Genome Editing and Engineering
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Module - 04
Targeted genetic modification
Lecture - 8
Targeted genetic modification-I

Welcome to module 4 of my course Genome Editing and Engineering. In this lecture, we are going to discuss about Targeted Genetic Modification.

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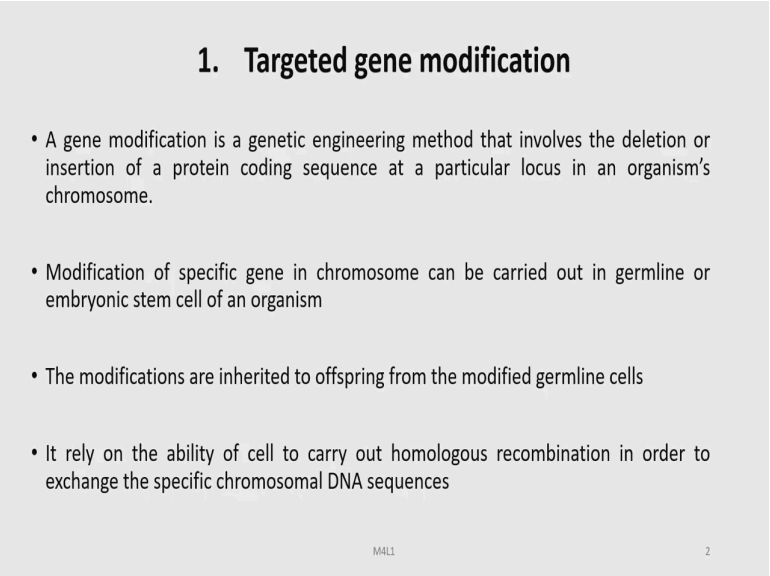

First let us learn what is targeted gene modification. A gene modification is a genetic engineering method that involves the deletion or insertion of a protein coding sequence at a particular locus in an organism. However, sometimes we may also include here a antisense RNA and which not necessarily may code for a protein. Modification of specific gene in chromosome can be carried out in germ line or embryonic stem cell of an organism.

The modifications are inherited to offspring from the modified germline cells and these modifications rely on the ability of cell to carry out homologous recombination in order to exchange the specific chromosomal DNA sequences. We have learned about the homologous recombination in our last lectures.

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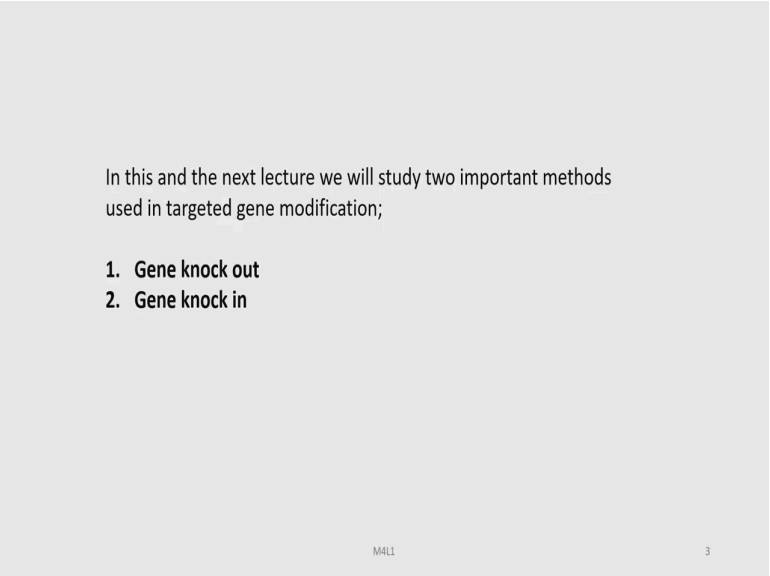

In these and the next lecture we will study the two important methods used in targeted genetic modification. The first one is the gene knockout and the second one is the gene knock in.

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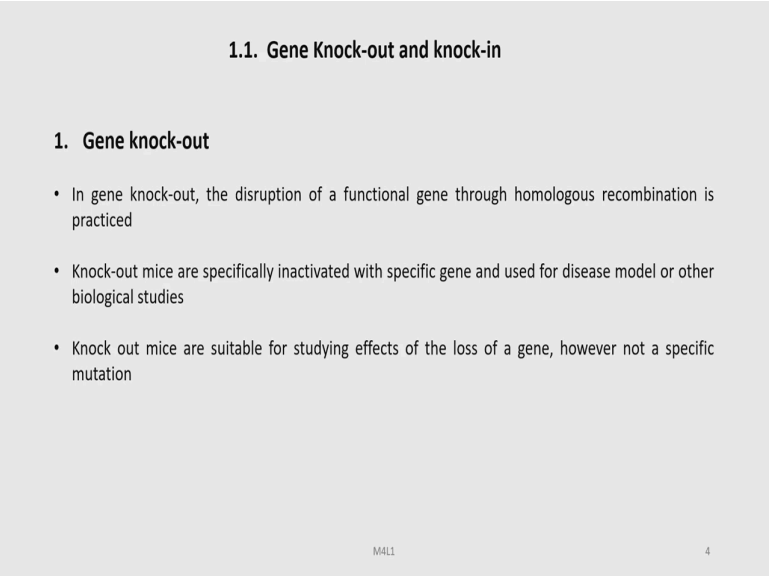

What is gene knockout and gene knock in? In gene knockout, there is a disruption of a functional gene through homologous recombination. Knockout mice are specifically inactivated with specific gene and used for disease model or other biological studies.

