MSc2 Biotechnology

Stemgenering

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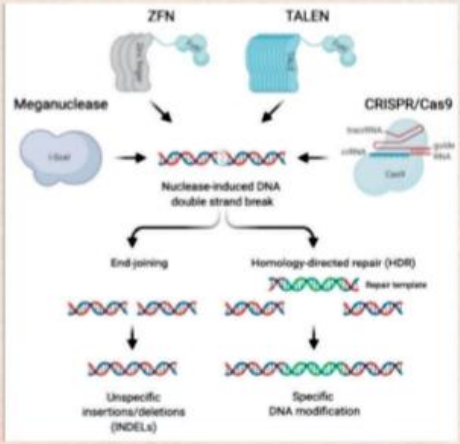
HISTORY

The first genome editing technologies were developed in the late 1900s. More recently, a new genome editing tool called CRISPR, invented in 2009, has made it easier than ever to edit DNA. CRISPR is simpler, faster, cheaper, and more accurate than older genome editing methods.

- Genetic engineering started with transgenesis, the deliberate transfer of genetic information from one organism to another. In 1974, Rudolf Jaenisch demonstrated that viral DNA injected into blastocysts led to stable integration in the genome of mice. This is considered to be the first transgenic animal generated.
- Genome editing with engineered nucleases, i.e., all three major classes of these enzymes–zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and engineered meganucleases–were selected by Nature Methods as the 2011 Method of the Year. The CRISPR-Cas system was selected by science as 2015 Breakthrough of the Year.
- As of 2015 four families of engineered nucleases were used: meganucleases, zinc finger nucleases (ZFNs), transcription activator-like effector-based nucleases (TALEN), and the clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) system. Nine genome editors were available as of 2017.
- Zinc fingers are small protein motifs with sequence specific DNA binding capacity. They were first discovered in 1985 as part of a transcription factor in frog oocytes but are in fact present in many species, including humans.

- The latter study introduced the term 'genome editing' in 2005 for the first time as an analogy to word processing.
- Another major discovery in the field of designer nucleases arose with the construction of transcription activator-like effector nucleases (TALENs) in 2010.



In 2007, the Nobel Prize for Physiology or Medicine was awarded to Mario Capecchi, Martin Evans and Oliver Smithies "for their discoveries of principles for introducing specific gene modifications in mice by the use of embryonic stem cells."

In 2020, the Nobel Prize in Chemistry was awarded to Emmanuelle Charpentier and Jennifer Doudna for "for the development of a method for genome editing"

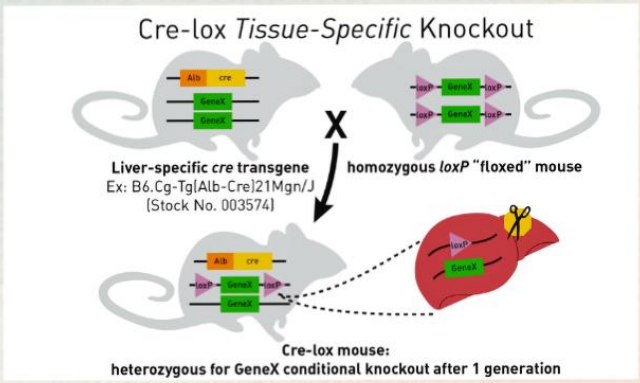
-Vanshika lokhande

THE CRE–LOXP SYSTEM:

A Versatile Tool for Targeting Genes in a Cell- and Stage-Specific Manner.

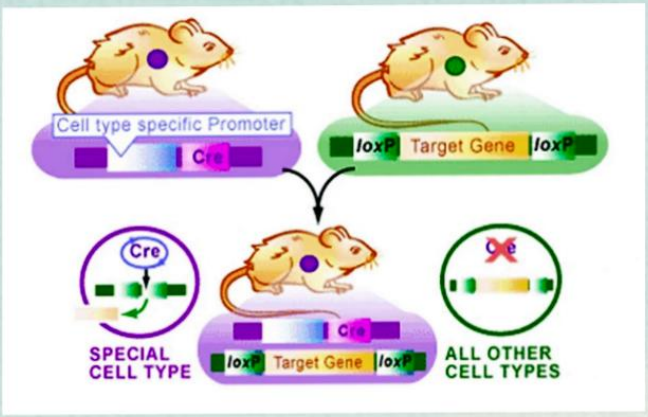
One cannot step into the same river twice, one cannot find anything fixed in stem cell research, where one will find, instead, that revelations and still other revelations are ever flowing. Accordingly, much has changed since the original revelation, the almost four-decade-old discovery of embryonic stem cells in mice. Subsequent studies led to the isolation of human embryonic stem cells, which were then grown in the laboratory, as well as the development of reprogramming techniques and the generation of induced pluripotent stem cells (iPSCs). More recently, advances in biotechnology and genome editing have fostered a modern evolution of stem cell advances that shows no signs of slowing.

