patient for treatment."

Despite this promising conclusion, it is still a matter of debate as to whether cells from older donors have accumulated more damaging mutations than those of younger donors. "This seems logical," says Elisabeth Strässler, co-author of the study. "There is also the issue of whether such mutations persist during the transformation to stem cells, or whether they are repaired."

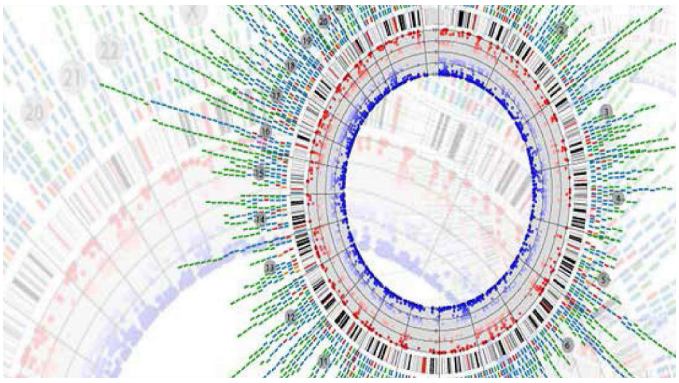
Journal Reference:

Age Is Relative—Impact of Donor Age on Induced Pluripotent Stem Cell-Derived Cell Functionality. Frontiers in Cardiovascular Medicine, 2018; 5 DOI: 10.3389/fcvm.2018.00004

Scientists generate Atlas of the human genome using stem cells

April 23, 2018

Scientists from the Hebrew University of Jerusalem have generated an atlas of the human genome using a state-of-the-art gene editing technology and human embryonic stem cells, illuminating the roles that our genes play in health and disease.



The researchers analyzed virtually all human genes in the human genome by generating more than 180,000 distinct mutations. To produce such a vast array of mutations, they combined a sophisticated gene-editing technology (CRISPR-Cas9 screening) with a new type of embryonic stem cells that was recently isolated by the same research group. This new type of stem cells harbors only a single copy of the human genome, instead of two copies from the mother and father, making gene editing easier thanks to the need of mutating only one copy for each gene.

The researchers show that a mere 9% of all the genes in the human genome are essential for the growth and survival of human embryonic stem cells, whereas 5% of them actually limit the growth of these cells. They could also analyze the role of genes responsible for all hereditary disorders in early human development and growth. Furthermore, they showed how cancer-causing genes could affect the growth of the human embryo.

Another key finding of the study was the identification of a small group of genes that are uniquely essential for the survival of human embryonic stem cells but not to other cell types. These genes are thought to maintain the identity of embryonic stem cells and prevent them from becoming cancerous or turning into adult cell types.

"This study creates a new framework for the understanding of what it means to be an embryonic stem cell at the genetic level," said Dr. Atilgan Yilmaz, PhD, postdoctoral fellow and a lead author on the paper. "The more complete a picture we have of the nature of these cells, the better chances we have for successful therapies in the clinic."

Journal Reference:

Defining essential genes for human pluripotent stem cells by CRISPR–Cas9 screening in haploid cells. Nature Cell Biology, 2018; DOI: 10.1038/s41556-018-0088-1

New process to differentiate stem cells

April 17, 2018

As scientists try to find early therapy options to fight degenerative disc disease, there has been considerable interest in harnessing stem cells to restore nucleus pulposus, or NP. Previous research shows human induced pluripotent stem cells (hiPSCs) -- generated directly from adult cells -- can express markers for a wide variety of cells, including those that secrete NP.

Setton's lab exposed the hiPSCs to a variety of different growth factors and culture media to coax them into first developing markers for, and then fully forming into, notochord cells. Once the scientists had the notochord cells, they used a similar chemical exposure process to develop those into NP-type cells. The lab tracked the differentiating process using fluorescent cell imaging, which tested for the necessary markers during each step.

"You can think of it as a push-pull," Setton said. "You can push it in one direction, but you have to pull it from the other direction as well. I could push it toward a nerve, but I have to pull it from becoming bone. We didn't know what combination would work. It's like cooking in the kitchen, and you have to add things to the gravy. It took us a really long time to figure out that perfect recipe. But now that we did, it's very repeatable."

Setton says the multistep process her lab used to derive NP-type cells from the hiPSCs provides the necessary quality control as scientists seek additional uses for stem cell therapies. Setton says the research's next steps include assessing environmental cues -- such as the stiffness of the culture surface, cell topography and how a cell attaches -- and observe their effects in transforming hiPSCs.

Journal Reference:

Differentiation of human induced pluripotent stem cells into nucleus pulposus- like cells. Stem Cell Research & Therapy, 2018; 9 (1) DOI: 10.1186/s13287-018-0797-1

Incompatible donor stem cells cure adult sickle cell patients

April 25, 2018

Doctors at the University of Illinois Hospital have cured seven adult patients of sickle cell disease, an inherited blood disorder primarily affecting the black community, using stem cells from donors previously thought to be incompatible.

With the new protocol, patients with aggressive sickle cell disease can receive stem cells from family members if only half of their human leukocyte antigen (HLA) markers match. Previously, donors had to be a family member with a full set of matching HLA markers, or a "fully-matched" donor.

"We have made great strides curing adults with sickle cell disease with stem cell transplants, but the unfortunate truth is that the majority of these patients have, until now, been unable to benefit from this treatment because there are no fully-matched HLA-compatible donors available in their family," said corresponding author Dr. Damiano Rondelli, the Michael Reese Professor of Hematology and director of the Blood and Marrow Transplant program at the University of Illinois at Chicago.

The doctors screened 50 adult sickle cell patients as candidates for a half-matched stem cell transplant between January 2014 and March 2017. Ten patients received a transplant. Following two unsuccessful transplants, the doctors adopted the new treatment protocol, which included modifications to a process first developed at Johns Hopkins University.

"We modified the transplant protocol by increasing the dose of radiation used before the transplant, and by infusing growth factor-mobilized peripheral blood stem cells instead of bone marrow cells," Rondelli said. "These two modifications helped ensure the patient's body could accept the healthy donor cells."

Of the eight patients who underwent the revised transplant, one experienced chronic graft-versus-host disease following the transplant and died of unknown causes. The other seven patients are alive and maintain 95 percent or higher stable engraftment --

acceptance of donor cells -- with improved blood work at least 12 months following the transplant.

"These patients are cured of sickle cell disease," Rondelli said.

"The takeaway message is twofold. First, this transplant protocol may cure many more adults patients with advanced sickle cell disease," he said. "Second, despite the increasing safety of the transplant protocols and new compatibility of HLA half-matched donors, many sickle cell patients still face barriers to care -- of the patients we screened, only 20 percent underwent a transplant."

Rondelli says that medical insurance denial accounted for 20 percent of the lack of access to the transplant.

Journal Reference:

Haploidentical Peripheral Blood Stem Cell Transplantation Demonstrates Stable Engraftment in Adults with Sickle Cell Disease. Biology of Blood and Marrow Transplantation, 2018; DOI: 10.1016/j.bbmt.2018.03.031

Identity of brain stem cells clarified

May 4, 2018

Unfortunately, when brain cells are damaged by trauma or disease they don't automatically regenerate. This can lead to permanent disability. But within the brain there are a small number of stem cells that persist into adulthood, offering a possible source of new cells to repair the damaged brain.

Work by researchers at the University of Calgary Faculty of Veterinary Medicine sheds new light on the identity of the brain cells that exhibit neural stem cell function.

One type, astrocyte neural stem cells, can self-renew and generate new neurons, particularly following brain injury. The other type -- called ependymal cells -- provide a supportive lining between the brain and the fluid that bathes the brain.

"Importantly, ependymal cells that line the caverns of the brain also sit right next to neural stem cells, suggesting that they might be important regulators of neural stem cell function,

"However, several high-profile studies have suggested that ependymal cells can actually become neural stem cells themselves, when activated by an injury to the brain. Our work provides evidence this is not the case and provides new insight into how they might contribute to brain function."

In the study, the researchers developed a process allowing them to specifically label ependymal cells within the adult brain, while avoiding astrocyte stem cells. Biernaskie says the research not only clarifies the identity of the adult neural stem cell, it also provides a new model to study the function of ependymal cells and their role in maintaining normal brain function.

Journal Reference:

Single-Cell Transcriptomics and Fate Mapping of Ependymal Cells Reveals an Absence of Neural Stem Cell Function. Cell, 2018; 173 (4): 1045 DOI: 10.1016/j.cell.2018.03.063

Fasting boosts stem cells' regenerative capacity

May 3, 2018

The age-related loss of stem cell function can be reversed by a 24-hour fast, according to a new study from MIT biologists. The researchers found that fasting dramatically improves stem cells' ability to regenerate, in both aged and young mice.

In fasting mice, cells begin breaking down fatty acids instead of glucose, a change that stimulates the stem cells to become more regenerative. The researchers found that they could also boost regeneration with a molecule that activates the same metabolic switch.

"Intestinal stem cells are the workhorses of the intestine that give rise to more stem cells and to all of the various differentiated cell types of the intestine. Notably, during aging, intestinal stem function declines, which impairs the ability of the intestine to repair itself after damage," Yilmaz says. "In this line of investigation, we focused on understanding how a 24-hour fast enhances the function of young and old intestinal stem cells."