Knockout mice are suitable for studying effects of the loss of a gene; however, not a specific mutation.

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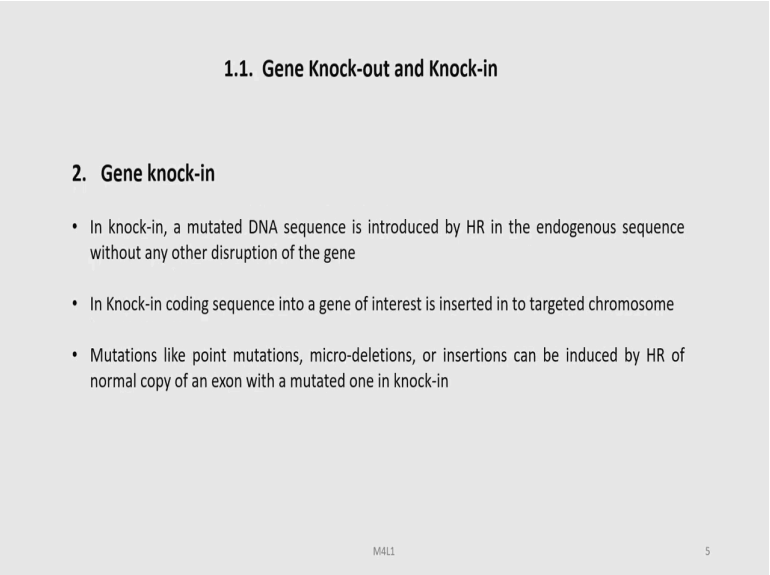

On the other hand in gene knock-in, a mutated DNA sequence or a foreign DNA sequence is introduced by homologous recombination in the endogenous sequence without any other disruption of the gene. In knock-in coding sequence into a gene of interest is inserted into targeted chromosomes. Mutations like point mutations, micro deletions or insertions can be introduced by homologous recombination of normal copy of an exon with a mutated one in the knock-in procedure.

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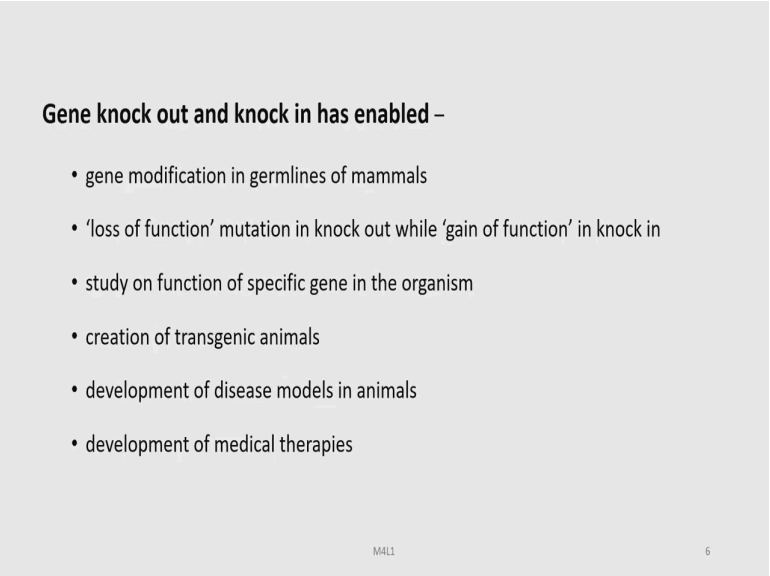

These are very very enabling technologies and due to gene knockout and knock-in, we can do genetic modification in germ lines of mammals, loss of function mutation in knockout and gain of function in knock-in, study on function of specific chain in the organism, creation of transgenic animals, development of disease models in animals and development of medical therapies. In this course we will learn some of these applications in the later lectures.

