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New Tools for Gene Therapy

... think scientifically, act scientifically... think scientifically, act scientifically... think scientifically, act...

Of cold weather and thicker skins ..

Dr. R. Gopichandran

The last few weeks have seen typically colder than during the rest of the year. People wear thick heavy clothes for all the warmth they need. Thicker fabrics and logically extended thicker skins reduce free space between elbows when sitting alongside and this is especially so on aircrafts. Elbowing becomes a way of life even in rarefied atmospheres. Pun not intended in all these expressions! Over the years, I have learnt a great deal about the determinants of the degree of indifference exhibited by fellow travellers. I have no hesitation in saying, less than a handful of fellow travellers are naturally sensitive enough about others' spaces. Many tend to also tuck the cell phone under the pants and in this process, expand further into neighbour's spaces. Legs wider apart; they inflict discomfort on the knees too. A double whammy!! I wonder at the investment needed to sensitise these indifferent folks even on such small courtesies. We obviously have a long way to go on decent behaviour in public spaces. Is someone talking of mindsets!!

The science communicators meet at the Indian Science Congress was in less harsh climes. Warm it was at Tirupati, reflective of the manner in which the main heads of the event were finally warming up to the meet. The President designate for the 2018 Congress evinced keen interest on organising a "more elaborate" science communicators meet scheduled for Bhubaneshwar. Our sincere thanks to the President of the 2017 Congress for setting the context this year. Good signals indeed. Will it be a good idea to interact with science communicators at the grass roots in every State as a lead up to the 2018 Congress's meet? Can we have interesting experience sharing meetings as focussed sessions at the 2018 event?

Experts at the 2017 meet righty highlighted important barriers in science communication that have to be tackled on a priority basis if the meet at Bhubaneshwar and its outcome should not be trivialised to even the extent seen this far; and consistently so. Sincere thanks to Drs Gita Bamezai of the Indian Institute of Mass Communication, Dinesh Sharma, and TV Venkateswaran of Vigyan Prasar, Sarita Ahlawat of IIT Delhi, Anil Manekar DG NCSM, and Manoj Patairiya Head CSIR NISCAIR in this context. All the elements of the science of science communication deliberated by the Academies of Science, Engineering & Medicine in the USA, indicators stated by the NSF in 2016 and the NRC Framework and the NGSS, to cite a few; were addressed by the experts.

The objective of referring to these inputs is to attract the attention of decision makers in science and technology in our country to the fact that we as a team engaged in various aspects of science communication

- Are aware of trends, deliberations and insights that pervade thinking in this field world over.
- Have internalised these and are poised to add value to the process of science communication aligned with the specific needs of our fellow citizens and systems.
- Wish to develop a compendium of practices on science and technology communication that will showcase locally adapted communication strategies. This will confer much-needed attention on the variety of approaches and less visible communicators. Can we then develop a community of practitioners to partner communication initiatives to deliver appropriate information in a timely manner? This may also help trash presumptions that communicators and communication are too trivial and that it is too dense out there in our country! You know what I mean…

As a communicator with some grass root experience I dare assume that the spread and depth of communication content and approaches will be quite rich and is only waiting to be documented on the basis of an inclusive framework. Mark the word inclusive. I only prompt with fond hope that reality checks that will help document such initiatives (one day soon after all) are guided by a vision that is open-ended and not self-perpetuating. Most importantly, communicators should stick to the agenda of science and not even inadvertently push their own agenda in the process/garb of communication. Science culture does not elbow out. Science communicators should practise this as a value. Greater warmth is needed to get out of thick superimposed skins and tackle cold climates.

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New Tools for Gene Therapy

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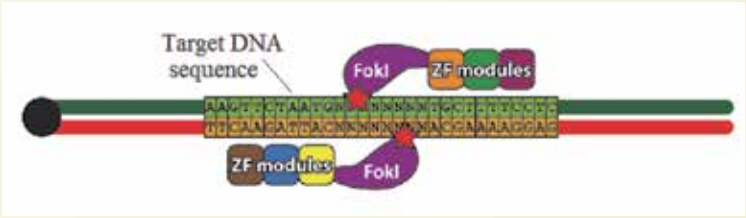
If you are down with fever caused by bacterial infection, doctor will treat you with a suitable antibiotic and in a week's time you are cured. So it is with more serious diseases like pneumonia. These are acquired diseases. What about diseases that we are born with – like haemophilia, cystic fibrosis, sickle-cell anaemia and even some forms of cancer? These are due to faults in our genes. These diseases manifest either because a necessary protein is not produced or a modified protein is produced or certain cellular control functions are lost. Doctors cannot cure most of these diseases, but can only manage them. However, after the advent of biotechnology in the early 1970s, researchers are developing techniques to hit at the root of these diseases – the faulty genes. Appropriately called "Gene therapy", the procedure attempts to knock out the faulty gene or repair it or supplement its functions with a normal gene to provide a functional cure for the disease.

In the early 1970s the method was to use a normal gene, packed into a disabled virus or plasmid. When such a vector is delivered into the cell, the new gene integrates itself into the cell's genome to compensate for the defective gene. Though many clinical trials have been conducted and a few successes also scored, vector-based gene therapy has many drawbacks. The system can only insert a new gene, but cannot silence or repair the faulty gene. More importantly, the new gene is inserted rather randomly, instead of into a specific locus in the host genome. This may have uncontrolled effects by turning on or suppressing other genes, or the new gene in a different location may not work as expected. Hence, something more precise and reliable was needed.

In the past two decades researchers have devised three new tools for precisely engineering the faulty gene – to knock it out, to edit it or even to insert a new gene at a specific site. All the three systems have some common mechanistic features. Each has two domains – one to grab the DNA sequence in the genome where the changes have to be introduced and the other to cut the DNA at precise location in the target sequence to create a double-strand break, which is a prerequisite for further steps of gene-editing. Because of their precision these tools are dubbed as "molecular scissors". They are popularly known by their acronyms: ZFNs, TALENs and CRISPRs.

ZFNs (Zinc Finger Nucleases)

ZFNs, developed in the 1990s, are the first engineered tools to facilitate targeted genome-editing. The DNA-binding domain consists of a protein motif, in which each protein has a finger-like protrusion stabilised by zinc ions (hence the name). Each protrusion can bind to a sequence of three nucleotide bases in DNA. The binding specificity of a zinc finger protein (ZFP) can be altered by manipulating its amino acid sequence. Several ZFPs can be stitched together to improve binding specificity over a length of DNA. Generally, a motif of three to six ZFPs is engineered to bind to a specific sequence of nucleotide bases in the target DNA.



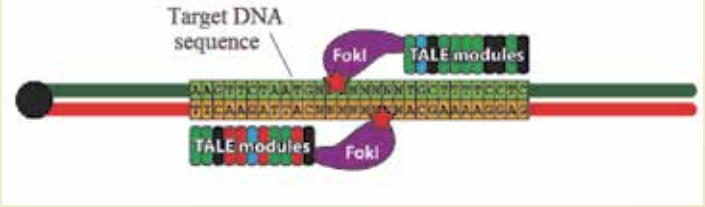
ZFNs gripping the target DNA

The DNA cleaving domain is a restriction enzyme called Fok I, which has the ability to non-specifically bind to DNA and cleave it. Fok I is a dimer. It works only when both parts are present. Therefore, two ZFN motifs with binding properties specific to the target DNA, each coupled to a Fok I enzyme form one unit of Zinc Finger Nuclease. (There is an interesting story on the genesis of ZFNs. See the Box).

When such a system is delivered to a cell, the two binding units flank the target site and the Fok I enzymes close in to nick the two DNA strands, producing a double-strand break.

TALENs (Transcription Activator-Like Effector Nucleases)

The DNA binding domain of a TALEN is composed of Transcription Activator-Like Effector (TALE) proteins secreted by *Xanthomomas* bacteria. These proteins were first observed by U. Bonas of the Institute of Biology, Martin Luther University, Germany in 2009. The *Xanthomonas* bacteria are plant pathogens, and they use the TALEs to activate specific genes in their hosts to maximise the susceptibility of the host to infection. The TALE protein consists of various numbers of tandem amino acid repeat domains and each domain binds to one specific nucleotide base in the DNA. Again, by changing the amino acid responsible for binding, the binding property of the protein can be altered to suit any DNA sequence. As in ZFNs, a pair of TALE proteins are coupled to the DNA-cleaving endonuclease Fok I dimer, form a TALEN unit, an effective gene-editing tool. Since each TALEN can bind to 17 or more nucleotide bases, it is easier to achieve a higher specificity than in the case of ZFNs.



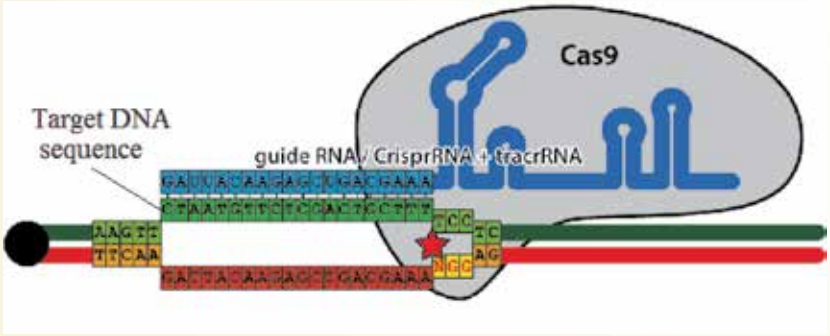
A pair of TALENs binding

CRISPRs (Clustered Regularly Interspaced Short Palindromic Repeats)

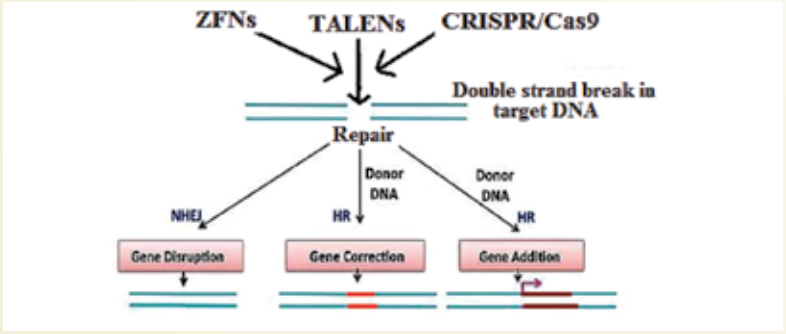
In the late 1980s, while sequencing the genome of bacteria *E. coli,* Japanese scientist Yoshizumi Ishine and his colleagues noticed an unusual DNA sequence with five identical but palindromic DNA repeat sequences, each made up of 29 nucleotide bases. These repeat sequences were separated from each other by seemingly random 32 base sequences called 'spacers'. Unlike the repeat sequences, each spacer had a unique base sequence. Later, other scientists discovered similar sequences in a wide variety of microbes. However, the biological significance of these sandwich sequences was not clear and they were just dubbed as "Clustered Regularly Interspaced Short Palindromic Repeats", or CRISPRs. It was also observed that CRISPR sequence was accompanied by a collection of genes, which were named CRISPR-associated genes, or Cas genes. These genes encoded DNA nucleases, which can cut the DNA strand. Other teams of scientists noticed that the spacer DNA resembled sections of viral genomes. Putting these disparate pieces of information together, scientists proposed that the bacteria may be using the CRISPR-Cas system as a defence against invading viruses and plasmids. When a virus attacks, the bacteria incorporate a piece of the viral DNA as a spacer in the CRISPR region. The next time the bacteria encounter that virus, they use the DNA in the spacer to make an RNA that recognises the matching sequence in the viral DNA and binds to it. A Cas protein attached to the RNA cuts up the viral DNA and prevents it from replicating.