After mice fasted for 24 hours, the researchers removed intestinal stem cells and grew them in a culture dish, allowing them to determine whether the cells can give rise to "mini-intestines" known as organoids.

The researchers found that stem cells from the fasting mice doubled their regenerative capacity.

Further studies, including sequencing the messenger RNA of stem cells from the mice that fasted, revealed that fasting induces cells to switch from their usual metabolism, which burns carbohydrates such as sugars, to metabolizing fatty acids. This switch occurs through the activation of transcription factors called PPARs, which turn on many genes that are involved in metabolizing fatty acids.

The researchers found that if they turned off this pathway, fasting could no longer boost

regeneration. They also found that they could reproduce the beneficial effects of fasting by treating mice with a molecule that mimics the effects of PPARs.

The findings suggest that drug treatment could stimulate regeneration without requiring patients to fast, which is difficult for most people. One group that could benefit from such treatment is cancer patients who are receiving chemotherapy, which often harms intestinal cells. It could also benefit older people who experience intestinal infections or other gastrointestinal disorders that can damage the lining of the intestine.

Journal Reference:

Fasting Activates Fatty Acid Oxidation to Enhance Intestinal Stem Cell Function during Homeostasis and Aging. Cell Stem Cell, 2018; 22 (5): 769 DOI: 10.1016/j.stem.2018.04.001

Experimental arthritis drug prevents stem cell transplant complication

April 24, 2018

An investigational drug in clinical trials for rheumatoid arthritis prevents a common, life-threatening side effect of stem cell transplants, new research from Washington University School of Medicine in St. Louis shows.

Studying mice, the researchers found the drug prevented what's known as graft-versus-host disease, a debilitating, sometimes lethal condition that develops when transplanted stem cells attack the body's own organs or tissues.

In past work, this research team defined the role of molecules called JAK1/2 kinases and their signaling pathways in immune cell activation and graft-vs-host disease. In the new study, these same researchers evaluated ruxolitinib and baricitinib, and found baricitinib to be the superior of the two drugs in reducing and preventing graft-versus-host-disease in mice. Both drugs belong to a class of pharmaceuticals called JAK inhibitors that are known for dialing down inflammation.

Surprisingly, baricitinib did more than shut down graft-versus-host disease. It actually boosted the ability of the donor T cells to fight the cancer.

"We don't know yet exactly how this happens, but we're working to understand it," said first author Jaebok Choi, PhD, an assistant professor of medicine. "We think at least part of the explanation is the drug strips the leukemia cells of their immune defenses, making them more vulnerable to attack by the donor T cells. At the same time, the drug also stops the donor T cells from being able to make their way to important healthy tissues, such as the skin, liver and gastrointestinal tract, where they often do the most damage."

In other words, the drug appears to stop graft-versus-host disease by simply keeping the donor T cells circulating in the bloodstream, away from vital organs. Simultaneously, the drug makes the leukemia cells more vulnerable to immune attack from the donor T cells, which are now mostly confined to the bloodstream, where the cancer is.

The drug also appeared to boost levels of specific immune cells that put the brakes on a runaway immune response that can make graft-versus-host disease worse. These apparently independent effects are specific to baricitinib and may explain why other JAK inhibitors did not work as well, according to DiPersio, who is also deputy director of Siteman Cancer Center at Barnes-Jewish Hospital and Washington University School of Medicine.

The researchers emphasized the finding that the drug not only prevented graftversus-host disease from developing in the mice but reversed established disease, suggesting possible options for patients already affected by it.

"We were surprised to achieve 100 percent survival of mice with the most severe model of graft-versus-host disease," Choi said. "We are now studying the multi-pronged ways this drug behaves in an effort to develop an even better version for eventual use in clinical trials."

Journal Reference:

Baricitinib-induced blockade of interferon gamma receptor and interleukin-6 receptor for the prevention and treatment of graft-versus-host disease. Leukemia, 2018; DOI: 10.1038/s41375-018-0123-z

Earth BioGenome Project aims to sequence genomes of 1.5 million species

April 23, 2018

An international consortium of scientists is proposing a massive project to sequence, catalog and analyze the genomes of all known eukaryotic species on the planet, an undertaking the researchers say will take 10 years, cost $4.7 billion and require more than 200 petabytes of digital storage capacity. There are an estimated 10-15 million eukaryotic species on Earth.

The proposed initiative, described in a paper in the Proceedings of the National Academy of Sciences, would require the cooperation of governments, scientists, citizen scientists and students from around the globe. The authors of the proposal compare it to the Human Genome Project, an international scientific research project from 1990 to 2006 that cost roughly

$4.8 billion in today's dollars and generated an estimated return-on-investment ratio of 141-to-1.

A similar initiative, the Earth Microbiome Project, has enlisted the support of more than 500 scientists to sequence bacterial and archaeal genomes across the globe.

The EBP project will support and promote international protocols for data storage and sharing. A coordinating council with members from Africa, Australia, Brazil, Canada, China, the European Union and the United States will head a global network of collaborators. The council also will include representatives of several current large-scale genomics projects including the Global Genome Biodiversity Network, the Global Invertebrate Genomics Alliance, the i5K Initiative to Sequence 5,000 Arthropod Genomes and the Genome 10K Project.

Journal Reference:

Earth BioGenome Project: Sequencing life for the future of life. Proceedings of the National Academy of Sciences, 2018; 201720115 DOI: 10.1073/pnas.1720115115

For how long will the USA remain the Nobel Prize leader?

May 9, 2018

Since first being awarded in 1901, most Nobel Prizes for science have gone to the USA, the United Kingdom, Germany and France. An empirical study by Professor Claudius Gros from the Institute for Theoretical Physics at the Goethe University in Frankfurt has now shown that the Nobel Prize productivity in these countries is primarily determined by two factors: a long-term success rate, and periods during which each country has been able to win an especially large number of Nobel Prizes.

For the study, Nobel Prizes for physics, chemistry and medicine were assigned proportionately, since up to three scientists can share the prize. The success rates were calculated on the basis of population figures. For France and Germany, the periods of increased scientific creativity occurred around 1900, whereas for the USA it occurred in the second half of the 20th century.

"The US era is approaching its end," states Claudius Gros. "Since its zenith in the 1970s, US Nobel Prize productivity has already declined by a factor of 2.4." According to his calculations, a further decline is foreseeable. "Our model predicts that starting in 2025 the productivity of the USA will be below that of Germany, and from 2028, below that of France as well."

With a nearly constant, very high success rate per capita, Great Britain occupies a special position with regard to Nobel Prizes. It remains uncertain, however, whether Great Britain will be able to maintain this success, especially in view of the increasing industrialization of research.

"National research advancement can undoubtedly also be successful independent of Nobel Prize productivity," Claudius Gros stresses. "Especially because new areas of research such as the computer sciences -- a typical US domain -- are not included." It therefore remains open whether the decline in Nobel Prize productivity is cause for concern, or merely an expression of a new orientation toward more promising research fields.

Journal References:

Claudius Gros. An empirical study of the per capita yield of science Nobel prizes: is the US era coming to an end? Royal Society Open Science, 2018; 5 (5): 180167 DOI: 10.1098/rsos.180167

Claudius Gros. Pushing the complexity barrier: diminishing returns in the sciences. Complex Systems, 2012; 21: 183

Genetic roadmap to building an entire organism from a single cell

April 26, 2018

Now, in three landmark studies Harvard Medical School and Harvard University researchers report how they have systematically profiled every cell in developing zebrafish and frog embryos to establish a roadmap revealing how one cell builds an entire organism.

Using single-cell sequencing technology, the research teams traced the fates of individual cells over the first 24 hours of the life of an embryo. Their analyses reveal the comprehensive landscape of which genes are switched on or off, and when, as embryonic cells transition into new cell states and types.

The researchers leveraged the power of InDrops, a single-cell sequencing technology developed at HMS by Klein, Kirschner and colleagues, to capture gene expression data from each cell of the embryo, one cell at a time. The teams collectively profiled more than 200,000 cells at multiple time points over 24 hours for both species.

To map the lineage of essentially every cell as an embryo develops, along with the precise sequence of gene expression events that mark new cell states and types, the teams developed new experimental and computational techniques, including the introduction of artificial DNA bar codes to track the lineage relationships between cells, called TracerSeq.

In the study co-led by Schier, the research team used Drop-Seq -- a single-cell sequencing technology developed by researchers at HMS and the Broad Institute of MIT and Harvard -- to study zebrafish embryos over 12 hours at high time resolution. Teaming with Aviv Regev, core member at the Broad, Schier and colleagues reconstructed cell trajectories through a computational method they named URD, after the Norse mythological figure who decides all fates.

Schier and colleagues profiled more than 38,000 cells, and developed a cellular "family tree" that revealed how gene expression in 25 cell types changed as they specialize. By combining that data with spatial inference, the team was also able to reconstruct the spatial origins of the various cells types in the early zebrafish embryo.