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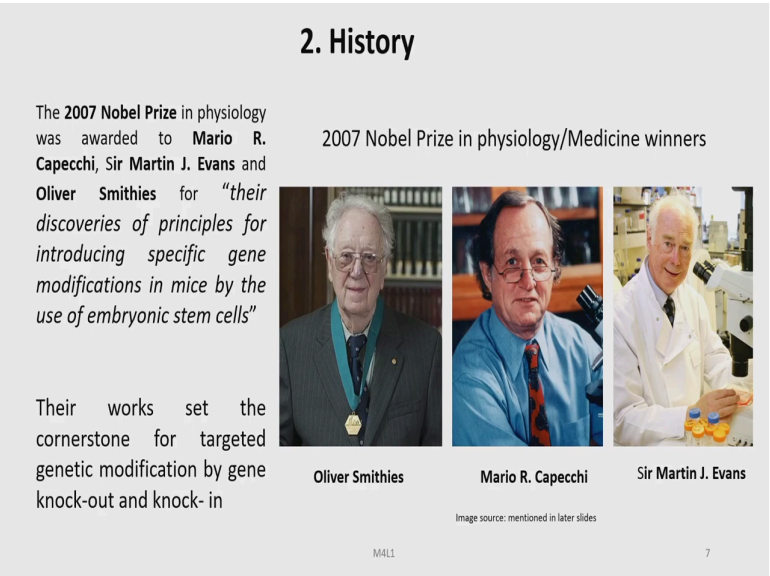

Let us look into the development of the various concepts regarding targeted gene modification. In 2007, the Nobel Prize in physiology was awarded to Mario Capecchi, Martin

J Evans and Oliver Smithies for "their discovery of principles for introducing specific gene modification in mice by the use of embryonic stem cells." They were recognized for their work which set the cornerstone for targeted genetic modification by gene knock-out and gene knock-in.

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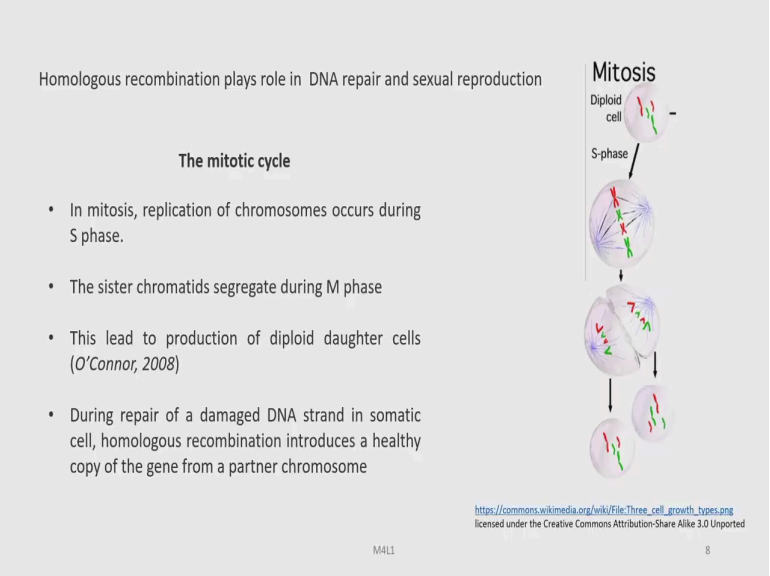

In this entire process, homologous recombination plays a role in DNA repair and sexual reproduction and let us first once again revisit the mitotic cycle which is known to many of you. In mitosis replication of the chromosomes occur during the S phase or the synthesis phase. The sister chromatids segregate during the M phase, this leads to production of diploid daughter cells. As a result, during a pair of a damage DNA strand in somatic cell homologous recombination introduces a healthy copy of the gene from a partner chromosome.

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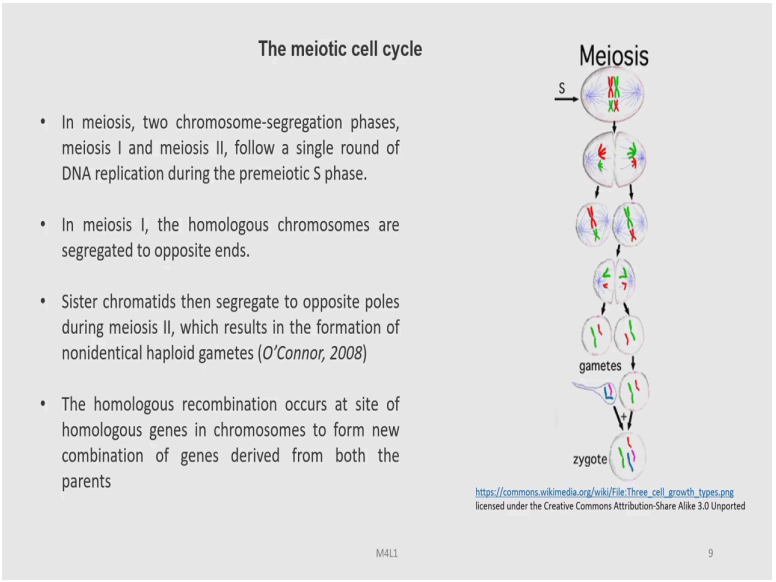

While in the meiotic cell cycle, two chromosome segregation phases are there. The meiosis 1 and meiosis 2 and they follow a single round of DNA replication during the premeiotic S phase.