Transgenic mice usually carry a transgene or gene modification (knockout gene) in all of their cells. However, it is helpful to have a process that selectively regulates the expression of a gene within a specific somatic tissue or cell type. The Cre–loxP recombination system, which is derived from genetic elements of bacteriophage P1, has been adapted for this purpose.



On the basis of these features, the Cre–loxP system was developed for producing cell-specific gene modifications in mouse cells. As a first step in the overall strategy, the cre gene is isolated and placed under the control of a cell-specific promoter. Transgenic mice with the cre gene construct are established, and the tissue specificity of the Cre activity is confirmed. Next, a loxP site with repeat sequences in the same direction is inserted on either side of a cloned DNA sequence, such as a cloned exon. The construct is integrated into a chromosome site of embryonic stem cells by homologous recombination.

Stem cell-derived synthetic embryos are mostly used for basic studies at this stage. But, in the future, if human synthetic embryos can be generated, Wu explains, they may be used for modeling disease and advancing preimplantation and implantation. Wu sums up the role of this work in advancing the stem cell field by quoting the Nobel-prize winning physicist Richard Feynman, PhD: "What I cannot create, I do not understand."



The Cre recombinase-loxP (Cre–loxP) system was developed to overcome this limitation as well as to confine the inactivation of the target gene in a cell- or tissue-specific manner. This system allows for the inactivation of the target gene in a single cell type, thereby allowing the analysis of physiological and pathophysiological consequences of the genetic alteration in mature animals.

A loxP site consists of two 13-base-pair (bp) inverted repeats that are separated from each other by an 8-bp spacer sequence. Briefly, Cre–loxP recombination entails the coming together of two remote loxP sites, each of which has two bound Cre recombinase molecules; cleavage by the Cre recombinase within the spacer regions between the repeat sequences; and the exchange and joining of DNA strands to form recombined DNA molecules. The outcome of the recombination event depends on the orientation of the repeats of the loxP sites.

These cells are selected, cultured, and used to establish a transgenic mouse line. Then, a transgenic mouse with the tissue-specific cre transgene is mated with a transgenic mouse with the integrated loxP-flanked sequence. The DNA between the two loxP sites is deleted after the cre transgene is expressed in double-transgenic organisms.In this way, the biological consequences of the loss of activity of a gene in a specific tissue can be monitored. The Cre–loxP recombination system can also be used to activate a transgene in a specific tissue. In this case, the sequence between the loxP sites prevents transcription. This construct is inserted between the promoter and the coding sequence of a transgene. When Cre is expressed in mice with the integrated construct, the DNA sequence that blocks transcription is excised, thereby enabling the expression of the transgene. By controlling expression of Cre, for example, by adding an inducer that activates the cre promoter to the drinking water of mice, the expression of a transgene can be controlled.This result mimics aspects of inherited retinitis pigmentosa in humans and is used for detailed studies of the pathophysiological effects on the retina. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3818143/