Journal References:

The dynamics of gene expression in vertebrate embryogenesis at single-cell resolution. Science, 2018; eaar5780 DOI: 10.1126/science.aar5780

Systematic mapping of cell state trajectories, cell lineage, and perturbations in the zebrafish embryo using single cell transcriptomics. Science, 2018

Single-cell reconstruction of developmental trajectories during zebrafish embryogenesis. Science, 2018; eaar3131 DOI: 10.1126/science.aar3131

Genomics is disrupting the healthcare sector

May 4, 2018

The independent report shows that genomics is already driving a remarkable paradigm shift in health practices and outcomes.

In the last 15 years, the cost of reading an individual's DNA sequence -- their genome -- has plummeted from hundreds of millions of dollars to around the cost of a shoulder MRI. This is ushering in a new era of precision healthcare, in which treatments, prevention strategies and health advice will reach the right person at the right time.

Applications of genomics in cancer, rare disease and reproductive services are booming, the report finds, with other clinical areas set to follow suit. The report shows that over 250 FDA-approved drugs are now labelled for prescribing based on the patient's genetics -- a number that has tripled since 2014.

A comprehensive resource, the report draws on patents, scientific publications, and clinical trials data to map out the emerging medical and consumer health applications of genomics.

The report shows that practical biomedical applications for genomics have stimulated the formation of hundreds of new companies globally -- particularly in the US. It surveys the diverse business models being used to transform fundamental discoveries into commercial products. It also ranks leading research organizations involved in genomic discovery and quantifies their R&D relationships with industry.

Story Source:

Materials provided by Garvan Institute of Medical Research.

Scientists discover gene controlling genetic recombination rates

April 21, 2018

Researchers hypothesize that crossover rates have evolved to balance the benefits of crossing over with the risks of selfish DNA.

Presgraves and PhD candidate Cara Brand recently accomplished an important milestone in learning about these evolutionary dynamics. By studying two species of fruit flies, they discovered a gene, MEI-218, that controls the rate of recombination. In a paper published in Current Biology, they explain how MEI-218 controls differences in the rate of crossing over between species and the evolutionary forces at play. "This is the first gene I know of that anyone has shown to be responsible for the evolution of recombination rates," Presgraves says.

The team focused on two closely related species of fruit flies -- Drosophila melanogaster and its sister species, Drosophila mauritiana -- because large differences have evolved in their rates of recombination: D. mauritiana does about 1.5 times more crossing over than D. melanogaster. When they compared genes in the two different species, the researchers found that the DNA sequences of MEI-218 were extraordinarily different.

Brand and Presgraves hypothesize that the change in recombination rates between D. mauritiana and D. melanogaster may have evolved because the species have different amounts of transposons in their genomes. The D. melanogaster genome has more transposons than D. mauritiana, so D. melanogaster may therefore have evolved a lower rate of crossing over in order to avoid the higherrisk of harmful crossovers between transposons.

This means, then, that the MEI-218 gene is constantly evolving to an ever-changing optimum. The evolution of MEI-218 is similar to genes involved in immunity, Presgraves says. "That should make some intuitive sense because genes involved in immunity are constantly adapting to the changing community pathogens that are challenging us all the time."

The MEI-218 gene has so far only been investigated in fruit flies, but the research into recombination has applications for humans. "During meiosis at least one crossover per chromosome, in general, is required to make sure the chromosomes separate properly," Brand says. "Either a lack of crossing over or crossing over in the wrong regions of the genome is what leads to many birth defects like Down Syndrome."

Journal Reference:

Molecular Evolution at a Meiosis Gene Mediates Species Differences in the Rate and Patterning of Recombination. Current Biology, 2018; DOI: 10.1016/j.ub.2018.02.056

Solving the structure of ATP synthase

April 17, 2018

"Understanding how the enzyme actually works requires the knowledge of its three dimensional molecular structure at the atomic level," said Dr. Mueller, principal investigator for the study that used cryo-electron microscopy (cryo-EM) to reveal the enzyme at near atomic resolution.

The first complete structure of ATP synthase provides evidence for the mechanism by which the drug oligomycin inhibits the enzyme and how disease-causing mutations disrupt the function of the molecule. Solving the structure overcomes a barrier to understanding its likely broader function in disease and drug mechanisms.

They used cryo-EM analysis to decipher the engineered ATP synthase, which was synthesized in yeast and reconstituted into nanodiscs to allow for structural analysis. While cryo-EM isn't new, advancements in technology have made it possible to solve the structure at near atomic resolution.

Journal Reference:

High-resolution cryo-EM analysis of the yeast ATP synthase in a lipid membrane. Science, 2018; eaas9699 DOI: 10.1126/science.aas9699

Researchers describe genetic clockwork in germ cell development

April 16, 2018

To reproduce, *C. elegans* must produce gametes, that is male sperm and female eggs. These develop from undifferentiated dividing stem cells. Extensive intracellular restructuring is required to realize these processes, which have to mesh faultlessly if the cells are to develop successfully," Eckmann continues. A highly intricate clockwork mechanism with many interlocking gears gives some idea of the level of sequencing complexity involved.

These processes are controlled by RNA-binding proteins. Outside of the nucleus, in the cytoplasm, these proteins regulate selective gene activation. For a germ cell to develop out of a stem cell, two specific RNA-binding proteins need to be destroyed to reorganise the cell's genetic programme. How, when and why the signal for this developmental switch is given was previously unclear. The researchers from Halle have now figured out that the already familiar MAP kinase signalling pathway plays a central role. Eckmann summarises the process as follows: "A protein degradation cascade is initiated via this molecular pathway, at the end of which the two target proteins CPB-3 and GLD-1 are recognised, inactivated, and destroyed."

The geneticists at MLU were able to demonstrate that this process already operates at a very early stage in meiosis and corresponds temporally to the sexual differentiation onset of female germ cells. The processes are thus optimally co-ordinated. According to Eckmann, "The special thing about these processes is that they involve known molecules with very long evolutionary histories, previously receiving attention as suppressors of tumour formation within the context of normal cell division. In C. elegans, these molecules were interleaved in an innovative way. The processes were adapted and temporally co-ordinated to facilitate optimized, rapid germ cell production." These findings of the MLU research group on Developmental Genetics suggest that the same genetic program may operate in germ cells of other, more complex organisms as well -- albeit in a timely less compressed form.

Journal Reference:

MAPK signaling couples SCF-mediated degradation of translational regulators to oocyte meiotic progression. Proceedings of the National Academy of Sciences, 2018; 115 (12): E2772 DOI: 10.1073/pnas.1715439115

New cell therapy to aids heart recovery -- without cells implant

Medical researchers have designed a creative new approach to help injured hearts regenerate by applying extracellular vesicles secreted by cardiomyocytes rather than implanting the cells. The study shows that the cardiomyocytes derived from human pluripotent stem cells (derived in turn from a small sample of blood) could be a powerful, untapped source of therapeutic microvesicles that could lead to safe and effective treatments of damaged hearts.

Cell-secreted microvesicles are easy to isolate and can be frozen and stored over long periods of time. Such an "off-theshelf" product has several major advantages over cell therapy -- 1) it can be used immediately in an acute-care setting, unlike cells that can take months to isolate and grow; 2) it does not cause arrhythmia (which often occurs when cells are transplanted); and 3) the regulatory path towards clinical application is much simpler than for a cell-based therapy.

It is well known from numerous clinical studies that most of the implanted stem cells are washed away within hours of the treatment, but there still are beneficial effects. This has led to the informal "hit-and-run" hypothesis, meaning that the cells deliver their cargo of regulatory molecules before leaving the site of injury. "Consistent with this hypothesis, we postulated that the benefits of cell therapy of the heart could be coming from the secreted bioactive molecules (such as micro RNAs), rather than the cells themselves,

"We reasoned that the cardiomyocytes would be the best source of molecules driving the recovery of injured heart, as it is well known that these cells can build muscle when used in tissue-engineering models,

The interdisciplinary team, which included bioengineers, clinicians, and systems biology scientists, derived cardiomyocytes from adult human stem cells and cultured these cells to allow them to secrete extracellular vesicles. The vesicles secreted by undiffereniated stem cells were used for comparison. The researchers then used next-generation sequencing to read their messages and instructions. They found that the extracellular vesicles from cardiomyocytes -- but not from stem cells -- contained cardiogenic and vasculogenic microRNAs that are very powerful regulatory molecules.

Building on the expertise of Vunjak-Novakovic's lab in biomaterials and hydrogels, the team encapsulated the vesicles in a collagen-based patch that slowly released them over the course of four weeks when implanted onto the injured heart in rat models of myocardial infarction. The researchers monitored the heart to measure blood-pumping function and look for any signs of arrhythmia.

"We were really excited to find that not only did the hearts treated with cardiomyocyte extracellular vesicles experienced much fewer arrhythmias, but they also recovered cardiac function most effectively and most completely," says Vunjak-Novakovic. "In fact, by four weeks after treatment, the hearts treated with extracellular vesicles had similar cardiac function as those that were never injured."