In 2012, Jennifer Doudna and her colleagues at the University of California, Berkeley designed a single RNA coupled to a particular enzyme known as Cas9 (one of the many Cas enzymes) which could slice a matching DNA sequence in test tubes. In the following year, Feng Zhang form MIT and George Church from Harvard Medical School, independently reported that the CRISPR/Cas9 could be used for gene editing in all types of animal cells too, including humans.

In practice, to use the CRISPR/Cas9 system to edit a gene, an RNA that matches a specific DNA sequence in the gene is synthesised and fused with the Cas9 enzyme. When this construct is delivered to the host cell, RNA guides the enzyme to the target sequence where the Cas9 enzyme produces a double-strand break in the DNA strand.



CRISPER/Cas9 binding to the target DNA



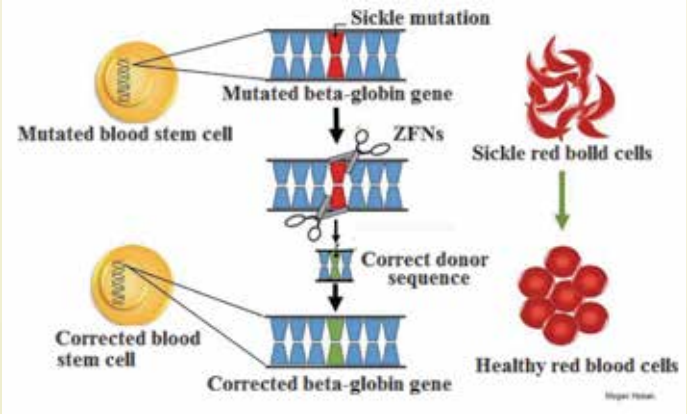
Genome modification fallowed by repair of double strand break caused by the gene-editing tools

And because the same cutting enzyme is used regardless of the target sequence, it is possible to simultaneously challenge multiple genes in the host cell by simply using Cas9 and the corresponding RNA guides.

Further steps in editing

Any damage to DNA stimulates natural repair processes in the cell. A double-strand break at the target site is repaired by a process known as 'non-homogenous end joining' (NHEJ), which joins the two broken ends. However, the process is imperfect resulting in insertions or deletions of some bases, leading to a dysfunctional gene. Alternatively another process, known as homologous recombination (HD) directed repair, can be stimulated to repair a gene instead of knocking it out. If a "donor" DNA sequence that represents the correct gene sequence is presented, then the cell can use it as a template to correct the original gene. On the other hand, if a whole new gene sequence is presented, the result is insertion of a new gene at a predetermined position in the host genome.

Generally, disabled viruses or plasmids are used as vectors to deliver the gene-editing tools into the cell. Some researchers even use electrophoresis – a low-voltage electric discharge – which punches holes in the cell membrane to admit the tool. ZFNs and TALENs are around for some years now and have been extensively tested in cultured human cells and various animal models to test for their efficacy, safety, possible side effects, etc. Following these, many pre-clinical trials are being conducted to treat diseases of the blood (like sickle-cell anemia) and immune system (HIV/AIDS). For example, researchers at the University of California at Berkeley treated cultured bone-marrow stem cells from patients with sickle-cell anaemia with ZFNs and successfully corrected the mutation. They



Correction of Sickle-Cell Disease Mutation in Human Blood Stem Cells

further demonstrated in mouse models that the corrected bone-marrow stem cells have the capability to replicate successfully and produce normal red blood cells. More recently, doctors from UK have successfully achieved remission in leukaemia (a form of blood cancer) in a 1-year-old girl by treating her with immune cells edited by TALENs to seek out and destroy cancer cells.

CRISPR/Cas9 system has been available for researchers only since 2013. However, because of the ease with which the tool can be engineered and used, compared with ZFNs and TALENs, it is catching up very fast. Another advantage with CRISPR/Cas9 is that it can be used to target more than one gene, which is of great advantage in treating diseases caused by mutations in multiple genes. Researchers at the MIT applied CRISPR/Cas9 tool to correct mutation in the FAH gene (that codes for the enzyme fumarylacetoacetate hydrolase) in a mouse model. In humans this mutation leads to a disease known as tyrosinemia in which excess tyrosine – an amino acid – accumulates in the body leading to liver failure and even death. Editas Medicine, a biotechnology company in Cambridge, Massachusetts hopes to use CRISPR/Cas9 to treat a rare retinal disorder caused by a mutated gene by as early as 2017.

In most of the cases, the strategy to treat diseases arising in blood cells is to harvest blood cell precursors called hematopoietic stem cells from the bone marrow of the patient, treat them with the chosen gene-editing tool to knock out/correct/insert a new gene, expand them in culture, check via genome sequencing to ensure successful editing, and then inject them back to the patient (*ex-vivo*). The premise is that corrective changes in a sufficient number of cells could provide a lasting curative treatment to the patient. In case of organs like liver where the cells cannot be taken out and put back, vectors containing the gene-editing tool are injected in sufficient numbers to the organ directly (*in-vivo*).

CRISPR/Cas9 is going one step further – to correct genetic defects even before birth through germ-line editing or embryo-editing. For example, a Chinese team has created genetically modified monkeys by injecting CRISPR-Cas9 construct into one-cell-stage embryos to rewrite two genes. In another development, a company called Recombinetics has developed hornless cattle using CRISPR-Cas9 technology by editing the sperm genome of bulls. This would improve the welfare of cattle by preventing injuries to other animals and to farm workers. In April 2015, another Chinese team reported attempts to edit human embryos which harboured a mutated beta-globin gene responsible for the inherited disease called beta-thalassemia, a blood disorder that reduces the production of haemoglobin.

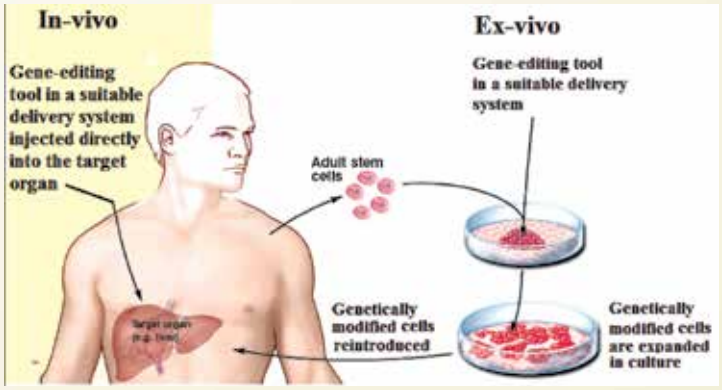
Gene-editing is thus no longer science fiction; it is becoming a reality.

When exploring the genetic code of the African clawed frog, scientists noted an odd protein tightly bound to the frog's DNA. When they mapped the 3D architecture of the protein, they were surprised to see elongated loops resembling fingers, grasping the frog's genes with extraordinary strength. Holding the loops together was a stable zinc ion. Because of its unusual hand-like structure, they named the protein zinc finger.

A decade later Srinivasan Chandrasegaran, then a postdoctoral fellow at Johns Hopkins University in USA, wondered how he could make practical use of the sticky proteins. The problem was that each zinc finger recognised only a tiny chunk of DNA, about three bases. This kept the collection from being specific enough to target genes in people. If he wanted to use the proteins to target a specific piece of DNA, he needed more length. Chandra, as his friends called him, had a simple solution. He stitched six of the proteins together so that instead of just three bases, he had eighteen, enough to recognise a fragment of a gene.

But binding the DNA wasn't enough. He also had to figure out a way to alter it. Chandra decided to borrow an enzyme used by bacteria to cut out viruses from their genetic code. Called restriction endonucleases, this clever defence mechanism is an ideal way to cut DNA. Chandra chose the FokI restriction enzyme, known for its ability to make a clean break. Chandra combined the DNA gripping ability of the zinc fingers with the DNA cutting enzyme. A zinc finger nuclease (ZFN) was born.

(From *Popular Science* – Posted 9 July 2014)



Strategy for gene therapy with the new gene-editing tools

Dr. M.S.S. Murthy retired as a senior scientist from the Bhabha Atomic Research Center, Mumbai in 1997. He is a popular science writer and authored a number of books.

Pascal's Triangle – An Interesting Number Pattern

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Patterns possess a very important place in the study of mathematics. The National Council of Teachers of Mathematics (NCTM) recommended in their publication in 2000 that "Instructional programs from pre-kindergarten through grade 12 should enable all students to understand patterns, relations and functions." The study of patterns can also address all the process standards recommended by the NCTM: problem solving, reasoning and proof, communication, connections and representation. One of the most interesting and beautiful number pattern in mathematics is Pascal's Triangle. Although it was well known and studied differently by Arabic, Chinese, European and Indian mathematicians, Blaise Pascal (1623-1662), a French mathematician, statistician and philosopher developed many applications out of it and for the first time wrote a treatise *Trait´e du Triangle Arithmetique* (Treaties on Arithmetical Triangle) in 1654, which was published posthumously in 1665.