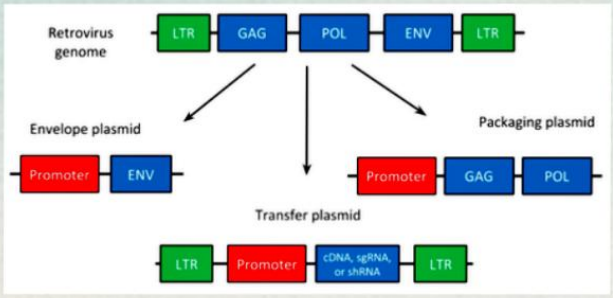
_Tejaswini.Khiratkar

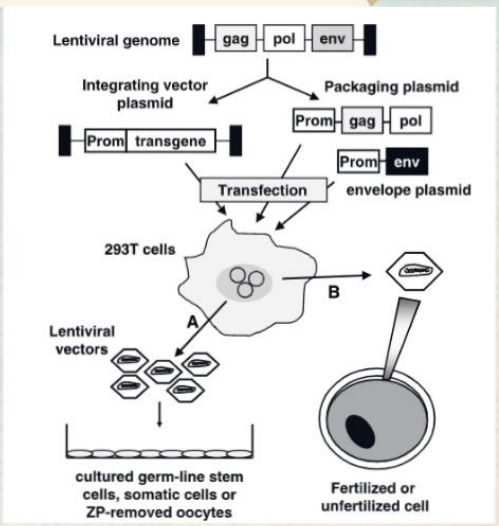
The Retroviral Vector Method

- **Vaishnavi Mitra (21169)**

Traditionally, the retrovirus is regarded as an enemy to be overcome. However, for the past two decades retroviruses have been harnessed as vehicles for transferring genes into eukaryotic cells, a process known as transduction. During this time, the technology has moved from being a scientific laboratory tool to a potential clinical molecular medicine to be used in gene therapy.

- **Vector technology** - A retroviral vector consists of proviral sequences that can accommodate the gene of interest, to allow incorporation of both into the target cells. The vector also contains viral and cellular gene promoters, such as the CMV promoter, to enhance expression of the gene of interest in the target cells. The most important advance in vector technology has been use of the packaging cell

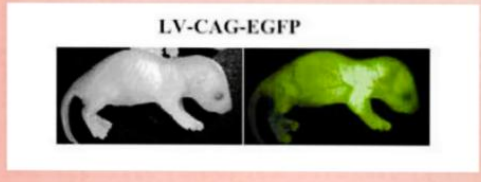


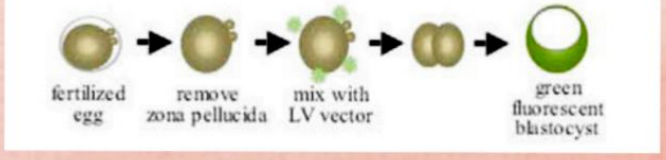


Of the various gene transfer methods, the use of retroviral vectors, has the advantage of being an effective means of integrating the transgene into the genome of a recipient cell. Retroviruses have RNA genomes that are used as templates for reverse transcriptase to synthesize a DNA copy that can be inserted into the host cell genome (see chapter 11). However, vectors derived from these viruses can transfer only small pieces (~8 kilobases [kb]) of DNA that, because of the size constraint, may lack essential adjacent sequences for regulating the expression of the transgene.

Generation of Transgenic Mice Using Lentiviral and Oncoretroviral Vectors

generation ofansgenic mice using viral vectors carrying EGFP under the control of the CAG and CMV promoters. After removal of the zona pellucida, we incubated fertilized eggs with viral vectors differentiated to blastocysts and implanted them into the uterus of pseudo-pregnant females.
a total of 60% (30/50) and 73% (22/30) of founder pups developed from embryos treated with oncoretroviral or lentiviral vector, respectively, were transgenic as determined by PCR analysis.When we placed founder pups under a fluorescence microscope, we observed EGFP expression only in lentiviral-mediated transgenic pups and not in the oncoretroviral-mediated transgenic pups





THURSDAY, 24/11/2022

NEWSLETTER

CREATIVE-BIOGENE

_Tejaswini.k

'WHAT NOW FOR HUMAN GENOME EDITING?'

CLAIMED CREATION OF CRISPR-EDITED BABIES TRIGGERS CALLS FOR INTERNATIONAL OVERSIGHT.

Few seemed more surprised by the tide of outrage unleashed by the claim that the first gene-edited babies had been created with the revolutionary lab tool called CRISPR than He Jiankui, the scientist responsible. On the eve of the International Summit on Human Genome Editing in Hong Kong, China, last week, He, a researcher at nearby Southern University of Science and Technology in Shenzhen, China, had dinner at the city's Le Méridien Cyberport with a few of the meeting's organizers. The news of He's claim had just broken, and shock waves were starting to reverberate. But the reports were still so fresh that the diners sat in the restaurant without being disturbed.