Journal Reference:

Cardiac recovery via extended cell-free delivery of extracellular vesicles secreted by cardiomyocytes derived from induced pluripotent stem cells. Nature Biomedical Engineering, 2018; DOI: 10.1038/s41551-018-0229-7

World's smallest optical implantable biodevice

April 25, 2018

Japanese researchers describe a new implantable device no bigger than the width of a coin that can be used to control brain patterns. The device, which can be read about in AIP Advances, converts infrared light into blue light to control neural activity and is both the smallest and lightest wireless optical biodevice to be reported.

The new device made by Tokuda's research team uses a complementary metal-oxide semiconductor that controls photovoltaic power. "We integrated two sets of photovoltaic cells onto semiconductor chips. Ten cells were integrated for powering, and seven cells for biasing," he said.

The device includes an InGan LED chip, which causes the device to emit blue light. A more distinguishing feature of the device, however, is that it can be activated with infrared light. Infrared is used in many light therapies, because it can penetrate deep in the body, whereas blue light cannot go much deeper than the surface. Therefore, the device can be implanted several centimeters into the body.

Journal Reference:

1 mm3-sized optical neural stimulator based on CMOS integrated photovoltaic power receiver. AIP Advances, 2018; 8 (4): 045018 DOI: 10.1063/1.5024243

3-D printed food now

April 24, 2018

Jin-Kyu Rhee, associate professor at Ewha Womans University in South Korea, discussed his new research and the potential of 3-D printing technology for food production at the American Society for Biochemistry and Molecular Biology annual meeting during the 2018 Experimental Biology meeting held on April 21-25 in San Diego.

"We built a platform that uses 3-D printing to create food microstructures that allow food texture and body absorption to be customized on a personal level," said Rhee. "We think that one day, people could have cartridges that contain powdered versions of various ingredients that would be put together using 3-D printing and cooked according to the user's needs or preferences."

3-D printing of food works much like 3-D printing of other materials in that layers of raw material are deposited to build up a final product. In addition to offering customized food options, the ability to 3-D print food at home or on an industrial scale could greatly reduce food waste and the cost involved with storage and transportation. It might also help meet the rapidly increasing food needs of a growing world population.

For the new study, the researchers used a prototype 3-D printer to create food with microstructures that replicated the physical properties and nanoscale texture they observed in actual food samples. They also demonstrated that their platform and optimized methods can turn carbohydrate and protein powers into food with microstructures that can be tuned to control food texture and how the food is absorbed by the body.

Story Source:

Experimental Biology 2018.

New take on early evolution of photosynthesis

April 24, 2018

A team of scientists from Arizona State University's School of Molecular Sciences has begun re-thinking the evolutionary history of photochemical reaction centers (RCs). Their analysis was recently published online in Photosynthesis Research and describes a new pathway that ancient organisms may have taken to evolve the great variety of photosynthetic RCs seen today across bacteria, algae, and plants.

There are two types of RCs that exist today: Type I RCs support metabolism by moving electrons to soluble proteins, while Type II RCs move electrons to membrane-associated molecules. However, evidence has been building in the lab of professor Kevin Redding that the RC from the heliobacteria may be able to perform both of these functions, making it a functional hybrid of the two RC types.

The heliobacterial RC is thought to be one of the simplest RCs still around today. It is homodimeric, meaning that its core is composed of two copies of the same protein. This contrasts with the two RCs from oxygen-producing organisms like plants whose core is heterodimeric, having their core composed of two similar, but not identical, proteins.

The team believes that the ancestral reaction center (ARC) was simpler than the versions that exist today. This ARC was probably homodimeric and interacted with molecules in the membrane, like the modern Type II RCs (and the heliobacterial RC), instead of with soluble proteins.

It is very difficult to reconstruct these evolutionary steps, which took over 3 billion years to occur. One way in which this is generally done is to compare the amino acid sequences of the proteins and note the number of differences between them, assuming that more similarity means that they are more closely related. In their study, however, the team cautions against relying heavily on this method for RCs. The sequence differences are just too numerous and too much time has passed to obtain reliable information from this method.

They instead compared the positions of protein structural elements and chlorophylls within the RCs.

The team envisions that the ARC, in its simplest form, was probably rather inefficient at its chemistry. Its job was to use the energy of sunlight to provide two electrons to a membrane-associated molecule called a quinone. However, the ARC likely could loosely bind two quinone molecules, one on each side of the core. With two identical-looking quinones, the ARC was not able to prioritize which quinone would get electrons, making it less likely that either would get the two it needed.

This problem was solved in two different ways. In the lineage that led to the modern Type II RCs, the core changed from homodimeric to heterodimeric, which allowed the RC to prioritize which quinone it gave electrons to, accelerating the chemistry. In the lineage that led to the modern Type I RCs, the core remained homodimeric, but a metal cluster was added so that the first electron would end up there, facilitating its delivery to the quinone that received the next electron.

Once the ARC had acquired the metal cluster, thus becoming the ancestor to all modern Type I RCs, more changes occurred to further direct electrons to a soluble acceptor, which resulted in extracting more energy for the cell's metabolism. These included changes in the positions and identities of the chlorophyll cofactors. Much of the later changes in the Type I RCs were driven by the need to deal with the presence of oxygen, as the unstable intermediates within RCs can react with oxygen to generate very damaging molecules. In the opinion of the ASU team, the heliobacterial RC retains clear vestiges of the changes leading to the early Type I RC and that understanding the fine details in how modern RCs work allows for informed hypotheses about how they evolved.

Journal Reference:

Gregory S. Orf, Christopher Gisriel, Kevin E. Redding. Evolution of photosynthetic reaction centers:

insights from the structure of the heliobacterial reaction center. Photosynthesis Research, 2018; DOI: 10.1007/s11120-018-0503-2

Newly improved microscopic glass slide works as a thermometers too

May 2, 2018

A new study describes how an updated version of the microscope slide can enable scientists to see tiny objects while also measuring their temperature. The advancement, made possible by a new transparent, has the potential to streamline and enhance scientific research worldwide, from clandestine government biology labs to high school chemistry classes. It may also have implications in computers, electronics and other industries.

The new coating is made of a layer of acrylic glass (the same material used in most eyeglasses) that's sandwiched between two layers of transparent gold. The gold is transparent because it's only 20 nanometers thick; a typical sheet of paper is 100,000 nanometers thick.

Engineers fabricated the coating so that "exceptional points" -- the sweet spots where unusual light behavior happens -- can develop within the tri-layered structure. The coating, which significantly enhances the slide's sensitivity to light detection, would be added to slides during the manufacturing process. Either the slide or cover slip could receive the coating.

To make use of the new coating, a laser is needed. Zhao says a common helium-neon laser, which can be seamlessly integrated with most microscopes, will do the job.

Common slides, which are often bought in bulk, typically cost around 5 cents. The new coating would likely add a few pennies to the cost, Zhao says.

Journal Reference:

Exceptional point engineered glass slide for microscopic thermal mapping. Nature Communications, 2018; 9 (1) DOI: 10.1038/s41467-018-04251-3

Biophysics -- lighting up DNA-based nanostructures

April 24, 2018

The term 'DNA origami' refers to a method for the design and self-assembly of complex molecular structures with nanometer precision. The technique exploits the base-pairing interactions between single-stranded DNA molecules of known sequence to generate intricate three-dimensional nanostructures with predefined shapes in arbitrarily large numbers. The method has great potential for a wide range of applications in basic biological and biophysical research. Thus researchers are already using DNA origami to develop functional nanomachines.

With the aid of a super-resolution technique called DNA-PAINT, the researchers are able to visualize nanostructures with unprecedented spatial resolution, allowing them to image each of the strands in the nanostructures.

The results obtained with the DNA-PAINT method revealed that variations in several physical parameters -- such as the overall speed of structure formation -- have little influence on the overall quality of the assembly process. However, although its efficiency can be enhanced by the use of additional staple strands, not all strands were found in all of the nanoparticles formed, i.e. not all available sites were occupied in all of the final structures. "When assembling nanomachines it is therefore advisable that the individual components are added in large excess and the positions of the modifications chosen in accordance with our mapping of incorporation efficiency," Strauss says.

The DNA-PAINT method thus provides a means of optimizing the construction of DNA nanostructures. In addition, the authors believe that the technology has great potential in the field of quantitative structural biology, as it will allow researchers to measure important parameters such as the labelling efficiency of antibodies, cellular proteins and nucleic acids directly.

Journal Reference:

Quantifying absolute addressability in DNA origami with molecular resolution. Nature Communications, 2018; 9 (1) DOI: 10.1038/s41467-018-04031-z

Witness forgery data fabrication and scientific misconduct in Calcutta University

Source: www.kashbiotech.com

Jayita Barua has accused assistant professor Anindita Ukil and her laboratory colleagues of fabricating data to generate scientific papers intended for submission to research journals and claimed she had also been part of "this game".

Barua had sent an email to the Journal of Biological Chemistry on April 12, seeking withdrawal of her name as co-author of the paper, claiming it contained fabricated data, and attaching raw data to back her claim. She also alleged in the email that her colleagues, responding to a request from the JBC's art editor, had used pencil marks to cover up the data fraud.