In meiosis 1 the homologous chromosomes are segregated to opposite ends. Sister chromatids then segregate to opposite poles during meiosis 2, which results in the formation of non-identical haploid gametes. And the homologous recombination occurs at site of homologous genes in chromosomes to form new combination of genes derived by both the parents, from both the parents. The meiosis 1 and meiosis 2 can also be altered in a way.

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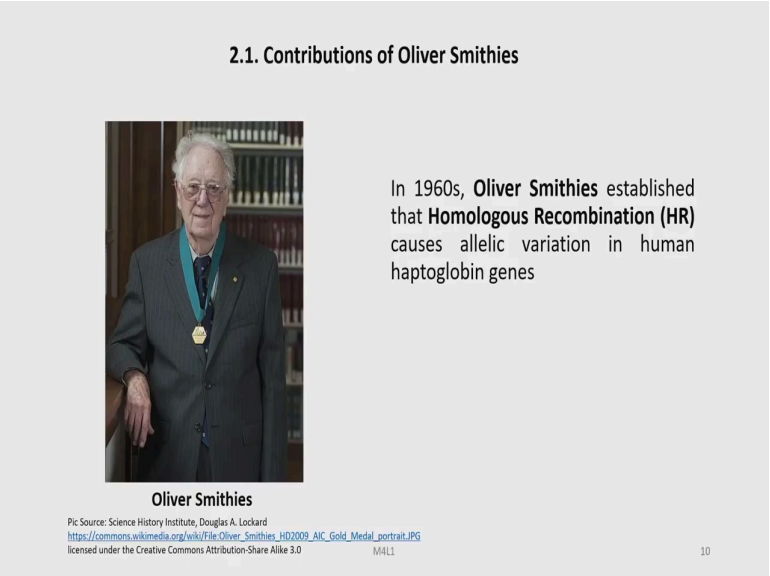

Now, let us look into the contribution of Oliver Smithies; Oliver Smithies in 1960s established that homologous recombination causes allelic variation in human haptoglobin genes.

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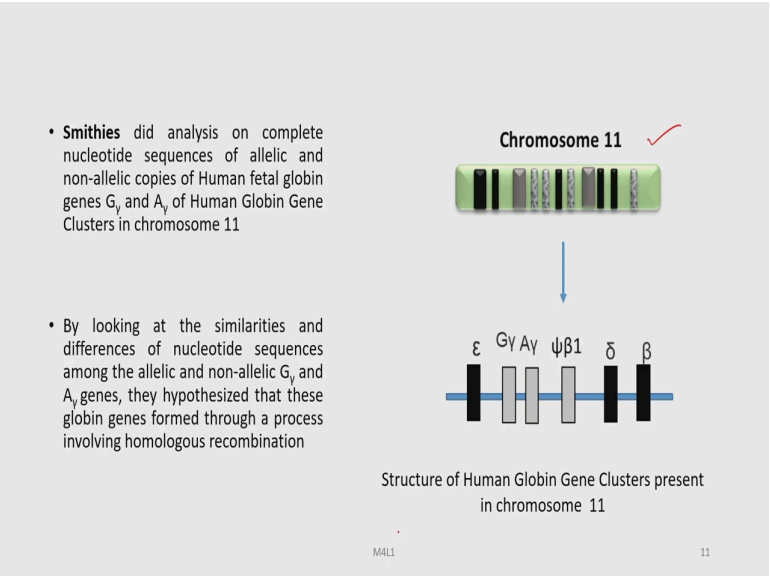

He analyzed complete nucleotide sequences of allelic and non-allelic copies of human fetal globin genes, G gamma and A gamma of human globin gene clusters in chromosome number 11. And by looking at the similarities and differences of nucleotide sequences among the allelic and non-allelic G gamma and A gamma genes, his team hypothesized that these globin

genes form through a process involving homologous recombination and you can see here the structure of human globin gene clusters in chromosome number 11.

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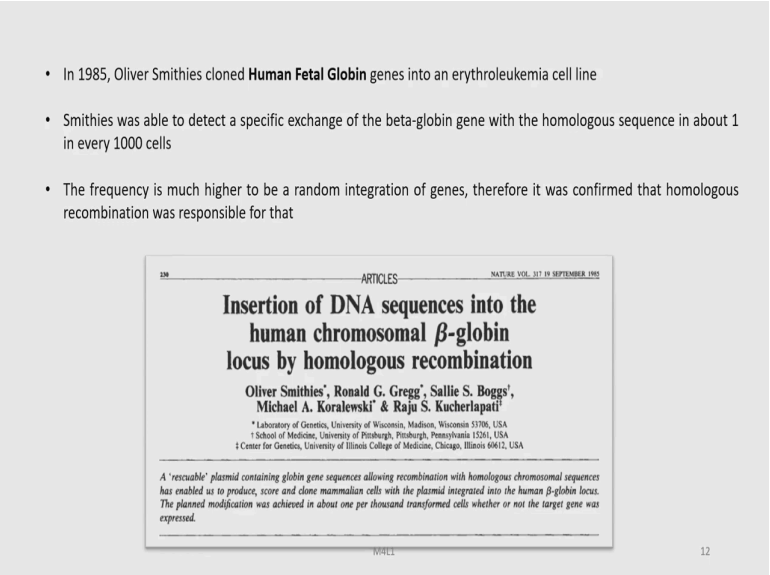