Blaise Pascal made many contributions to mathematics. He laid the foundation of probability, invented 'Pascaline' (the first mechanical calculator), discovered an important theorem in geometry, worked with cycloids, developed theory of convergent and divergent series, and planted the seeds of calculus, but he is more famous for 'Pascal's Triangle', which is a fascinating resource in mathematics. This triangle is mathematics' paradigm of Nature. It has been stated that the Pascal's Triangle can be alluded to "either a gold mine or an iceberg – the former because the riches are there, but some ingenious labour is often needed to unearth it, the latter because we shall perhaps never see more than a small percentage of the mass." Martin Gardner, an American popular mathematics and science writer, in his book *Mathematical Carnival* describes Pascal's Triangle as "so simple that a 10-year old can write it down, yet it contains such inexhaustible riches and links with so many seemingly unrelated aspects of mathematics, that is surely one of the most elegant of number arrays."

Let us have a look at its mathematical beauty for many internal patterns and properties.

Pascal's Triangle is a triangular array of binomial coefficients.

C(n,r)=n!/r!(n-r)! is a binomial coefficient, where n is a whole number; i.e., n= 0,1,2,3,4,5,6,……, 0 ≤r ≤n, n!=1*2*3*……*n, r!=1*2*3*……*r, 0!=1

In other words, we may say that, to build this triangle, start with '1' at the top and then continue placing numbers below it in a triangular pattern. Each number is just the sum of the two numbers above it except for the edges, which are all '1'. By convention, we start numbering the rows from n=0.

```
                1
              1   1
            1   2   1
          1   3   3   1
        1   4   6   4   1
      1   5  10  10   5   1
    1   6  15  20  15   6   1
  1   7  21  35  35  21   7   1
1   8  28  56  70  56  28   8   1
1 9  36  84 126 126  84  36  9   1
```

Internal Patterns & Properties

1) All are positive natural numbers

We may observe that Pascal's Triangle can be extended by including additional 1's and by adding existing positive natural numbers. Thus we have all numbers in Pascal's Triangle are positive natural numbers.

2) Number of elements in a row

The number of elements in a particular row is one more than the number of that row; i.e., the nth row of the Pascal's Triangle has (n+1) number of elements. Thus an even numbered row has odd number of elements and an odd numbered row has even number of elements; e.g., the 7th row has 8 elements and the 8th row has 9 elements.

3) First increase, then decrease

In each row, the numbers first increase from 1 to a maximum value, then decrease back to 1 in a similar manner. For even numbered row the maximum value occurs once and for odd numbered row the maximum value occurs twice.

4) Row number is the 2nd number

The 2nd number and the 2nd last number in the nth row is n, except the 0th row (the 0th number of every row is 1).

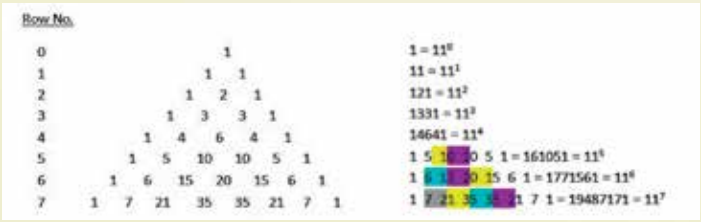
5) Sum of the numbers of the rows

The sum of the numbers of a row doubles each time. It is in the form of power of 2. The sum of the numbers of the nth row is equal to 2^n; where n=0, 1, 2, ……

Row No.		Row Sum
0	1	$1 = 2^0$
1	1 1	$1+1 = 2 = 2^1$
2	1 2 1	$1+2+1 = 4 = 2^2$
3	1 3 3 1	$1+3+3+1 = 8 = 2^3$
4	1 4 6 4 1	$1+4+6+4+1 = 16 = 2^4$
5	1 5 10 10 5 1	$1+5+10+10+5+1 = 32 = 2^5$
6	1 6 15 20 15 6 1	$1+6+15+20+15+6+1 = 64 = 2^6$

6) Powers of 11

The value of a row, if each entry is considered a decimal place (and numbers larger than 9 carried over accordingly) is a power of 11. Thus for nth row, it is 11^n.



7) Sum of the squares of the numbers of a row

The sum of the squares of the numbers of the nth row equals to the middle number of (2n)th row; e.g., the sum of squares of the 1st row is $1^2+1^2=2$, which is the middle number of the 2nd row, that of the 2nd row is $1^2+2^2+1^2=6$, which is the middle number of the 4th row, that of the 3rd row is $1^2+3^2+3^2+1^2=20$ which is the middle number of the 6th row.

8) Sum is always zero

We may observe that if we alter the signs of the alternate numbers in any row and then add them together, the sum is always zero;. e.g., for 5th row this sum = 1-5+10-10+5-1=0

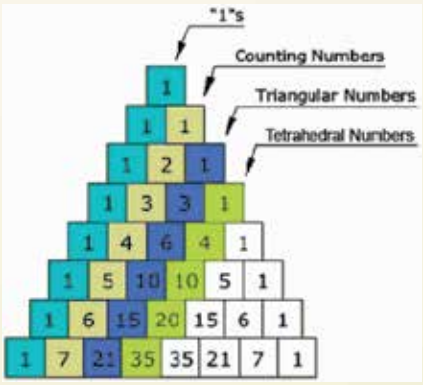
For 8th row this sum =1-8+28-56+70-56+28-8+1=0

9) Multiples of prime numbers

In case of a prime numbered row or a row where the 1st element is a prime number (the 0th element of every row is 1), all the numbers in that row (except the 1's) are multiples of that prime number; e.g., in the 7th row the 1st number 7 is a prime number and all the terms except 1's are 7 21 and 35 which are multiples of 7.

10) Only the number 1's

The diagonals joined along the left and right edges contain only the number 1's.

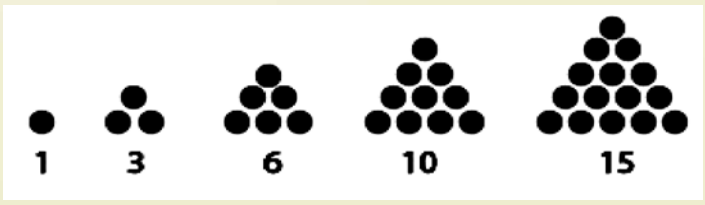


11) Natural numbers

The diagonals next to the edge diagonals; i.e., both the 2nd diagonals contain the natural numbers in order i.e. 1 2 3 4 5 6 7 .....

12) Triangular Number

The next pair of diagonals; i.e., both the 3rd diagonals contain the triangular numbers in order viz., 1 3 6 10 15 21 ..... A triangular number is the number of dots in an equilateral triangle uniformly filled with dots. It is a Figurate Number. (Figurate numbers are numbers that can be represented by a regular geometrical arrangement or sequence of evenly spaced points.)



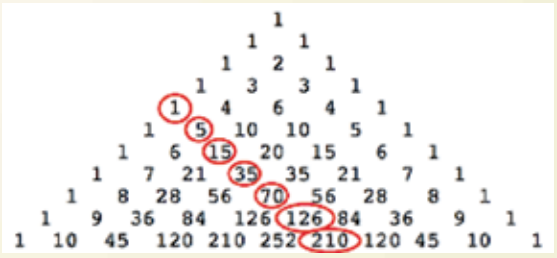
A triangular number is, equivalently, the sum of the 'n' natural numbers from 1 to n, $T_n=1+2+3+.....+(n-1)+n =n(n+1)/2$

13) Tetrahedral Number

Both the 4th diagonals contain the tetrahedral numbers in order; viz., 1 4 10 20 35 ..... Tetrahedral numbers are the sum of consecutive triangular numbers. We know that 1 3 6 10 15 21 ..... are triangular numbers. So the sum of the consecutive triangular numbers 1, 1+3=4, 1+3+6=10, 1+3+6+10=20, ... are the tetrahedral numbers.

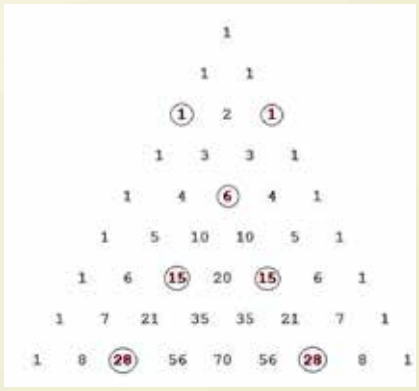
14) Pentatope Number

A pentatope number is a number in the fifth cell of any row of Pascal's Triangle starting with the 5-term row 1 4 6 4 1 either from left to right or from right to left. Both the 5th diagonals contain the pentatope numbers in order; i.e. 1 5 15 35 70 126 ..... The nth pentatope number can be found by the formula $P_n=n(n+1)(n+2)(n+3)/24$, where 'n' is a natural number.



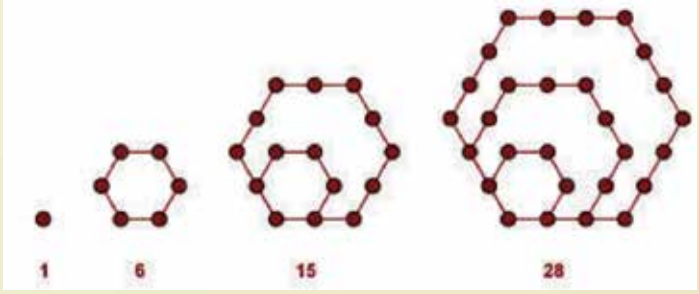
15) Hexagonal Number

Both the 3rd diagonals contain hexagonal numbers; viz., 1 6 15 28 45,.....



The nth hexagonal number H_n is the number of distinct dots in a pattern of dots consisting of the outlines of regular hexagons with sides up to n dots, when the hexagons are overlaid so that they share one vertex. The nth hexagonal number can be found by the formula, $H_n= n (2n-1)$, where n is a natural number.

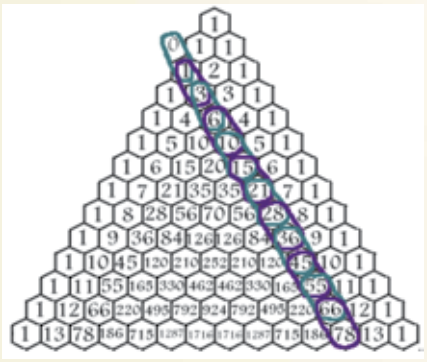
These hexagonal numbers are also known as "cornered hexagonal numbers."