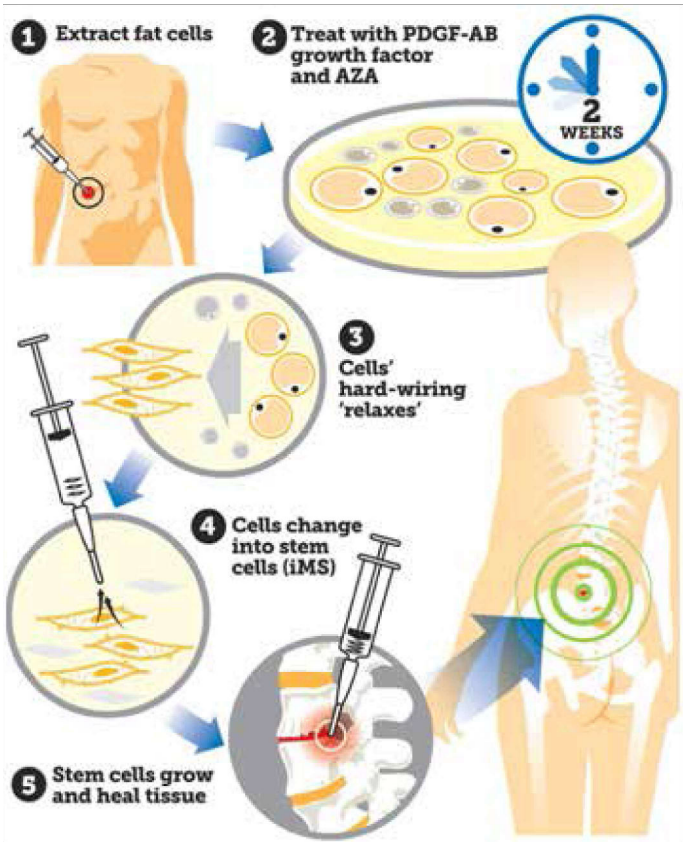
STEM CELL TECHNOLOGY FOR TISSUE REPAIRING

S.Archana and C.V.Karthikeyan
RVS Padmavathy College of Horticulture, Sempatti

Stem cell is an undifferentiated cell of multicellular organism that is capable of giving rise to identify more cells of the same type. These cells are undifferentiated biological cells that can differentiate into more specialized cells and divide through mitosis to produce stem cells called as self-renew. There are mostly found in multicellular organism. The cells arise from two main sources that include embroyinc stem cell and adult tissues. Embryos formed during the blastocyst phase of embryological development are known to embryonic stem cells. The adult tissue includes cell capable to convert red blood cells, white blood cells, platlets, nerve cells and liver cells.

Stem cell therapy is the use of stem cells to treat or prevent the disease or any physical conditions. Stem cell therapy is used in treatment for neurodegenerative disease, diabetes and heart disease. Stem cells are also used to cure stroke, traumatic brain injury, learning defects, Alzheimer disease, Parkinson disease, missing teeth, wound healing, bone marrow transplantation, spinal cord injury, osteoarthritis, Rheumatoid arthiritis, baldness, blindness, deafness, amyotrophic lateral sclerosis, myocardial infraction, muscular dystrophy, diabetes and cancer.

Over 30 years, bone marrow has been used to treat cancer patients with leukemia and lymphoma. This is the only form of stem cell therapy that uses cytotoxic agents. The agents however cannot discriminate between leukemia or neoplastic cells and Hematopoietic stem cell within bone marrow. The stem cell therapy involves replacement of cells lost in the host system. The transplanted cells generate immune response through serious of mechanisms that helps in curing cancer cells. This process has disadvantage of causing side effects. Various new stem technologies are being developed through scientists at universities that are found to be more strategic without any side effects. Under the



assistance of a growth factor, new stem cell techniques not only can regenerate the injured tissue but also can be applied as regenerative medicine.

The new technology employs growth factor and reprogrammed bone and fat cells to induce multipotent stem cells. The method involves culturing of fat or bone cells with Azacitidine (AZA) along with naturally occurring platelet- derived growth factor (PDGF-AB). The cells are grown for two days. The experiment is further repeated with treating with growth factor for further three weeks. AZA induces cell plasticity that is crucial for reprogramming cells. The compound relaxes hard wiring of cells

transforming the bone and fat cells into IMS cells. When the cells are inserted into damaged tissue site, they multiply promoting growth and healing. The bone and fat cells are switched off the memory and converted to stem cells so as to repair different types of cells for replacement. Most importantly it is proved effective on initial animal experiments. The clinical trial on human is set to be launched before 2018 making therapy an advanced version over the presently available stem cell therapies.

This methodology has a great potential to treat many diseases that are thought to be incurable. Patients suffering from Parkinsons disease, Schizophrenia, Alzhemier diseas, cancer, spinal cord injuries and diabetes have commendable improvements. Applying of common fat cells to make the multipotent stem cells makes therapy easier to be accessed and can be applied in many practical situations like bone pain to replace the ineffective spinal implantations. The method has decreased the dependencey on donor organs and the problems of timely availability of organs. Organs such as kidney and liver can be grown, extracted from their own body. The greatest advantage is that organ rejection from immune system is avoided. It has opened new doors in the field in clinical research as doctors can study the potential of new drugs without testing them on animals or humans. It has also helped to study all the different developmental stages in human embryos, pregnancy loss, infertility and birth defects. This could help get rid of diseases and abnormalities present during birth (fetal anomalies) and treat them at early stages. Scientists believe this methodology to hold key to reverse aging and may help to prolong life.

Genes alone don`t tell our whole story. Here`s why!

By Deblina Dam

Two women who are identical twins having the same hair and eye colour can have a difference in possessing colour blinding defects for a particular colour. That is one of the sisters turn out to be unlucky while the other is normal. How is that possible, you think? Their genes hold the answer. The differences in their genes have arrived from mutations, which are changes to the genetic sequence that may have occurred many generations ago! While some of these changes have no effect, some may cause disease and some lead to advantageous adaptations. The result of having two versions of each gene is that we display a combination of our biological parent`s traits.

A study of women heterozygous for colour deficiencies, by G. Jordan and J.D. Mollon, gives us an insight into the X chromosome inactivation in women (heterozygous for anomalous trichromacy and ought to have at least four types of cone in their retinae). The 23rd pair (Sex Chromosome) is called unique because it is responsible for such differences and is the secret behind the one colour blind twin. Biological males with an XY chromosome pair only gets one copy of all these X chromosome genes, so the human body has evolved to function without duplicates. This creates a problem for people with two X chromosomes. If both X chromosomes produced proteins, as is normal in other chromosomes. Development of the embryo would be completely impaired. The solution is X inactivation!

This happens early in development when an embryo with two X chromosomes is just a ball of cells. Each cell inactivates one X chromosome. There`s a certain degree of randomness to this process. One cell may inactivate the X chromosome from one parent, and another the chromosome from the other parent. The inactive X shrivels into a clump called a Barr body and goes silent. Almost none of its genes order proteins to be made. When these early cells divide, each passes on its X inactivation. So some clusters of cells express the maternal X chromosome, while others express the paternal X. If these chromosomes carry different traits, those differences will show up in the cells.

So, here goes the explanation for colour blind twins…

Both sisters inherit one mutant copy of the green receptor gene and one normally functioning copy. The embryo split into twins before X inactivation. So each twin ended up with different inactivation pattern. In one, the X chromosome with the normal gene was turned off in the cells that eventually became eyes. Without those genetic instructions that person now cannot sense green light and is colour blind.

Thus, disorders those are associated with mutations of X chromosome genes, like colour blindness, or haemophilia, are often less severe in individuals with two X chromosomes. That`s because, in someone with one normal and one mutant copy of the gene, only some of the cells would be affected by the mutation. This severity of the disorder depends on which X got turned off and where those cells were. On the other hand, all the cells in someone with only one X chromosome can only express the mutant copy of the gene, if that`s what they inherited. But it still makes us curious about how some genes on the X chromosome escape in activation and why inactivation isn`t always random.

USFDA Form 483 Obseravtions in Biocon's Bengaluru Unit and Associated Share price effect

It was news all around in major newspapers about USFDA's latest inspection of Biocon's Bengaluru manufacturing facility and so was time of great activity on stock market as we would see Biocon's share price on BSE is 52 week high at 1188 Rs. which is an exception and for very short time. The average price of share is around 330-350, which is current price on BSE (333.35 as on 7th September 2017).