It seems unlikely history will view He in the same light as Edwards, who won a Nobel Prize, but the Chinese researcher certainly is a disrupter. His claim has triggered widespread calls for mechanisms to prevent others from germline editing humans until there's an international consensus that the CRISPR technology has matured and there's a convincing medical need. And it has sparked concern that his actions could set back less problematic applications of gene editing: treating diseases by editing nongerm cells, which do not pass their DNA to future generations.

"He arrived almost defiant," says Jennifer Doudna, who did landmark **CRISPR** work at the University of California (UC), Berkeley. She and the other conference organizers politely asked He questions about the scientific details and rationale of his work, the permissions he had secured to conduct it, and how he recruited hopeful parents to participate and informed them about risks. He asked them whether his planned talk 2 days later should include data about the twin girls, who had a gene altered to make them resistant to HIV infection. "We were all like, 'Uh, yes,'" Doudna says.

Another human genome editing summit is planned for 2021. It will be hard to surpass Hong Kong's drama. "One of our concerns was this was going to be a really boring summit," Charo says. "**Everything you look at here, the closer you get, the stranger this whole story becomes.**"

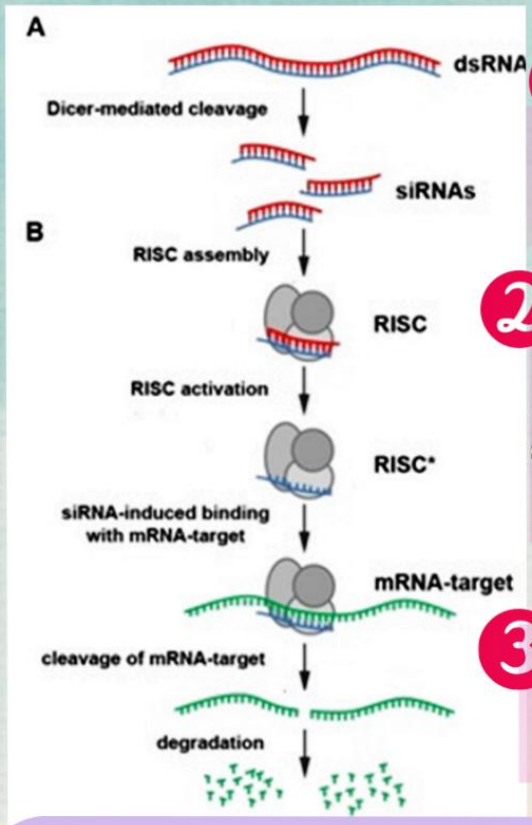
After more than an hour of questioning, He had had enough. "He just seemed surprised that people were reacting negatively about this," Doudna says. "By the end of the dinner he was pretty upset and left quite abruptly."

Reference:https://www.science.org/doi/10.1126/science.362.6419.1090

RNA Interference (RNAi)

Tanmayi Bhosale (21167)

RNAi is a method to silence gene expression in specific cells to study biological processes. This technique specifically brings the decrease in the expression of a target gene by preventing translation of messenger RNA (mRNA). It was 1st discovered in Caenorhabditis elegans. The mechanism of RNAi essentially involves double-stranded RNA (dsRNA)-mediated degradation of target mRNA.

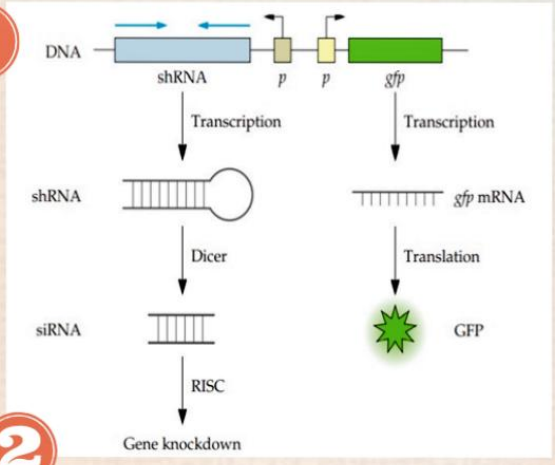


1. In RNAi, double-stranded RNA is recognized by a ribonuclease (RNase) called Dicer that cleaves the RNA into smaller double-stranded RNA molecules known as small interfering RNA (siRNA).