16) Square Number

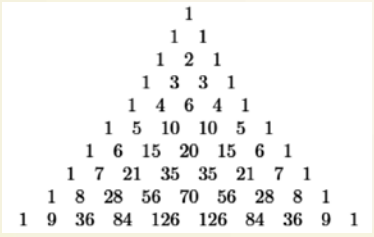
In the 3rd diagonal, if we add two consecutive triangular numbers continuously, we get square numbers in order. By doing this we have 1 1+3 3+6 6+10 10+15 15+21 21+28 28+36 36+45 45+55 .....

i.e., 1, $4=2^2$ $9=3^2$ $16=4^2$ $25=5^2$ $36=6^2$ $49=7^2$ $64=8^2$ $81=9^2$ $100=10^2$ .....



17) Square numbers in groups of four

In Pascal's Triangle, if we add four numbers in an order like below, we can get the square numbers in groups of four; e.g.,



$1+2+3+3=9=3^2$

$3+3+6+4=16=4^2$

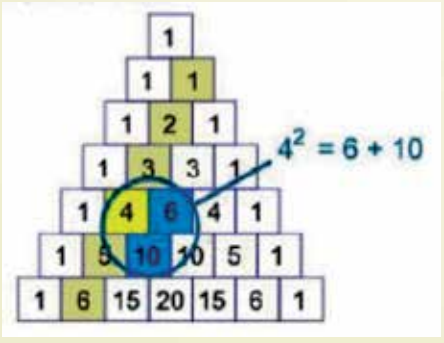
$6+4+10+5=25=5^2$

$10+5+15+6=36=6^2$ ..... etc.

18) Square of the numbers of 2nd diagonals

In case of the 2nd diagonal, the square of a number is equal to the sum of the numbers next to it and below both of those.

$2^2=1+3$ $3^2=3+6$ $4^2=6+10$ $5^2=10+15$



19) Sum of the natural numbers serially

We may note that each number in the 3rd diagonal of Pascal Triangle is the sum of the natural numbers serially in the preceding diagonal; i.e., 2nd diagonal.

20) Symmetrical

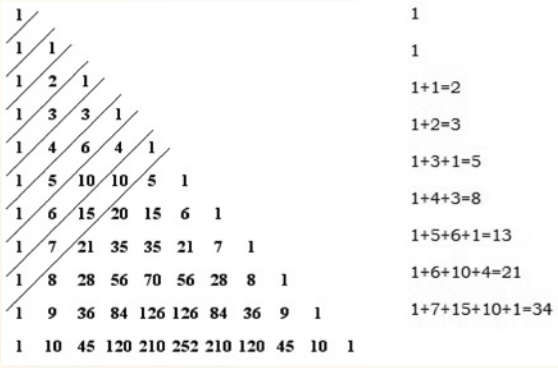
Pascal's Triangle is symmetrical. It is symmetric about the middle of each row. If a line is drawn vertically down through the middle of the Pascal's Triangle, it is like a mirror image, the numbers on the left side have identical matching numbers on the right side, excluding the centre line.

21) Fibonacci Sequence

The Fibonacci sequence is hidden in Pascal's Triangle. If the rows of the Pascal's Triangle are left-justified and we take the sum of the numbers in the left-justified triangle like below, we can detect the Fibonacci Numbers.

The Fibonacci sequence starts "1, 1" and then continues by adding the two previous numbers, for example:

1 1 2 (=1+1) 3(=1+2) 5(=2+3) 8(=3+5) 13(=5+8) .....



22) Hockey-Stick Pattern

The sum of the numbers, starting with any '1' at the border sides of the triangle, extended diagonally into the triangle and ending on any number inside the triangle is equal to the number below the last number of the diagonal, which is not on the diagonal. This is called the 'Hockey Stick Pattern' of Pascal's Triangle as the numbers involved form a long straight line similar to the handle of the hockey stick and the sudden turn at the end where the sum appears is like the part that contacts the puck.

Some examples of this are:

1+3+6=10, 1+6+21+56=84, 1+12=13, 1+7+28+84+210+462 +924=1716

23) Sierpinski's Triangle

Let us colour all the odd numbers in a Pascal's triangle with black and let the rest (the evens) left blank (white). This odd-even Pascal's triangle results in an interesting pattern of triangles within triangles. This pattern is similar to the Sierpinski's Triangle, first described by the Polish mathematician Waclaw Sierpinski in 1915.

From the above discussion we have seen how Pascal's Triangle is an interesting number pattern due to its many internal patterns and properties. Besides these, Pascal's Triangle is a beautiful number pattern due to its wide applications in various fields of mathematics. Pascal's Triangle is used in the theory of probability, theory of combination, theory of figurate numbers, in dividing the stakes in games of chance, in finding the powers of binomial expressions. Pascal's Triangle is useful in solving some well-known puzzles such as the Tower of Hanoi and maximizes one's winning in the Plinko game. With the help of Pascal's Triangle, the numbers of Lucas, Catalan, Fermat, Stirling and others may be derived.

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Topological Phases and Exotic States of Matter

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Matter that we come across in daily life are mostly three-dimensional, which have certain fixed properties. But when present at the surface or inside extremely thin layers that can be considered two-dimensional, matter shows strange properties such as superconductivity and superfluidity, especially at extremely low temperatures. The physics that takes place in two-dimensional matter is very different from that we recognise in the world around us. Two-dimensional systems such as thin fluid films or single-layer materials can exhibit surprising effects, such as frictionless liquid flows or unconventional electrical behaviour, and one-dimensional systems can be equally strange. New collective phenomena are being continually discovered in such two-dimensional matter, and condensed matter physics is now one of the most vibrant fields in physics.

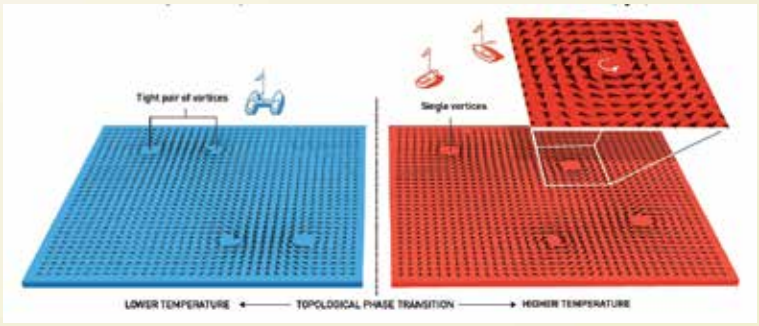
Michael Kosterlitz

David James Thouless

Duncan M. Haldane

The Nobel Prize in Physics for 2016 has been awarded jointly to David J. Thouless of the University of Washington, Seattle, USA, F. Duncan M. Haldane of Princeton University, NJ, USA, and J. Michael Kosterlitz of Brown University, Providence, USA, for their theoretical explanations of strange states of matter in two-dimensional materials, known as topological phases. Thouless will get half the prize amount and Haldane and Kosterlitz will share the other half. The work of the three laureates has given new insights into the behaviour of matter at low temperatures, and has laid the foundations for the creation of new materials called topological insulators, which could allow the construction of more sophisticated quantum computers.

The most common phases of matter are gaseous, liquid and solid. However, at extremely high or low temperatures matter assumes other, more exotic states. For many decades, scientists have been examining how matter behaves in extreme circumstances, such as when it is cooled to within a few degrees of absolute zero. These experiments have turned up a slew of exotic phases where matter behaves in strange ways and show unusual properties like superconductivity and superfluidity. In superconductivity, certain materials when cooled to a few kelvins allow electrons to pass through with almost no resistance. In superfluidity, certain liquids flow without any resistance and may crawl up the sides of a container defying gravity. Helium shows superfluidity when cooled to a few kelvins. In these strange phases, scientists can see quantum mechanical effects at work in materials, unencumbered by the random motions of atoms.



Phase transition occurs when phases of matter transition between each other, such as when ice melts and becomes water. Using topology, Kosterlitz and Thouless described a topological phase transition in a thin layer of very cold matter. In the cold, vortex pairs form and then suddenly separate at the temperature of the phase transition. This was one of the twentieth century's most important discoveries in the physics of condensed matter. (Credit: nobelprize.org)

The role of topology in condensed matter physics was established in the early 1970s, when theorists were debating phase transitions in two-dimensional systems. Early work showed that conventional transitions (like those between water and ice) could not happen in two dimensions, but it was clear that some sort of abrupt change was occurring in, for example, liquid films that exhibited superfluidity below a critical temperature.

To settle the debate, Kosterlitz and Thouless conceived of a new form of phase transition based on vortices and other so-called topological defects. A vortex is a point in a magnetic film, for example, around which the magnetic spins of the atoms orient in a circular pattern. A related structure, called an antivortex, has a more complicated pattern, with spins pointing inward along two directions – say, east and west – and outward along north and south. At high temperature, vortices and antivortices are plentiful, and the spins are disordered. However, Kosterlitz and Thouless showed that at extremely low temperatures, vortices pair up with antivortices, largely cancelling out their effect. As a result, the spins throughout the two-dimensional material are able to align with each other to a certain degree. This alignment is a form of "topological order" that also applies more

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Pulses for Food and Nutritional Security

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India has already enjoyed five decades of post-green revolution bumper crops, although there has not been significant gains pulses production, which has created several problems including that of protein malnutrition and insecurity of quality food. Demand of pulses being much higher than the availability, there is frequent increase in prices of pulses often making them unaffordable by consumers. The projected pulses requirement by the year 2030 in India is estimated at about 32 million tonnes. Currently the country needs to produce 4-5 million tonnes of additional pulses to meet the domestic requirement.

Pulses play a pivotal role in enhancing livelihood security, nutritional security, food security, soil health, farm profit and environmental sustainability. Thus pulses are premier crops cultivated in the Indian sub-continent for better soil health, farm profit and environmental sustainability. The Indian population being predominantly vegetarian, pulses and its products provide a rich source of essential nutrients like protein, minerals and vitamins. Pulses can easily meet the protein requirement of a vegetarian diet. Mixing grains of pulses with other cereals enhances the nutritive value of the food. Pulses are also a cost-effective alternative for ameliorating energy- protein/ nutrient deficiency in the country. Several serious diseases in humans can be prevented by regular intake of pulses. The current per capita availability of pulses is only 37 g/day as against 54 g/day required to fulfil the protein demand.