In 1985, Smithies cloned the Human Fetal Globin genes into one erythroleukemia cell line and was able to detect the specific exchange of the beta-globin gene with the homologous sequence in about 1 in every 1000 cells. The frequency is much higher to be a random integration of genes, therefore it was confirmed that homologous recombination of responsible for this phenomena.

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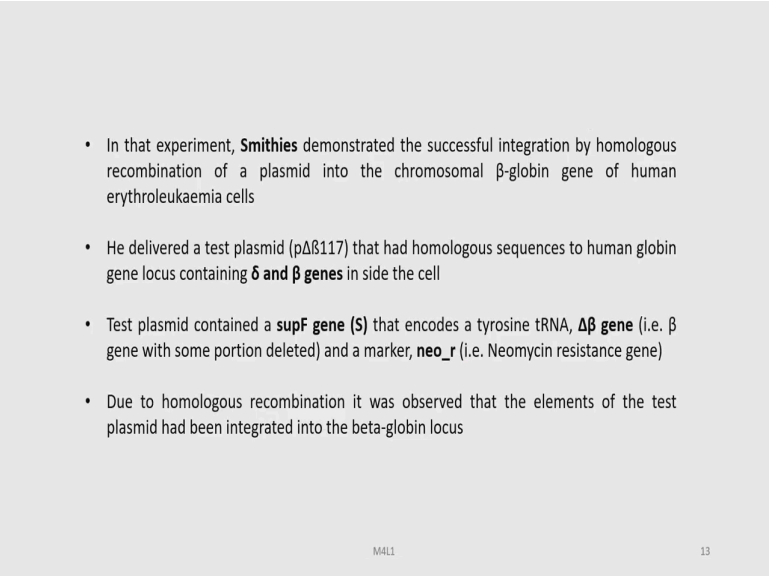

In this experiment, he demonstrated the successful integration by homologous recombination of a plasmid into the chromosomal beta globin gene of a human erythroleukemia cell. He delivered a test plasmid that had homologous sequences to human globin gene locus containing delta and beta genes inside the cell. Test plasmid contain a supF gene that encodes a tyrosine tRNA, delta beta gene which is a beta gene with some portion deleted and a marker neo_r which is neomycin resistance gene.

Due to homologous recombination, it was observed that the elements of the test plasmid was integrated into the beta globin locus.

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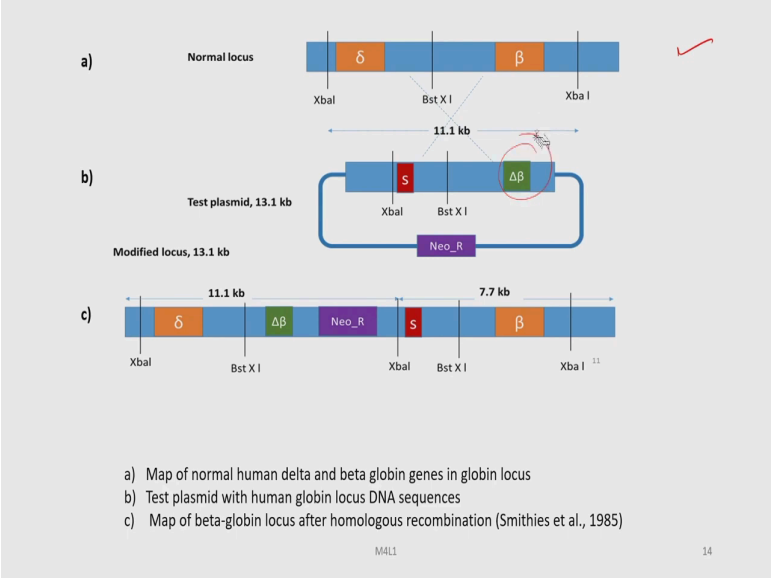

Then you can see here a, map of normal human delta and beta globin genes in the globin locus. And in b, you can see the test plasmid with human globin locus DNA sequences and you can see here the delta B which is having some kind of deletion and the supF gene over here. And here in number c, you can see the map of beta globin locus after homologous recombination.

So, all these elements has been transferred into the construct at c and this was due to homologous recombination.