2. A large nuclease complex called RISC (RNA-induced silencing complex) separates the strands of siRNA, and the single-stranded RNA products, together with RISC, bind to homologous sequences on mRNA molecules. The nuclease component of RISC then degrades the mRNA, which prevents the encoded protein from being synthesized.

3. Short endogenous RNAs (micro-RNAs) transcribed from regions of the genomes of animals (and plants) are also recognized by Dicer and RISC and block translation of a target mRNA.

1. A transgenic construct encoding an shRNA to target specific mRNA for degradation and a green fluorescent protein marker (gfp), each under the control of its own promoter (p) (the direction of transcription is indicated by black arrows), is introduced into mouse embryonic stem cells by pronuclear injection or on a lentiviral vector.



2. Transgenic mice are identified by production of green fluorescent protein (GFP). Transcription of the transgene encoding short inverted repeats (blue arrows) separated by a spacer sequence yields an shRNA that is processed by the host cell Dicer and RISC proteins to reduce expression of a target gene.

FUTURE

The discovery of RNAi has had an enormous impact on experimental biology and in particular functional genomics. To date, siRNAs and shRNAs have been used extensively to investigate gene function in mammalian cell cultures and have been applied successfully in vivo. Furthermore, extensive siRNA and shRNA libraries for genome-wide screening have been produced. These types of high-throughput screens will have a very important role in decoding the enormous amount of sequence information that is available, especially for the mouse and human genomes. In addition to assigning biological functions to different genes using cell culture systems, it is anticipated that the RNAi pathway will ultimately be utilized to perform more complex genetic experiments in vivo.

The Ethics of using Transgenic Animals

- **Tanmayi Bhosale and Vaishnavi Mitra**

In recent years, new genome editing technologies have emerged that can edit the genome of non-human animals with progressively increasing efficiency.These animals carry and express genetic information not normally found in that species or organism. The purpose of much transgenic animal research involves protection from heritable disease or the improvement of domestic livestock productivity, applicable in biomedical research and for the resolution of health problems. However, the research and use of these animals remains controversial. Ethical and moral issues, animal welfare issues, patients´ interests and equity as well as economic, commercial and regulatory issues all need to be discussed if these new emerging technologies are to be used in ways which are socially acceptable.

 According to Personal beliefs about respect and consideration owed to other life forms, Transgenesis is seen to constitute a lack of respect for the natural dignity that an animal may warrant.
 In terms of a religious belief, transgenesis may be perceived by some as involving the creation of life forms by means not found in Nature.
 Species are commonly defined in terms of breeding barriers, and transgenesis is seen to breach those barriers.
 Genetically modified animals have been intentionally designed to suit human purposes using modern technology.

WHY TRANSGENIC

- Research on cloning in laboratory and farm animals is likely to add to our understanding of biological processes, may contribute to human well-being.
- In the field of medicine and medical research, (Gene therapy, physiological and disease related study)
- Cloning of adult animals of high performance, would reduce the number of transgenic animals needed and would allow human pharmaceuticals e.g. to be produced at a lower cost than would be possible otherwise.
- It is ethically only acceptable if carried out with strict regard to animal welfare, under the supervision of licensing bodies, etc.

ETHICAL ISSUES

Researches are only acceptable only when the aims and methods are ethically justified and when it is carried out under ethical conditions that include :

& The duty to avoid or minimize animal suffering since unjustified or disproportionate suffering is unacceptable.
& The duty of reducing, replacing and when possible refining (3Rs) the experimentation adopted for the use of animals in research
& The lack of better alternatives
& Human responsibility for animals, nature and the environment.
& International movement of cloned and genetically modified animals should be monitored to prevent smuggling, maintain consumer choice, and protect the environment

ISSUES OF CONCERN

1. One area of concern is the moral status of animals, animal rights and animal welfare.
2. Another area of concern is the boundary constructed between what is considered ‘natural’ and ‘unnatural’.
3. Where transgenic animals are concerned, it remains important to ‘expect the unexpected’. Even with the best information, and the best of intentions, it is not possible to predict with certainty how they will impact upon the experimental animal.