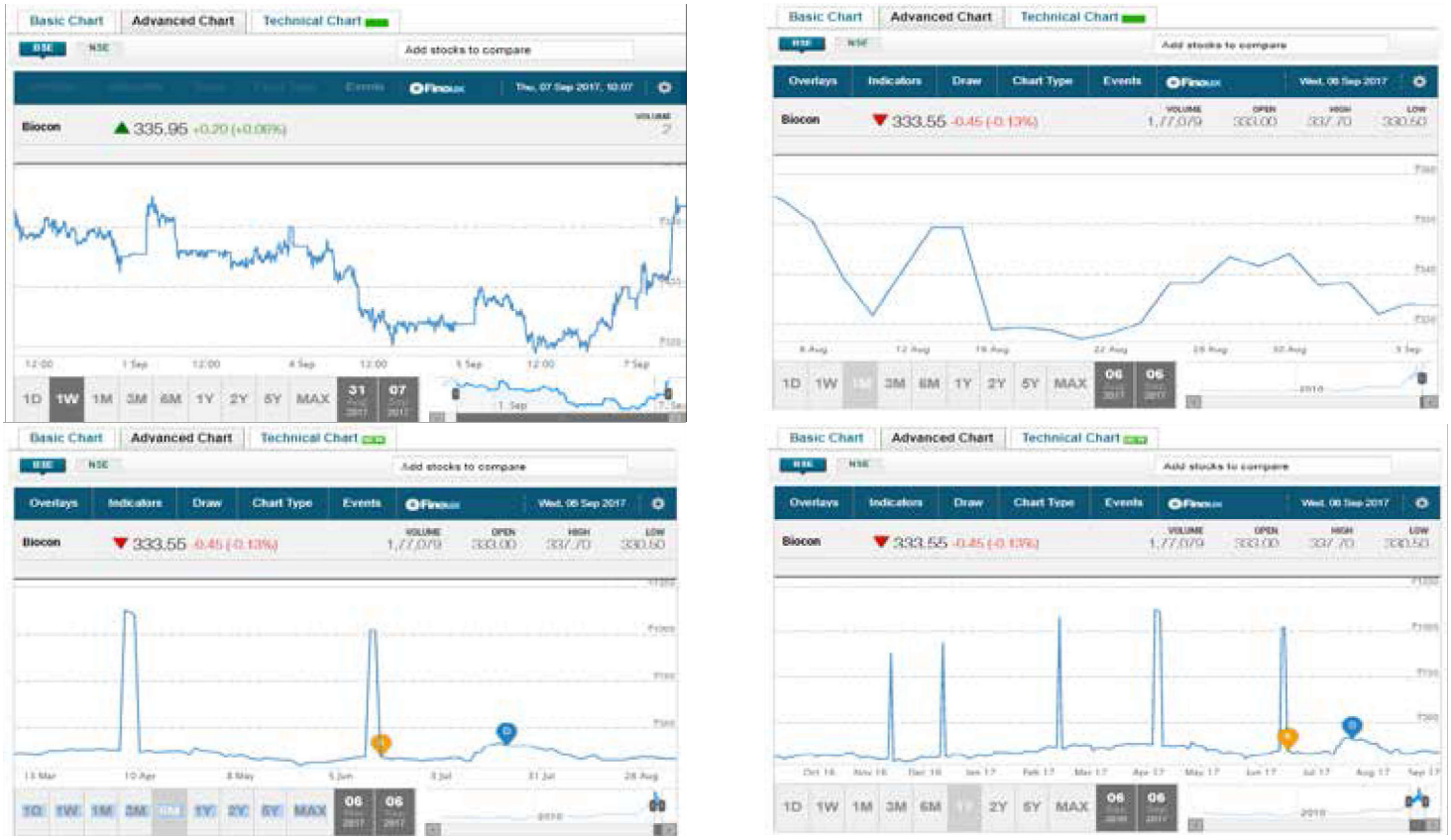


FIG: Figure shows high fragility on short term investments(1,2) whereas its almost same throught in long term investments except the peaks which shows not exponential but factorial surge (3,4). (Images Source: Moneycontrol)

We could see this factorial surge in June because it was pre USFDA decision but couldn't see stationary phase because this time USFDA and European Pharma Regulatory authorities did not give relief to Biocon. The reason was serious; they found major irregularities in plant.

According to article Published online on 4th August in ET and The Hindu Business Line, The FDA inspection took place on May 25, June 1 and June 3. USFDA observed inadequate procedures by the company to prevent microbiological containment of drug products, unexplained discrepancy in batches of the same product, no complete records of data derived from all tests to assure compliance with standards, lack of authority and responsibility of the quality control unit to accept or recent drug, among others. USFDA said that the employees engaged in the manufacture and processing of a drug product lack the training and experience required to perform their assigned functions. "They are inspectional observations and do not represent a final agency determination regarding your compliance," USFDA said in a note (gave some relief).

Details of Observations

USFDA releases Form 483 if their investigators witness any untoward conditions which in their judgement may constitute violations of the US Food Drug and Cosmetics Act (FD&C) and related laws.

Form 483 listing violations in current Good Manufacturing Plant (cGMP) have been issued by USFDA to Biocon following an inspection between May 25 and June 03 2017. USFDA noticed 10 observations in Biocon's small molecule injectable plant in Bengaluru.

The observations listed in the U.S. FDA document are as follows:

Observation 1: Investigations of an unexplained discrepancy did not extend to other batches of the same drug product and other drug products that may have been associated with specific failure or discrepancy

Observation 2: Procedures designed to prevent microbiological contamination of drug products purporting to be sterile do not include adequate validation of sterilization process

Observation 3: Aseptic processing areas are deficient regarding the system for monitoring environmental conditions

Observation 4: Procedures designed to prevent microbiological contamination of drug products purporting to be sterile are not established, written or followed

Observation 5: Laboratory records do not include complete data derived from all tests, examinations and assay necessary to assure compliance with established specifications and standards

Observation 6: Procedures for the cleaning and maintenance of equipment are deficient regarding sufficient detail of the methods, equipment, and arterials used in cleaning and maintenance operation

Observation 7: The quality control unit lacks the responsibility and authority to approve and reject all components and drug products

Observation 8: Employees engaged in the manufacture and processing of a drug product lack the training and experience required to perform their assigned functions

Observation 9: Laboratory controls do not include the establishment of scientifically sound and appropriate specifications

Observation 10: Procedures describing in sufficient detail the controls employed for the issuance of labeling are not followed

This inspection was conducted in accordance with the periodic audit requirements for small injectable plant. USFDA is more stringent with injectable plants and issued Form 483 to same plant with 8 observations earlier this year in April 2017.

However, Biocon again has responded promptly with a Corrective and Preventive Action Plan (CAPA) and is determined to implement all the observations timely.

Additionally, french regulator "French National Agency for Medicines and Health Products Safety" as part of European Medicine Agency (EMA) reviewed company's Biosimilar's Marketing Authorization. The French Agency expressed their concerns over sterile products, biological medicinal products, packaging and quality control testing at the Biocon unit.

Source: https://www.bloombergquint.com/markets/2017/08/03/bicocon-gets-form-483-with-10-observations-from-us-fda-for-bengaluru-plant,

https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/CDERFOIAElectronicReadingRoom/UCM557440.pdf

http://igmpiindia.org/USFDA-Issues-Form-483-to-Biocons-Bengaluru-Unit.html

The agriculture mission:How the Modi government is shaping the future of farming and farmers

August 6, 2018, 2:00 am IST MS Swaminathan in TOI Edit Page | Edit Page, India | TOI

Under then agriculture minister Rajnath Singh, in 2004 for the first time in the history of independent as well as colonial India, a National Commission on Farmers (NCF) was set up by the Government of India for looking into the problems of farm families and suggesting methods for making farming more remunerative as well as attractive to the younger generation.

This commission's report in 2006 not only contained suggestions for the advancement of agriculture but also for the economic wellbeing of farming families. An important goal set for farmers' welfare by NCF is to improve the economic viability of farming by ensuring that farmers earn a "minimum net income" and that agricultural progress is measured by the advance made in improving that income.

Other significant goals include mainstreaming the human and gender dimension in all farm policies and programmes and giving explicit attention to sustainable rural livelihoods; completing the unfinished agenda in land reforms and initiating comprehensive asset and aquarian reforms; and developing a social security system and support services for farmers.

Furthermore, protecting and improving the land, water, biodiversity and climate resources essential for sustained advances in the productivity, profitability and stability of major farming systems by creating an economic stake in conservation. Strengthening the biosecurity of crops, farm animals, fish and forest trees would safeguard the work and income security of farmer families, and the health and trade security of the nation. Likewise fostering community-centred food, water and energy security systems in rural India would help ensure nutrition security at the level of every child, woman and man.

In terms of the goal of attracting youth to farming, NCF suggests making it both intellectually stimulating and economically rewarding, by conferring the power and economy of scale to small and marginal farmers both in the production and post-harvest phases of farming. Emphasis is also put on restructuring agricultural curriculums and pedagogic methodologies for enabling every farm and home science graduate to become an entrepreneur and to make agricultural education gender sensitive.

Finally there is the goal of making India a global outsourcing hub for the production and supply of inputs needed for sustainable agriculture, and products and processes developed through biotechnology and ICT.

But although the NCF report was submitted in 2006 very little action was taken until the present government headed by Prime Minister Narendra Modi

took office. Fortunately over the last four years, several significant decisions have been taken to improve the status and income of farmers.

Designation of the agriculture ministry as the agriculture and farmers' welfare ministry has stressed keeping farmers' welfare as the measure of agriculture progress. Issuance of soil health cards to all farmers has been critical because soil health is basic to plant health and plant health is basic to human health.

Both budgetary and non-budgetary resources have been allocated for promoting micro-irrigation through the Pradhan Mantri Krishi Sinchayee Yojana. Conservation and sustainable use of indigenous breeds of cattle is being encouraged through a Rashtriya Gokul Mission. The Prime Minister also inaugurated the first ever International Agrobiodiversity Congress.