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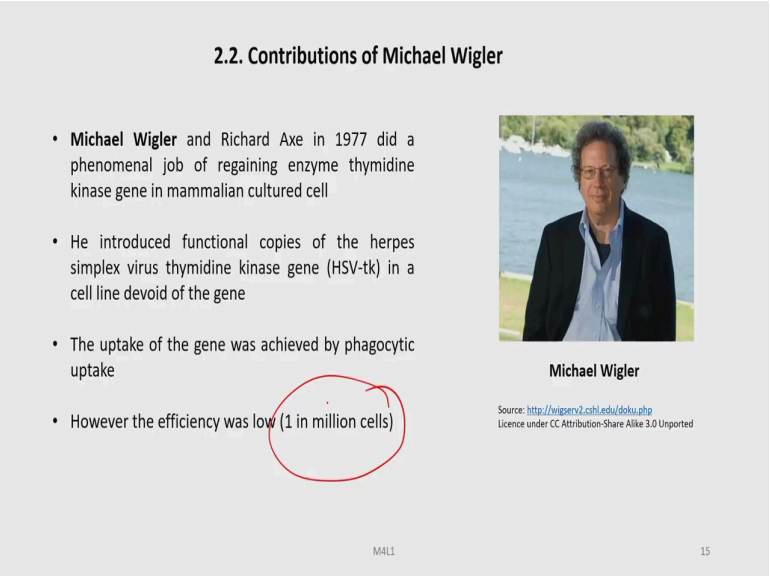

Another person who contributed in the development of this field is Michael Wigler and Richard Axe who in 1977 did a phenomenal job of regaining enzyme enzyme thymidine kinase gene in mammalian cultured cell. He introduced functional copies of the herpes simplex virus thymidine kinase gene or HSV-tk in a cell line devoid of the gene.

So, he made HSV-tk negative cell line, HSV-tk positive. The uptake of the gene was achieved by phagocytic uptake; however, the efficiency was low 1 in million cells.

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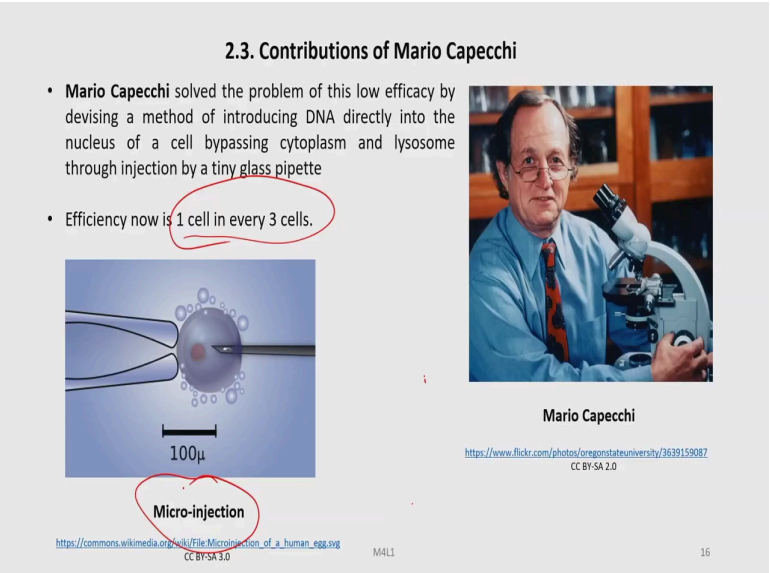

Also remarkable are the contribution of Mario Capecchi who solved the problem of this low efficacy by devising a method of introducing DNA directly into the nucleus of a cell, bypassing cytoplasm and lysosome through injection by a tiny glass pipette. You can see here this tiny glass pipette over here and this is a cell which is being held firmly with the help of suction and then this is the scale of this cell around 100 micron.

And by using these pointed glass pipette, Capecchi introduced DNA directly into the nucleus of the cell here. So, these efficiency immediately improved from 1 in million cells in the process developed by Wigler to one cell in every three cells, but this is being done with a special apparatus and human intervention and this process is known as the micro injection.

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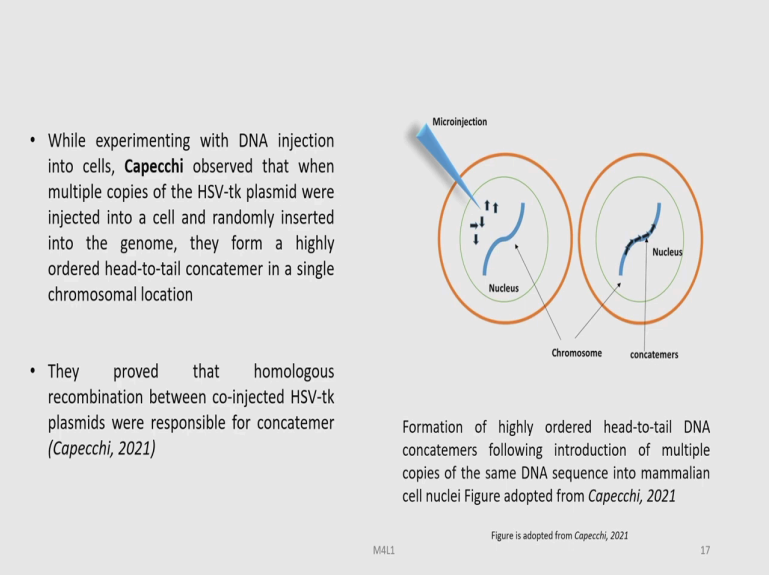