India has only 3 percent of the world's land resources and 5 percent of water resources. Yet, Indian agriculture system supports 18 percent of the world population. The production of pulses in India has remained insufficient, making us dependent on imports. The demand for these food commodities is expected to increase substantially in future. India is the world's largest producer, importer and consumer of pulses. Our national, annual import bill on pulses is as high as Rs.10,000 crores. Thus there is a great need for increasing production of pulses. Under the present changing climate scenario, more emphasis needs to be given on achieving the target of 24 million tonnes of pulses by 2020 in order to make the country self-sufficient and reduce the burden of import bill substantially. This can be achieved through development and promotion of new improved/hybrids varieties, balanced fertiliser use, irrigation management and timely insect pest control. More emphasis also needs to be given on production of sufficient quality of seed pulses through farmers' participatory approach.

In the changing climate scenario, biotic and abiotic stresses have become major production limiting factors. Further, deteriorating soil quality and declining productivity are major concerns for pulse production. Due to these factors, pulses output went down from 19.24 million tonnes to 17.20 million tonnes during 2014-15 leading to the crisis of unprecedented price rise of these commodities. The prices of pulses have been rising abnormally in recent times due to reduction in domestic production as well as shortage globally, mainly in case of pigeon pea. Efforts to nail in hoarders and black marketeers did not yield the desired effect. During the year the government raised the minimum support price of major pulses by Rs.275 per quintal. With production estimates for 2015-16 still lower than the bumper crop of 2013-14, the government has decided to create a buffer stock of 1.5 lakh tonnes of pigeon pea and black gram, which will be procured directly from farmers at market rates. Keeping in view the above-mentioned facts, agronomists, plant breeders and natural resource management scientists need to play an important role in enhancing production and productivity of pulse crops in the long run. There is need of the best management practices to increase pulse production per unit area as the arable land is decreasing day

Table 1: Nutritional value of different pulses grown in India (Per 100 gm)

Pulses	Moisture (g)	Protein (g)	Fat (g)	Minerals (g)	Fibre (g)	Carbohydrate (g)	Calcium (mg)	Phosphate (mg)	Iron (mg)
Gram	9.8	17.1	5.1	3.0	3.9	60.9	202	312	4.6
Cowpea	13.4	24.1	1.0	3.2	3.8	54.5	77	414	8.6
Moong	10.4	24.0	1.3	3.5	4.1	56.7	124	326	4.4
Lentil	12.4	25.1	0.7	2.1	0.7	59.0	69	293	7.58
Moth	10.8	23.6	1.1	3.5	4.5	56.5	202	230	9.5
Pea	16.0	19.7	1.1	2.2	4.5	56.5	75	298	7.05
Rajma	12.0	22.9	1.3	3.2	4.8	60.6	260	410	5.1
Soybean	8.1	43.2	19.5	4.6	3.7	20.9	240	690	10.4
Arhar	10.5	19.3	4.5	3.4	7.4	55.5	280	301	12.3

Source: National institute of food and nutrition, Hyderabad

by day due to urbanisation, industrialisation and other development activities.

Pulses occupy an important place in Indian agriculture. In India pulses are grown over an area of 23.8 million hectares with a total production of 18.6 million tonnes. The average yield of pulses in India is about 735 kg/hectare. Green gram, black gram, pigeon pea and cowpea are the most important and leading pulse crops of India grown during the rainy season. Chick pea, lentil, grass pea, field pea, and kidney bean are the important pulse crops grown during the winter season. Pulses belong to Leguminosae family and are generally grown in irrigated as well as rain-fed areas. Main growing areas of pulses in India are Madhya Pradesh, Uttar Pradesh, Gujarat, Maharashtra, Karnataka and Rajasthan. Karnataka is the major pulses growing state in the country; it is the largest producer of pigeon pea.

A large number of children below the age of three suffer from protein deficiency. As pulses and pulse products are the main and chief source of protein and minerals for more than fifty percent of our population, to address malnutrition in children, food grains must be bio-fortified with quality protein and micronutrients. Recently several national and international research institutes have developed iron and zinc-rich lentil varieties through molecular breeding (the application of molecular biology tools in plant breeding). These varieties/technologies should reach the farmers immediately for alleviating malnourishment in women and children. Government of India are also giving more emphasis on pulse production and have allotted Rs.500 crore in central budget 2016-17 for increasing pulse production. Nowadays there is a great need to increase the productivity and total production of pulses to fulfil the demand of burgeoning population of India. Hence, there is need to concentrate on new varieties and situation-specific technologies, managing demonstrations to harvest maximum yield advantages.

Realising the importance of pulses as a rich and economical source of protein in vegetarian diet, the United Nations General Assembly has declared the year 2016 as the "International Year of Pulses" (IYOP) to highlight and create awareness of the problem of hunger and protein malnutrition worldwide, seek solutions to nutritional security problem, call for changes to our agriculture and food supply systems, and make the world free from hunger and malnutrition. Having a UN dedicated year is expected to raise the level of awareness about pulses globally and also about the important role pulses can play in advancing health and nutrition, food security and environmental sustainability. It provides an unprecedented opportunity to raise awareness about the role of pulses in feeding the world and to give additional research attention to pulses they deserve. South Asia already has the highest number of food insecure people, with 300 million undernourished – India accounts for 250 million of them. Over the years, while the country has accumulated a huge surplus of wheat and rice, pulses remain in short supply. Consequently, the per capita availability of pulses has progressively declined from 65 g a day in 1961 to merely 39.4 g in 2011, whereas, availability of cereals has gone up from 399.7 to 423.5 g. For a country that faces persistent protein inflation and has preference for vegetarian diet, pulses are the most economical source of vegetable protein. Higher consumption of pulses will help address the scourge of pervasive malnutrition caused by protein deficiency among large sections of the Indian population.

Pulses research and development

Recently a large number of improved varieties/hybrids of pulses have been developed suitable for unconventional areas that can boost pulse production in future. Efforts are also going on to develop synchronous-maturity hybrids and varieties of pigeon-pea, black gram, green gram and chickpea. In pigeon pea, several genotypes with early maturity, determinate growth habit and amenability to mechanical spraying and harvesting have been developed. Recently five new varieties, namely Pusa 1371 of Mung bean; DC 15, TPTC 29 and PCP 0306-1 of Cowpea and RMB 2251 of Moth bean were recommended for identification.These technologies will enhance the productivity of pulses to meet our domestic requirements fully. There is urgent need to provide such improved varieties of pulse crops to the farmers immediately.

National Food Security Mission (NFSM) and pulses

Government has started National Food Security Mission for food and nutritional security and to promote of cultivation of pulses and other food grains. More states are now covered under the National Food Security Mission. Pulses cultivation has been started in Jammu & Kashmir, Himachal Pradesh, Uttarakhand and all the North-East states. Salient points of the National Food Security Mission are as given as below.

1. Seven crops – Rice, wheat, pulses, jute, sugarcane, cotton, and coarse cereals covered under NFSM.
2. 50% NFSM has been dedicated for development of pulses.
3. Cultivation of pulses under NFSM has been started in J&K, HP, UK, and all the North-Eastern States.

Inclusion of pulse crops in cropping system

Cultivation of pulse crops is a must once in a year for every farmer for maintaining soil fertility (as the root bacteria in these pod-bearing crops can directly fix atmospheric nitrogen), betterment of their livelihood, increased farm income and enhancing nutritional security. After harvest of sorghum, barley, wheat and maize, farmers should grow gram, red gram, green gram and lentil. Pulse crops should be grown along with food and cash crops in a cropping sequence. Small and marginal farmers can also increase soil fertility of their farmland by growing short-duration pulse crops and applying crop residue in the soil. Thus soil biomass can also be enhanced, which is the main source of energy and food for several beneficial microorganisms involved in oxidation and reduction process in soil. Farmers could be motivated to think about inclusion of pulse crops in crop rotation for achieving sustainable harvests. Likewise, after harvesting wheat farmers should grow green gram in their fields and after getting two pickings of mature pods, the green gram crop residue should be incorporated into the soil. This would enhance the soil biomass which later on decomposition could supply primary as well as secondary and micro-nutrients to the succeeding crops. This would increase the soil fertility and improve the soil health too. Thus the water-holding capacity and water availability for crops can also be increased.

Soil protection and pulses

Due to soil rejuvenation qualities such as release of soil-bound phosphorous, build up soil fertility through atmospheric nitrogen

fixation, recycling of soil nutrients and addition of organic matter and other nutrients make pulses an ideal crops of sustainable agriculture in the tropical and sub-tropical regions of India. Besides, pulses have the capability to protect the soil from wind and water erosion in arid and semi-arid tropics. The roots of pulse plant have Rhizobium nodules that work for nitrogen fixation in the soil. For better nitrogen fixation, suitable species of Rhizobium should be applied for different pulse crops. Pulses are rich source of protein and can be easily grown under rice-wheat cropping system in North-West India. Pulses improve soil fertility by fixing atmospheric nitrogen and hence the farmers need to adopt this technology in the region.

Use of biofertilisers in pulse cultivation

Bio-fertilisers are not only eco-friendly and cost-effective but also help increase production and productivity of various pulse crops. The method of application is easy and simple in pulse crops production. Bio-fertilisers are also easily available at various research institutes at low cost. Use of bio fertilisers such as Rhizobium, Azospirillum, phosphate solubilising bacteria (PSB) and Trichoderma has also resulted in significant increase in all growth and yield parameters in pulse crops. Apart from this it has a potential role in saving of chemical fertilisers in pulse crops cultivation. Biofertilisers such as PSB and mycorrhiza fungi significantly increase the yield and phosphorus content in pulse crop. Similarly, the growth attributes and nutrient uptake in pulse crops also increased due to application of Rhizobium, PSB, Azotobacter and Azospirillum compared to control.

Plant protection measures

There is great need for plant protection measures for getting higher yield and yield attributes in pulses cultivation, because pulses are highly sensitive and susceptible to insect-pest infestation, particularly at pod formation and grain-filling stages. There is also a matter of concern over problems of yellow mosaic virus, pod borer and white fly in pulse crops and also need to develop varieties resistant to viruses.

Publicity and awareness drive

Agricultural extension has to be adequately strengthened organisationally and financially to support the chain of transfer of technology from the research institutions to farmer's fields in a cost-efficient manner. Farmers should adopt the scientific technology for sustainable yield of pulses. Advanced techniques regarding pulse crops cultivation should be disseminated to encourage farmers and extension workers increase pulses production in future.