Promotion of the electronic national agriculture market is helping bring together different agriculture markets. Likewise the creation of Gramin Agriculture Markets will provide scope for direct sales to consumers in both retail and bulk form. Notable in this context is also the introduction of the Agricultural Produce and Livestock Marketing Act, 2017 and Agricultural Produce and Livestock Contract Farming Services Act, 2018 supported by electronic Negotiable Warehouse Receipt system for increased institutional credit to the farm sector.

Also notable is the determination of MSP on the basis of NCF recommendations and assured procurement at MSP of more crops. Integration of protein rich pulses and nutri-rich millets into welfare programmes including PDS, mid-day meals and ICDS is important too.

Activities like apiculture, mushroom cultivation, bamboo production, agro-forestry, vermicomposting and agro-processing are being promoted to generate additional jobs and income for farm families. The prime minister has also suggested that we should develop methods by which farmers' income can be doubled within five years. Plus several corpus funds are being set up to complete ongoing irrigation productions, modernise the infrastructure in dairy cooperatives, and strengthen the adoption of inland and marine aquaculture.

Above all, the recent announcement of a remunerative price based essentially on the recommendation of NCF is a very important step to ensure the economic viability of farming. To underline, government has ensured in its notification that from kharif 2018 onwards MSP of the notified crops would be minimum of 150% of the cost of production; it ranges from 150-200% for coarse cereals.

As for farmers' agitations still continuing, a major demand is the waving of loans and the implementation of the NCF recommendations on MSP. Both these problems are now receiving attention and appropriate action.

These are only some of the steps being taken to realise the concept of Jai Kisan. If all the above schemes are implemented effectively by the state and central governments, the future of farming and farmers can be shaped to also help India become a leader in both food and nutrition security. In addition the Prime Minister has launched a National Nutrition Mission with a three year budget of Rs 9,000 crore. His emphasis on agriculture as the prime industry of rural India urges doing everything possible to make agriculture both a source of income and the pride of our nation.

Hazardous Effects of Lead (Pb) in the Environment

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1. Introduction

Global industrialization and urbanization in the past decades have resulted in the generation of huge quantities of toxic wastes. These wastes include a variety of organic and inorganic compounds which pose serious threats to the environment. Organic contaminants include various compounds such as heavy metals, petroleum hydrocarbons, polychlorinated benzenes (PCBs) and other pollutants like radionuclides (Oncel *et al*, 2000). There are 35 metals that concern because of occupational or residential exposure; 23 of these are the heavy elements or "heavy metals". The term heavy metal refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations.

Lead (Pb) is one of the most widespread heavy metal contaminant in the environment. It is highly toxic to living organisms and one of the main sources of environmental pollution (Mahaffay, 1990). Lead forms a variety of compounds and exists in the environment in various forms. Organic lead compounds, which cross the skin and respiratory tract easily, affect the central nervous system predominantly.

2. Distribution & occurrence of Lead

Lead is ubiquitous and widely distributed as metallic lead, inorganic compounds & organometallic compounds. Metallic lead usually found in ore with zinc, silver and copper (most abundantly) and is extracted together with these metals. The main lead mineral is galena (PbS), which contains 86.6 % lead by weight. Other common varieties are cerussite ($PbCO_3$) and anglesite ($PbSO_4$).

Lead has been known since ancient times and is relatively abundant in the earth's crust (13 g/ton, ranking 36th), where it is found in galena (PbS). Lead having 82 atomic number in the periodic table and its atomic weight 207.19. The lead crystal has a cubic structure with centered faces. Lead. This metal is highly resistant to corrosion because of this property; it is used to contain corrosive liquids (for example, sulfuric acid) because lead is very malleable and resistant to corrosion. It is extensively used in building construction- for example in the external coverings of roofing joints.

3. Sources of lead

Lead can be found in many products and locations. Some you might never have thought of, including some imported candies, toys and traditional medicines. Lead was used in household paint until 1978 and was also found in leaded gasoline, some types of batteries, water pipes, and pottery glazes. Lead paint and pipes are still found in many older homes and lead is sometimes also found in water, food, household dust and soil. There are various sources of lead in the environment-

3.1 Paint Industry

Now a day's various paint industries are using lead in the manufacturing of paints. Lead was used in paint to add color, improve the ability of the paint to hide the surface it covers and to make it last longer. However, when such paint is peeling, flaking, chipping or otherwise deteriorating it can create lead contaminated dust and paint chips that pose a potential health risk, especially to children. Lead based paint is also a potential hazard if it is disturbed during remodeling or repainting activities that create lead contaminated dust.

3.2 Dust and soil

Lead dust is the most common way that people are exposed to lead. Inside the home, most lead dust comes from chipping and flaking paint or when paint is scraped, sanded or disturbed during home remodeling. Young children usually get exposed to lead when they put something with lead dust on it into their mouths. Lead dust may not be visible to the

naked eye. Homes near busy streets may contain higher levels of lead in the soil because lead used to be used in gasoline. Today, lead still comes from metal smelting, battery manufacturing and other factories that use lead. This lead gets into the air and then mixes with the soil. Lead-based paint mixing with soil is a problem during home remodeling if

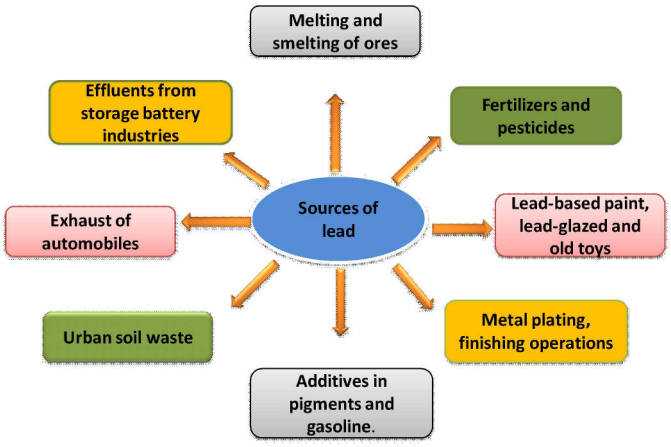

Figure 1: Sources of lead in the environment.

3.3 Drinking Water

Lead seldom occurs naturally in water supplies like rivers and lakes. Lead enters into drinking water primarily as a result of the corrosion or wearing away, of materials containing lead in the water distribution system and household or building plumbing. These materials include lead-based solder used to join copper pipe, brass and chrome plated brass faucets and in some cases, pipes made of lead that connect houses and buildings to water mains. In 1986, Government banned the use of lead solder containing greater than 0.2% lead, and restricted the lead content of faucets, pipes and other plumbing materials to 8.0%. Older construction may still have plumbing that has the potential to contribute lead to drinking water.

3.4 Sources of Airborne Lead

Amount of lead in the air has increased markedly due to anthropogenic activities. Atmospheric lead concentration in urban areas are 50 times higher than rural areas. Lead in air comes mainly comes from automobile emissions & industrial sources (e.g., smelters, waste incinerators, and lead-acid battery manufacturers). Atmospheric Lead can be in the form of gaseous compounds or particulate matters. Gaseous emissions are by combustion of tetraethyl lead and tetramethyl lead in automobile engines.

Sources of water-borne lead pollution

The source of lead was found to be lead pipe used in interior and exterior plumbing. The hard waters contain carbonate and sulphate ions which react with lead to form a water-insoluble protective coating of $PbCO_3$ and $PbSO_4$. Many industries utilizing lead releases lead contaminated water in the near by water-bodies leading to water pollution.

Lead-containing ceramic glazes have been a serious source of lead poisoning when used on containers of foodstuffs. It has been found that highly acidic liquids such as apple juice may dissolve the glaze and release lead into the liquid.

3.5 Children's jewelry and toys

Lead has been found in inexpensive children's jewelry sold in vending machines and large volume discount stores across the country. It also has been found in inexpensive metal amulets worn for good luck or protection. Some costume jewelry designed for adults has also been found to contain lead. It is important to make sure that children don't handle or mouth any jewelry.

3.6 The workplace and hobbies

People exposed to lead at work may bring lead home on their clothes, shoes, hair or skin. Some jobs that expose people to lead include: home improvement; painting and refinishing; car or radiator repair; plumbing; construction; welding and cutting; electronics; municipal waste incineration; lead compound manufacturing; manufacturing of rubber products, batteries, and plastics; lead smelting and refining; working in brass or bronze foundries; demolition; and working with scrap metal.

3.7 Lead-glazed ceramics, china, leaded crystal

Lead is used in industries for glazing ceramic pots. It may get into foods or liquids that have been stored in ceramics, pottery, china, or crystal with lead in it. Lead-glazed dishes usually come from other countries.

3.8 Imported food in cans that are sealed with lead solder

In 1995 the United States banned the use of lead solder on cans. But lead solder can still be found on cans made in other countries. These cans usually have wide seams and the silver-gray solder along the seams contains the lead. Cans containing lead may be brought to the United States and sold. Over time the lead gets into the food. Foods that are acidic cause lead to get into the food faster. Food and liquids

stored in lead crystal or lead-glazed pottery or porcelain. Food can become contaminated because lead can leach in from these containers.

4. Uses of lead

Lead has a number of uses but many of these are currently being phased out because of growing awareness of its toxicity and of the damage that uncontrolled dispersion in the environment has already caused. Lead is used in applications where it's low melting point, ductility and high density and resistance from corrosion is an advantage.