While experimenting with DNA injection into cells, Capecchi observed that when multiple copies of the HSV-tk plasmid were injected into a cell and randomly inserted into the genome, they form a highly ordered head-to-tail concatemer in the single chromosomal location.

So, this is the micro injection through which these plasmids were injected into the cell and this is directly inside the nucleus and this is a chromosome you can see over here. Now, all these HSV-tk plasmid you can see here are aligned in a very systematic way. And in fact, they are getting connected to one another and forming a concatemer.

From these they got the evidence that homologous recombination between co-injected HSV-tk plasmids were responsible for this concatemerization and here you can see formation of highly ordered head to tail DNA concatemers follow introduction of multiple copies of the same DNA sequence into mammalian cell nuclei as shown by Capecchi.

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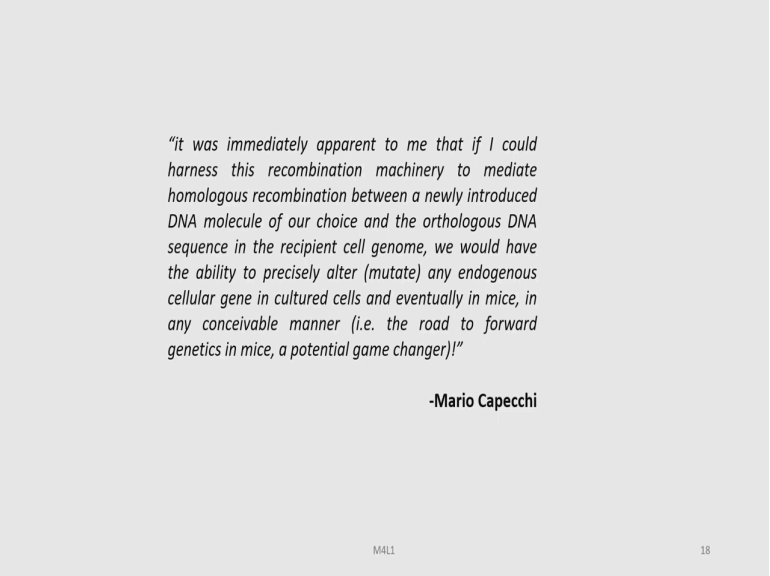

And with all these evidences, Capecchi came out with a view that "it was immediately apparent to me that, if I could harness this recombination machinery to mediate homologous recombination between a newly introduced DNA molecule of our choice and the orthologous DNA sequence in the recipient cell genome, we would have the ability to precisely alter or mutate any endogenous cellular gene in cultured cells and eventually in mice, in any conceivable manner. That is road to forward genetics in mice a potential game changer. "

So, this is very important discovery by Mario Capecchi and these are the words he spoke about the potential of these technology.

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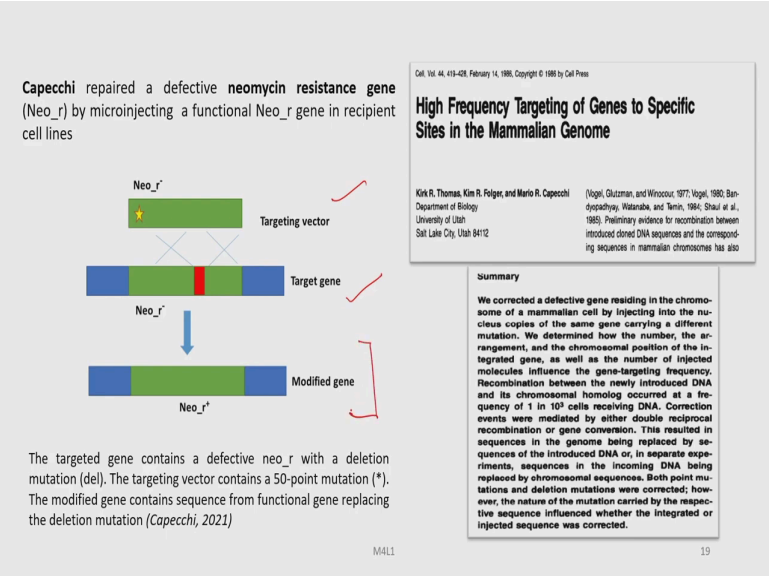

Capecchi then went on to repair a defective neomycin resistance gene by micro injecting a functional neomycin r gene in recipient cell lines. So, as in his earlier statement this technology has lot of potential and one of the potential that he tried was to repair a defective gene neomycin resistance gene.