Drip irrigation and fertigation (a method of injection of fertilisers, and other water-soluble products into an irrigation system) technologies may also be popularised among farmers for saving precious and costly irrigation water and other farm inputs in pulse crops cultivation in dry areas. Farmers must be made aware of the adverse effects of excess and unbalanced use of agrochemicals, mainly urea in pulses cultivation. For this, farmers' conferences, farmer-scientist interface, *Kisaan Mela*, field visits, and meetings can be organised for quicker spread of new technologies and information among the farming community. This will provide an exceptional opportunity for increasing productivity and profitability of pulses. Further, consumers would get fresh, cheap and better quality pulses at lower price. There is need to concentrate on new varieties and situation-specific technologies, managing demonstrations to harvest maximum yield advantages, promote farmer-to-farmer spread of the produce as quality seeds and encourage value addition of pulses at the farmers' level.

New initiatives and efforts

There has to be planned efforts including adequate financial investment to evolve pulse crop varieties which are high-yielding and resistant to diseases, drought, flood and salinity. Special efforts need to be initiated through scientists, subject matter specialists, extension workers, NGOs and farmers to make India self-sufficient in pulse production. In this connection, improved technologies for pulses cultivation should be demonstrated at different parts of the country, particularly in non-traditional areas by *Krishi Vigyan Kendras* (KVKs) to motivate pulse growing farmers. Besides, technology support, seeds of improved and hybrid varieties of pulses must be distributed among progressive farmers and extension workers to boost pulse production.

Processing, packing and storage

To overcome pulse crises in future, more emphasis needs to be given on farm processing and value addition of pulses and also better storage facilities, as pulses are easily damaged by insect-pests. Further, moisture percentage in the pulse grains needs to be brought down to 9 or less after sun drying and water-proof bags such as thick polyethylene bags used for packing and storage. These bags should be heat sealed. In case of higher seed moisture, jute bags are recommended. Pulses seeds being hygroscopic in nature, absorbs moisture from the atmosphere or loses moisture until the equilibrium is reached between the vapour pressure of seed and atmosphere. Therefore, efforts should be made that relative humidity in the seed storage space is kept as low as possible and any chance of absorbing moisture by the seed from atmosphere is avoided. More emphasis may also be given on pulses processing techniques and development of local markets for pulse produce.

Table 2: Recommended biofertilisers and their method of application in pulses

Biofertiliser	Dose/ha.	Method of application	**Comments**
Rhizobium spp.	500-800 gm.	Seed inoculation	Before sowing
Phosphate solubilising Bacteria (PSB)	1-2 kg.	Seed inoculation and soil treatment	Before sowing
Azotobacter	500-800 gm.	Seed inoculation and soil treatment	Avoid bacterial culture from sunlight
Mycorrhiza	1-2kg	Seed inoculation and soil treatment	Before sowing

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Safeguarding against Rabies — the 1-2-3 of post-exposure treatment

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Rabies is a fatal viral disease largely transmitted to human beings from bites by infected animals — predominantly from stray dogs. The disease is entirely preventable through immediate post-exposure prophylactic care to the bitten person and can be controlled through mass vaccination of dogs. However, thousands of human lives continue to be lost to rabies in developing countries annually, and the biggest sufferer is India, where more than 25,000 people die of the disease each year. Since a large number of victims die unreported at home particularly in areas with poor access to health services, these numbers may, in fact, be much larger.

Given that a large number of people die in the prime of their youth, the loss of productivity due to their premature death carries a huge economic burden both for the individual family and community. The price of man-hours lost in obtaining post-exposure prophylactic care, and the cost of its administration is also very considerable. A

greater focus on mass dog vaccination could eliminate the disease at source, reducing the need for costly prophylactic treatment and preventing the large and unnecessary burden of mortality on at-risk communities.

Immediate Post-Exposure Prophylactic Measures to Prevent Rabies

Type of contact with a suspected rabid animal	*Prophylactic measures*
Category I : Touching or feeding animals, licks on intact skin	None
Category II : Nibbling of uncovered skin, minor scratches or abrasions without bleeding	Immediate vaccination and local treatment of the wound
Category III : Single or multiple bites or scratches which break through skin; licks on broken skin; contamination of mucous membrane with saliva from licks; contact with wild animals	Immediate vaccination and administration of rabies immunoglobulin; local treatment of the wound

A three-pronged treatment

A person, scratched or bitten by a warm-blooded animal at-risk for carrying rabies, must receive immediate post-exposure prophylactic care to safeguard against rabies. Any delay in treatment is fraught with grave risk. Once the rabies virus finds its way into the central nervous system, there's little that can be done, and death is on the cards.

The rabies prophylaxis is a three-pronged treatment approach. Each measure is equally important and must be carried out simultaneously depending upon the kind of risk (see table).

In effect, if a person has a category II or III contact with a potentially rabid animal, s/he is at definite risk of developing rabies. This risk is increased if:

- the biting animal belongs to a mammalian species that could be harbouring the rabies virus;
- the animal looks sick or displays an abnormal behaviour;
- the saliva of the animal would have contaminated the wound or mucous membrane of the bitten person;
- the bite was unprovoked; or
- the animal is not vaccinated.

The 1-2-3 steps

The three key steps, which must be initiated as soon as possible after an exposure, must include:

- Local treatment of the wound
- Active immunization against rabies — by employing a course of potent and effective rabies vaccine; and
- Passive immunization against rabies — by administering rabies immunoglobulin in type III wounds.

A timely and optimal post-exposure rabies prophylaxis virtually guarantees protection from the disease. The administration of vaccine, and immunoglobulin if required, must be conducted by, or under the direct supervision of, a physician.

Step 1: Local treatment of the wound

Cleaning of the wound

Since the rabies virus finds its way into the human body through a contact with a rabid animal, be it a scratch or bite, the immediate first-aid step is to clean the wound thoroughly. This simple measure helps remove the rabies virus contained in the animal saliva from the wound.

Due care must be taken about how the wound is handled. A vigorous scrubbing can worsen the injury to the tissues. So, be

gentle. Since the rabies virus can persist and multiply at the site of a bite for quite a long time, a thorough flushing and washing of wound with detergent, or soap and water, and running water for a minimum of 15 minutes can do a host of good and can save the life of the bitten person.

If faced with a situation where soap or detergent cannot be found, simply wash the wound with running water. The key thing is: do not delay, act quickly.

Use of antiseptics

Once a wound has been thoroughly washed, apply a chemical viricidal agent, such as povidone iodine or alcohol povidone. These substances can kill the rabies virus.

Do not use chilies and mustard oil

Use of irritants like chilies, mustard oil, lime, spices or local remedies is a common practice in many parts of the country. It offers no help and can worsen the damage to injured tissues. If a wound is pasted with such remedies, wash it off with plentiful water.

Using rabies immunoglobulin

If a wound is of category III, the risk can be checked by infiltrating rabies immunoglobulin in the depth of and around the wound. This measure helps kill the virus lurking in the wound.

Stitching of the wound

The wound must not be stitched as far as possible. If stitches are unavoidable, care must be taken to first carry out a thorough cleaning of the wound, and rabies immunoglobulin must be infiltrated in the depth of and all around the wound. It is still best to stitch a few hours later. This would allow diffusion of antibodies in the tissues. However, if a wound is bleeding, minimum loose sutures may be applied to arrest the bleeding.

Tetanus and antibiotic prophylaxis

A shot of tetanus vaccine may be given to check against the risk of tetanus. A suitable antibiotic may also be given to prevent wound sepsis.

Step 2: Active immunisation

The modern rabies vaccines of cell-culture or embryonated-egg origin are much safer and more effective than the older vaccines, which were produced in animal brain tissue. These modern rabies vaccines are now available in almost all major urban centres, and can be administered either through the intra-muscular or intra-dermal route.

Intramuscular regimen

A five-dose regimen is commonly used for post-exposure vaccination. The five doses are administered on days 0, 3, 7, 14 and 28 into the upper arm (deltoid muscle). The day 0 is the date of first dose administration of anti-rabies vaccine and may not be the date of rabies exposure/animal bite. Depending on the choice of vaccine, each time 0.5ml or 1ml of the vaccine is injected.

Currently, the following vaccines are to be had in India for intramuscular administration:

Cell Culture Vaccines

Human Diploid Cell Vaccine (HDCV): liquid (adsorbed), dose: 1ml. These are produced locally in the private sector.

Purified Chick Embryo Cell Vaccine (PCECV): dose: 1ml. These are also produced locally in the private sector.

Purified Vero Cell Rabies Vaccine (PVRV): dose: 0.5ml and 1ml. These are imported and also produced locally in public and private sector vaccine manufacturing units.

Embryonated-egg origin Vaccine

Purified Duck Embryo Vaccine (PDEV): dose: 1ml. These are produced locally in private sector and are currently being exported.

Site of injection

The upper arm (deltoid region) is ideal for the administration of these vaccines. Hip (gluteal region) is not recommended because the fat present in this region retards the absorption of antigen and hence impairs the generation of optimal immune response. In case of infants and young children, the front and outer part of the thigh is the preferred site.

Intradermal regimen

The use of cell-culture- and embryonated-egg-based rabies vaccines through the intradermal route offers a major advantage that only 0.1 ml dose has to be injected into the skin each time. Since the shots are given at two-sites each time, 0.2ml of the vaccine is sufficient for each dose. Shots are given at two sites on days 0, 3, 7 and 28.

However, this requires a carefully trained nurse or a doctor, who can administer the vaccine intradermally.

Precautions and contraindications

Modern rabies vaccines are well tolerated. Some people may, however, develop mild itching, redness, and rarely body ache and fever following a shot. They may need medical help.

A vaccinated person must be careful not to rub the injection site, or apply anything to the injection site.

Concurrent use of the anti-malarial medicine chloroquine can reduce the antibody response to intradermal application of cell-culture rabies vaccines. People who are in the midst of a malaria prophylaxis or treatment should therefore receive the vaccination by the intramuscular route.

Step 3: Passive immunisation

Human rabies immunoglobulin (HRIG) or equine rabies immunoglobulin (ERIG) or F(ab')2 fragments of equine anti-

rabies immune globulin can be used to obtain immediate passive immunization against rabies when the risk of rabies is more severe. Such a risk typically exists in the category III exposures (see table).