- Lead is used as electrodes in the process of electrolysis. Lead is used in solder for electronics, although this usage is being phased out by some countries to reduce the amount of environmentally hazardous waste. Lead is used in high voltage power cables as sheathing material to prevent water diffusion into insulation.
- Lead is used as shielding from radiation (e.g., in X-ray rooms). Molten lead is used as a coolant (e.g., for lead cooled fast reactors).
- Lead is added to brass to reduce machine tool wear. Lead, in the form of strips, or tape, is used for the customization of tennis rackets. Tennis rackets of the past sometimes had lead added to them by the manufacturer to increase weight.
- It is used to form glazing bars for stained glass or other multi-lit windows. Lead, or *sheet-lead*, is used as a sound deadening layer in some areas in wall, floor and ceiling design in sound studios where levels of airborne and mechanically produced sound are targeted for reduction or virtual elimination.
- Lead has many uses in the construction industry (e.g., lead sheets are used as architectural metals in roofing material, cladding, flashing, gutters and gutter joints, and on roof parapets). Detailed lead moldings are used as decorative motifs used to fix lead sheet. Lead is often used to balance the wheels of a car; this use is being phased out in favor of other materials for environmental reasons.
- Lead compounds are used as a coloring element in ceramic glazes, notably in the colors red and yellow. Lead is frequently used in polyvinyl chloride (PVC) plastic, which coats electrical cords.
- Lead is used in some candles to treat the wick to ensure a longer, more even burn. Because of the dangers, European and North American manufacturers use more expensive alternatives such as zinc.
- Some artists using oil-based paints continue to use lead carbonate white, citing its properties in comparison with the alternatives. Tetra-ethyl lead is used as an anti-knock additive for aviation fuel in piston-driven aircraft.

5. Environmental hazards of lead

5.1 Effects on plants :

Plants can take up high levels of lead from soils. Higher concentrations of lead can cause the negatively influence on the plant growth (Sharma et al., 2005). Through plant uptake, lead enters food chains. There are various effects which is caused by lead in the plants-

- The visual non-specific symptoms of Pb toxicity are rapid inhibition of root growth, stunted growth of the plant and chlorosis.
- Pb toxicity inhibits germination of seeds and retards growth of seedlings. Pb decreases germination percent, germination index, root/ shoot length, tolerance index and dry mass of roots and shoots.
- Pb phytotoxicity leads to inhibition of enzyme activities, disturbed mineral nutrition, water imbalance and change in hormonal status and alteration in membrane permeability.

A generalized view of the effects of Pb toxicity on key physiological processes in plants is presented in figure 2.

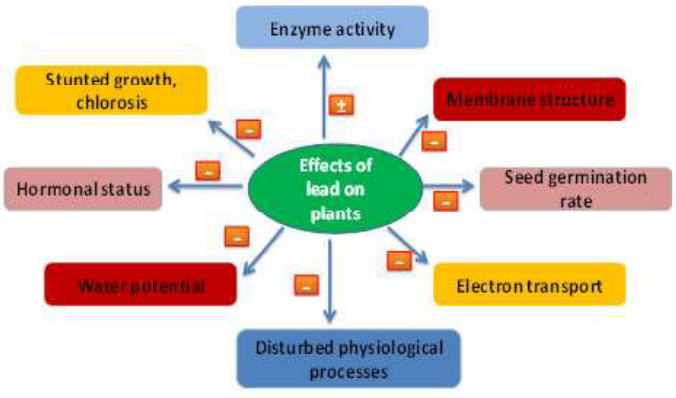

Figure 2. A generalized view of lead toxicity in plants. '+' and '–' signs indicate positive and negative effects respectively.

5.2 Effects on humans of lead

Lead interferes with a variety of body processes and is toxic to many organs and tissues including the heart, bones, intestines, kidneys, and reproductive and nervous systems. It interferes with the development of the nervous system and is therefore particularly toxic to children, causing potentially permanent learning and behavior disorders. Symptoms include abdominal pain, confusion, headache, anemia, irritability, and in severe cases seizures, coma, and death.

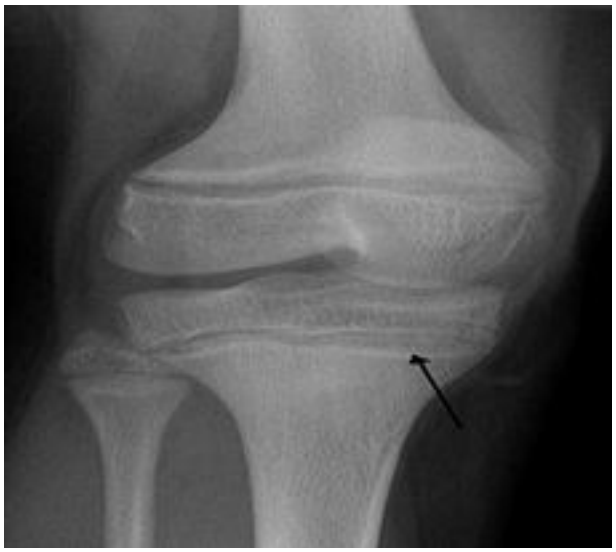

Table1: Effects of lead toxicity on plants, humans and animals. (a) chlorosis on plant leaf, (b) head pressing behavior in cattle, (c) lead infected vultures and (d) characteristic finding of lead poisoning in humans- dense metaphyseal lines.

- Plumbism- Lead poisoning or plumbism is defined as a toxic condition caused by the ingestion or inhalation of the metallic element lead, which is found in many places, including the air, soil, water, houses, ceramic cookware, and solder used in metal cans and pipes. Lead poisoning occurs when blood lead levels are equal to or greater than 10 ?g/dl (micrograms per deciliter). These enter the body by respiration (of dust, fumes, or sprays) or by ingestion of food or other substances that contain lead.
- Neurotoxicity: Lead uptake through the blood-brain barrier and into the brain proceeds at an appreciable rate, consistent with its action as a potent central neurotoxin. The effects of lead on the brain, including mental retardation and cognitive deficit, are mediated by its interference with three major neurotransmission systems: the dopaminergic, colinergic and glutamatergic systems (Dart et al., 2004; Needlemann, 2004).
- Children with high levels of lead in their bodies can suffer from damage to the brain and

nervous system, behavior and learning problems, such as hyperactivity, slowed growth hearing problems and headaches (Chisolm and Harrison, 1956).

- Adults with higher level of lead can suffer from reproductive problems (in both men and women), high blood pressure and hypertension, nerve disorders, memory and concentration problems, muscle and joint pain, anemia, constipation and abdominal spasm.

5.3 Effect of lead on animals

Humans are not alone in suffering from lead's effects; plants and animals are also affected by lead toxicity to varying degrees depending on species. Animals experience many of the same effects of lead exposure as humans do, such as abdominal pain, peripheral neuropathy, and behavioral changes such as increased aggression.

5.3.1 Wildlife

Lead, one of the elements that causes toxicity in waterfowl which has been known to cause death of wild bird populations. When hunters use lead shot, waterfowl such as ducks can ingest the spent pellets later and be poisoned; predators that eat these birds are also at risk. Cattles show head pressing behavior. Turkey vultures "Cathartes aura" and California condors can be poisoned when they eat carcasses of animals shot with lead pellets. Other threats to wildlife include lead paint, sediment from lead mines and smelters, and lead weights from fishing lines. Lead in some fishing gear has been banned in several countries (Buekers et al., 2009)

5.3.2 Aquatic Birds:

Lead poisoning in aquatic birds may occur when spent lead shot is mistaken for gravel (which is normally consumed to aid in digestion) and ingested. Birds may also be exposed to lead when feeding on fish attached to lead fishing gear such as sinkers or jig heads. In addition to loons, frequent victims of lead poisoning include swans, pelicans, geese, ducks, cormorants, cranes, and herons.

6. Mechanism for environmental cleanup

6.1 Phytoremediation using hyperaccumulator plants

Phytoremediation is an eco-friendly technology of using plants, grown in polluted soil and water to remove metals. The use of plants provides a number of advantages compared to common remedial technologies such as excavation and offsite disposal, thermal desorption, incineration, and physical and chemical degradation. There are many plant species reported which can absorb the heavy metals from soil and water. These plants are known as hyperaccumulator plants like Brassica juncea, Zea mays, Tagetes erecta L., Thlaspi caerulescens, Amaranthus, Helianthus annuus, Brassica chinensis, maize, willow, poplar, water hyacinth plant, Moringa sps. have been identified as phytoremediator plants. Phytoremediation represents a set of innovative technologies (phytotechnologies) that takes advantages of the specific extractive and metabolic capabilities of plants.

6.2 Bioremediation using microbes

A diversity of bacteria, fungi, and algae has been characterized as to their capacity to degrade lead. Researchers have endeavored to utilize microbes to facilitate the removal of both organic and inorganic contaminants from the environment, especially from soil. There are some of lead resistance bacteria such as Escherichia sp., Sphingomonads, Pseudomonas sp., Bacillus subtilis, Arthrobacter and Ochrobactrum which can remove the metals.

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