The shot of passive immunisation should be administered just before or shortly after administration of the first dose of cell-culture or embryonated-egg rabies vaccine given in the post-exposure prophylaxis regimen. However, if it is not immediately available, passive immunisation can be administered until the seventh day after initiation of the primary series of post-exposure prophylaxis vaccine.

Rabies immunoglobulin is in short supply worldwide and may not be easily available even in major urban centres.

Dosage and administration

The dose for HRIG is 20 IU/kg body weight and for ERIG and F(ab')2 products 40 IU/kg body weight. The full dose of rabies immunoglobulin, or as much as is anatomically feasible, should be administered into and around the wound site. Any remainder should be injected intra-muscular at a site distant from the site of active vaccine administration.

Multiple needle injections into the wound should be avoided. If the correct dose of rabies immunoglobulin is too small to infiltrate all wounds, as might be true of a severely bitten individual, it can be diluted in physiological buffered saline to ensure greater wound coverage.

Post-exposure Prophylactic Care in the Vaccinated

Individuals who have previously received a complete series of cell-culture or embryonated-egg rabies vaccine as a part of post-exposure prophylactic care need to take two booster doses of the vaccine.

Ideally, the first dose should be administered on the day of exposure and the second 3 days later. This should be combined with thorough wound treatment. Rabies immunoglobulin is not required for patients who have previously received a complete vaccination series.

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Topological Phases and Exotic States of Matter (continued from page 28)

generally to two-dimensional systems of atoms (or electrons) that align an aspect of their quantum states.

The work of Thouless and Kosterlitz also showed that when the temperature rises past a certain thermal threshold the vortice-antivortice pairs drift apart and there is topological phase transition, also known as "KT transition" (for "Kosterlitz-Thouless"). The topological phase transition is not an ordinary phase transition, like that between ice and water. The leading role in a topological transition is played by small vortices in the flat material. At low temperatures they form tight pairs. When the temperature rises, a phase transition takes place: the vortices suddenly move away from each other and sail off in the material on their own This "KT transition" is universal, and has been used to study superconductivity in thin films as well as to explain why superconductivity dissipates at higher temperatures. As such, the KT transition explains the emergence of both superfluidity and superconductivity in two dimensions.

KT transition has proved to be very successful in explaining the exotic states of superconductivity, superfluidity and magnetism in extremely thin materials that could be regarded as two-dimensional. At low temperatures, due to the vortices remaining tightly bound to each other, the phenomena of superconductivity or superfluidity is observed. But, when phase transition takes place with rising temperature, the materials lose the property of superconductivity or superfluidity as the vortices scatter away.

In the 1980s, Thouless and Haldane each studied how the conductivity of electricity in quantum systems followed topological rules. Thouless was able to describe theoretically, using topology, the mysterious phenomenon known as the quantum Hall effect. The phenomenon was discovered in 1980 by the German physicist Klaus von Klitzing while studying a thin conducting layer between two semiconductors, where the electrons were cooled to a few degrees above absolute zero and subjected to a strong magnetic field. He was rewarded with the Nobel Prize in 1985.

Another breakthrough occurred in 1988, when Haldane discovered that topological quantum fluids, like the one in the quantum Hall effect, can form in thin semiconductor layers even when there is no magnetic field. He said he had never dreamed of his theoretical model being realised experimentally but, as recently as 2014, this model was validated in an experiment using atoms that were cooled to almost absolute zero.

Together, the insights from Thouless's and Haldane's work have proved crucial in developing and understanding topological insulators, novel substances that block the flow of electrons in their interiors while simultaneously conducting electricity across their surfaces.

The research of the three laureates has opened up new areas of frontline research. Topological insulators, topological superconductors and topological metals are examples of areas which, over the last decade, have defined frontline research in condensed matter physics, mainly because of the hope that topological materials will be useful for new generations of electronics and superconductors, or in future quantum computers. Current research is now revealing the secrets of matter in the exotic flatlands discovered by this year's Physics Nobel Laureates.

Recent Developments in Science and Technology

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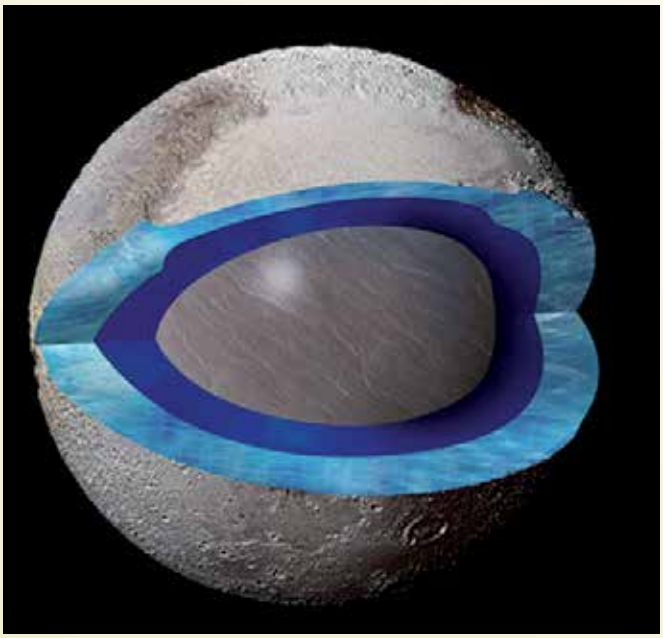
An ocean found under Pluto's surface

Ever since NASA's *New Horizons* planetary probe flew past Pluto on 14 July last year and sent back spectacular images of its surface, scientists have learnt a lot about Solar System's former ninth planet, which has revolutionised our ideas about this tiny distant world. Close-up images sent back by *New Horizons* during the flyby have revealed curious surface features – from a dark shadowy whale figure to a bright heart-shaped region that has been named Tombaugh Regio. A close-up image of an equatorial region near the base of Tombaugh Regio showed a mountain range with peaks as high as 3,500 metres above the surface of the icy body. The close-up of Pluto also makes clear that the dwarf planet has water ice that is as hard as rock. According to mission scientists, the mountains on Pluto were likely formed no more than 100 million years ago, making them among the youngest mountains – younger than the Himalayas – in a 4.56-billion-year-old Solar System.

It has been a couple of decades since Hubble Space Telescope images revealed a persistent bright spot on Pluto situated on the hemisphere facing away from its big moon Charon, although it was impossible to image it with enough resolution to determine its shape. Last year's flyby of Pluto by *New Horizons* revealed this intensely white spot, now called Sputnik Planitia, to be the western half of the heart-shaped Tombaugh Regio. Strangely, in the Hubble images it was found that the bright spot always aligned almost exactly opposite Charon, Pluto's largest moon, as it orbited the dwarf planet. But the scientists did not have a "convincing explanation" for this strange orientation of Pluto's bright feature aligning with the position of its largest moon. Now the mystery appears to have been solved.

Detailed analysis of the images and data received from *New Horizons* by a team of scientists led by Francis Nimmo at University of California, Santa Cruz, USA has now revealed the presence of liquid ocean lying deep beneath Pluto's frozen surface, which they say is the "best explanation" for features revealed by the *New Horizons* spacecraft (*Nature*, 16 November 2016 | DOI: 10.1038/nature20148). The finding also provides a possible mechanism for the apparent shift of Pluto's bright spot to align with Charon's position in orbit. The idea that Pluto has a subsurface ocean is not new, but the latest study provides the most detailed investigation yet of its likely role in the evolution of key features such as the vast, low-lying plain known as Sputnik Planitia, which forms one side of the Tombaugh Regio.



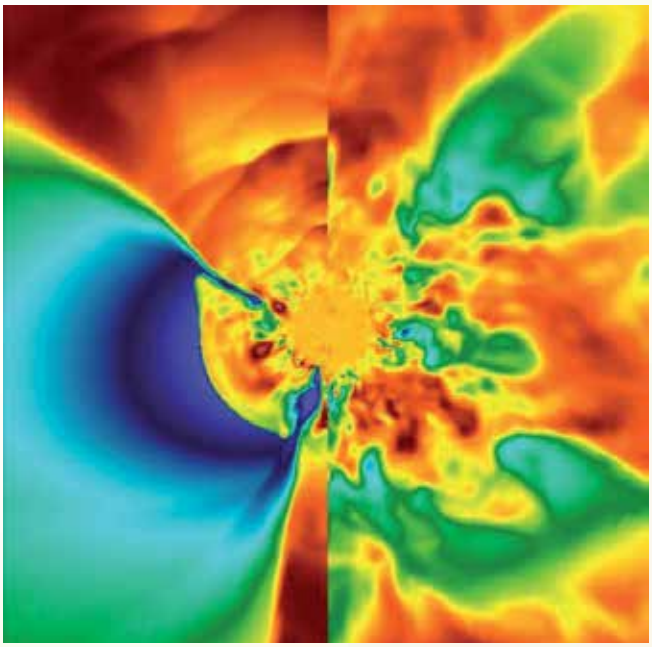
This cutaway image of Pluto shows a section through the area of Sputnik Planitia, with dark blue representing a subsurface ocean and light blue for the frozen crust. (Credit: Pam Engebretson)

From the new analyses the scientists conclude that Sputnik Planitia lies almost directly opposite Charon not by accident but because it literally dragged the crust of Pluto around to the current arrangement. Geophysicists refer to this crustal shifting as true polar wander. It is the tendency of spinning objects to reorient themselves so that locations with mass excesses end up on the equator and mass deficiencies at the poles. According to the scientists, the evidence seems strong that Pluto's big, ice-filled heart has been on the move, and maybe the equator-ward migration will continue, slowly, over time. And this wouldn't have been possible without a liquid ocean underneath.

But if Pluto does have an ocean, the question arises: How has it managed to avoid freezing up entirely over the past 4.5 billion years? Nimmo has an answer. He says, "Pluto is big enough that it may have retained a substantial amount of internal heat, and the dwarf planet's water may contain significant amounts of ammonia or other substances that act as an antifreeze".

Formation of solar system was triggered by low-mass supernova

There are many theories of formation of our solar system. According to the most widely accepted theory the process began some 4.6 billion years ago with the gravitational collapse of a small part of a giant molecular cloud. Scientists have previously suggested that the explosion of a nearby dying star (supernova) could be strong enough to have triggered the collapse of a cloud of gas and dust, but there was no conclusive evidence to support this theory. Now they seem to have found one. A research team, led by Projjwal Banerjee of School of Physics and Astronomy, University of Minnesota, USA used computer modelling and evidence from meteorites to show that a low-mass supernova – a star exploding at the end of its life-cycle – would have generated the energy needed to compress a gas cloud and trigger the gravitational collapse that ultimately led to the formation of the solar system (*Nature Communications*, 22 November 2016 | doi:10.1038/ncomms13639). The gravitational collapse formed the proto-Sun



Supercomputer model of a low-mass supernova. (Credit: Bernhard Mueller, MNRAS 453, 287-310 (2015))

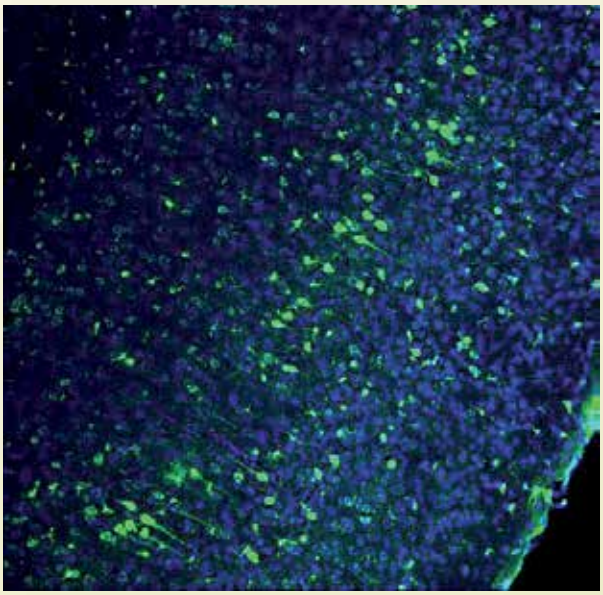
with a surrounding disc where the planets were born.

The research team focussed their study on short-lived nuclei like Beryllium-10 found in meteorites, which are considered as debris from the formation of the solar system. As debris from the formation of the solar system, meteorites are comparable to the leftover bricks and mortar in a construction site. They tell us what the solar system is made of and in particular, what short-lived nuclei the triggering supernova provided.

It must be stated here that previous efforts at understanding the formation of the solar system were focussed on a high-mass supernova trigger, but the researchers say that a high-mass supernova trigger wouldn't have left evidence on meteorites the same way that low-mass supernova did and so would not provide any evidence. But a low-mass supernova, about 12 times heavier than our Sun, could explain the meteoritic record. According to the scientists, the mass of exploding stars affects the types of nuclei forged during supernovae. Low-mass supernovae yield different nuclei than medium- or high-mass supernovae. Beryllium-10 is a short-lived nucleus that has four protons (hence the fourth element in the periodic table) and six neutrons and thus weighs 10 mass units. This nucleus is formed in low-mass supernova and is widely distributed in meteorites. According to the scientists, "the presence of Beryllium-10 is the forensic evidence needed to explain how the solar system was formed and it points to a low-mass supernova as the trigger". In addition to explaining the abundance of Beryllium-10, the low-mass supernova model, they say, would also explain the presence of the short-lived nuclei Calcium-41, Palladium-107, and a few others found in meteorites. According to the researchers, Beryllium-10 can be readily synthesised in such supernovae by neutrino interactions.

Novel DNA-editing breakthrough restores vision in blind rats

Retinitis pigmentosa is a group of genetic disorders that affect the retina's ability to respond to light. This inherited disease causes a slow loss of vision, beginning with decreased night vision and loss of peripheral (side) vision, eventually leading to blindness. In persons with retinitis pigmentosa, cells in the retina called rods and cones die. With most forms of the disorder, rods – which are mainly responsible for our peripheral, and night vision and makes up the outer regions of the retina and are – degenerate first. With time, the cones are affected, resulting in loss of colour perception and reading vision, leading to total blindness. At present there is no cure for the disorder.



Part of the adult mouse brain. Cell nuclei are blue and genome-edited neurons are green. (Credit: Salk Institute)

But there is hope. Scientists from Salk Institute for Biological Studies, La Jolla, California, USA have recently used a unique gene-editing technology to restore partial vision to blind rats afflicted with retinitis pigmentosa, paving the way for revolutionary new treatments that alter DNA in damaged parts of the body. It is the first time scientists have precisely modified DNA in non-dividing cells of the sort that make up most adult organs and tissues (*Nature*, 16 November 2016 | DOI: 10.1038/nature20565).

Until now, techniques that modify DNA – such as the CRISPR-Cas9 system – have been most effective in dividing cells, such as those in skin or the gut because they used the cells' normal copying mechanisms. But the new Salk technology presents the first time scientists have managed to insert a new gene into a precise DNA location in adult cells that no longer divide. Prime examples would be the cells of the eye, brain, pancreas, or heart, opening up new possibilities for therapeutic applications in these cells. According to the Salk scientists the new technology is "ten times more efficient than other methods at incorporating new DNA into cultures of dividing cells", making it a promising tool for both research and therapy.

The Salk researchers achieve this breakthrough by targeting a DNA-repair cellular pathway called NHEJ or "non-homologous end-joining". This pathway repairs routine DNA breaks by re-joining the original strand ends. The researchers paired this process with existing gene-editing technology to successfully place new DNA into a precise location in non-dividing cells. They worked on optimising the NHEJ machinery for use with the CRISPR-Cas9 system to insert DNA at very precise locations within the genome and then created a custom insertion package made up of a nucleic acid cocktail, which they call HITI, or "homology-independent targeted integration". Then they used

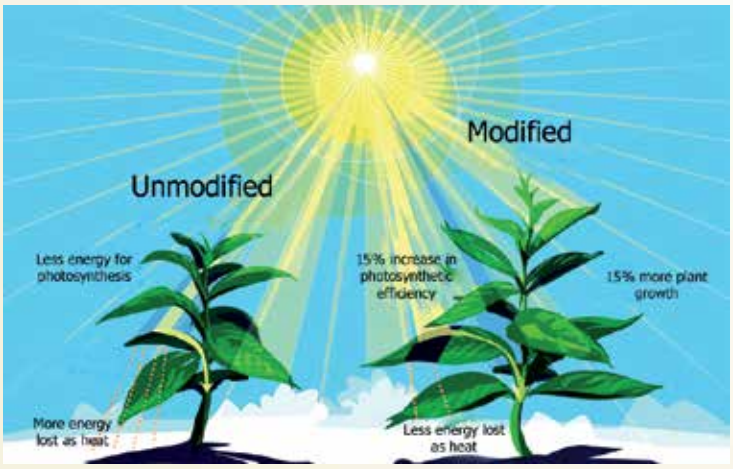
an inert virus to deliver HITI's package of genetic instructions to neurons derived from human embryonic stem cells.

To explore the possibility of using HITI for gene-replacement therapy, the team tested the technique on a rat model for retinitis pigmentosa. This time, the team used HITI to deliver to the eyes of 3-week-old rats a functional copy of Mertk, one of the genes that is damaged in retinitis pigmentosa. Analysis performed when the rats were 8 weeks old showed that the animals were able to respond to light and passed several tests indicating healing in their retinal cells. According to the researchers the new technique will open new avenues for basic research and a variety of treatments, such as for retinal, heart and neurological diseases.

Genetic modification can boost photosynthesis and increase production

Photosynthesis is a well-known biochemical process that plants use to convert carbon dioxide and water in presence of sunlight catalysed by chlorophyll into biomass, which is used for food, fuel and fibre. It is the key to global food production. But, too much sunlight is bad for plants as it can damage the chloroplasts. To prevent that from happening plants have a built-in mechanism to protect them from excessive exposure to sunlight. Inside chloroplasts, they have a system called NPQ, or non-photochemical quenching, which comes into play when there is more sunlight than the plants can safely use and allows the excess energy to be dissipated as heat. Using this mechanism crop leaves in full sunlight dissipate damaging excess absorbed light energy as heat. However, while plants switch on the quenching mechanism almost instantaneously (similar to the way in which the pupil in the human eye contracts in bright light), it takes much longer – up to half an hour – for it to switch off again and use the available light efficiently thus reducing photosynthesis. Calculations have shown that this delay could reduce production of field crops by up to 30 per cent of their potential yield.

To remedy the situation a team of plant biologists at the U.S. Department of Energy's Lawrence Berkeley National Laboratory, the University of California, Berkeley, and the University of Illinois resorted to genetic modification of three genes involved in NPQ to increase their expression and thus speed up recovery from photoprotection. By boosting the expression of three genes involved in NPQ, they showed that NPQ turned off more quickly, and the efficiency of photosynthesis in the shade was higher. By increasing the expression of the specific genes, increase of 14-20 per cent in the productivity of genetically modified tobacco plants in field experiments was observed. The researchers grew seedlings from multiple experiments and then tested how quickly the engineered plants responded to changes in available light. Two of the modified plant lines consistently showed 20 percent higher productivity, and the third was 14 percent higher than the unaltered tobacco plants. (*Science*, 18 November 2016 | DOI: 10.1126/science.aai8878). According to the researchers, half of crop photosynthesis occurs in the shade, so any improvement in speeding up recovery from photoprotection could have a big benefit. They say this is a critical step towards increasing crop production to feed a growing global population.



As computer models predicted, genetically modified plants are better able to make use of the limited sunlight available when their leaves go into the shade. (Credit: Julie McMahon)

Genetic modification techniques have been used in the past to produce crops that are pest-resistant, disease-resistant or less sensitive to herbicides, but this was one of the first demonstrations of a crop's basic efficiency being improved through genetic modification. According to Krishna K. Niyogi, a faculty scientist in Berkeley Lab's Division of Molecular Biophysics and Integrative Bioimaging, who was involved in the study, "Tobacco was used as the model crop plant in this study because it is easy to work with, but we're working to make the same modifications in rice and other food crops. The molecular processes we're modifying are fundamental to plants that carry out photosynthesis, so we hope to see a similar increase in yield in other crops".

This research was supported by the Bill and Melinda Gates Foundation. Any new technology licensed from this work will be made freely available to farmers in poor countries in Africa and South Asia.

Biman Basu is a former editor of the popular science monthly Science Reporter, published by CSIR, He is a winner of the 1994 'NCSTC National Award for Science Popularisation'. He is the author of more than 45 popular science books.