And this is the publication which came out in 1986 in cell where he described about the high frequency targeting of genes to specific sites in mammalian cells. And he summarized that he corrected a defective gene residing in the chromosome of a mammalian cell, by injecting into the nucleus copies of the chain same gene carrying a different mutation.

So, in brief, here you can see a targeting vector and you can see here a target gene and this is the outcome, the modified gene. So, let us see briefly what happen the targeted gene contains a defective neo_r neo mycin resistance with a deletion mutation, del. The targeting vector contains a 50 point mutation shown with this star here. The modified gene will contain sequences from the functional gene replacing deletion mutation.

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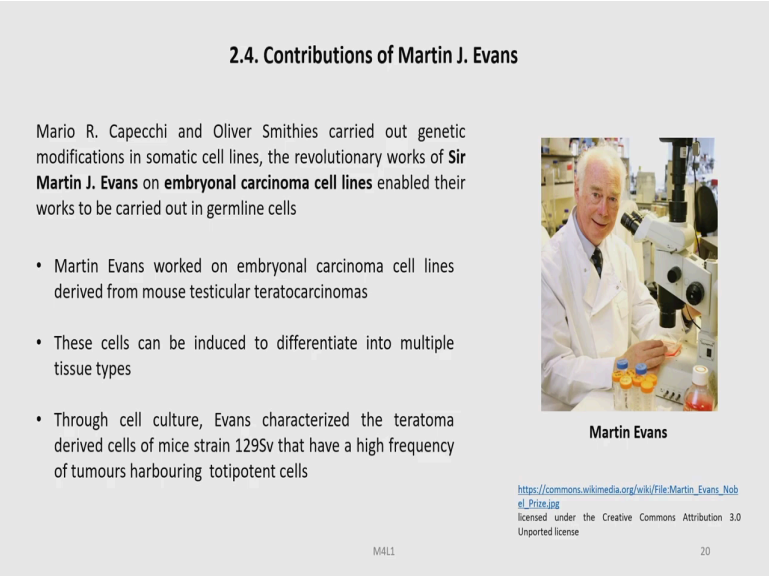

Another person who contributed in the development of these technology is Martin J Evans. Mario Capecchi and Oliver Smithies carried out genetic modifications in somatic cell lines. The revolutionary work of Martin J Evans was on embryonic carcinoma cell lines which enable their work to be carried out in stem line cells.

Martin Evans worked on embryonic carcinoma cell lines derived from mouse testicular teratocarcinomas. These cells can be induced to differentiate into multiple tissue types. Through cell culture Evans characterized the teratoma derived cells of mice strain 129 Sv that have a high frequency of tumours harbouring the totipotent cells.

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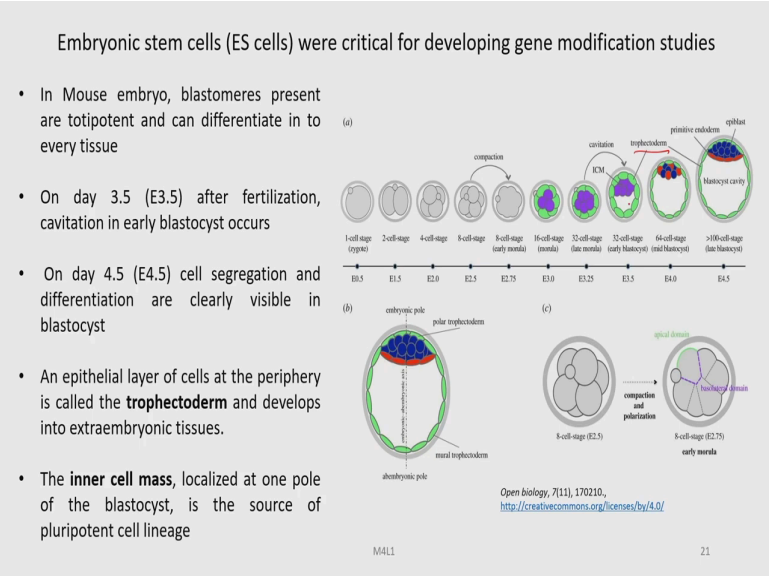

So, let us see here the cell division, you can see here one cell stage zygote which divides and become a two cell stage zygote and a four stage cell zygote and so on and so forth. And then you will see after a 8 cell stage you have the early morula stage, then you have the morula stage. Then at 32 cell stage, you have the late morula followed by the transformation into early blastocyte, then mid blastocyte and finally, late blastocyte with over…which is over 100 cell stage.

So here, other important things we need to remember is that trophectoderm and also there is a primitive endoderm and you have here the epiblast, ok? So, let us now discuss one by one exactly what happens in such a case. In a mouse embryo, blastomeres present are totipotent and it can differentiate into many kinds of tissues, almost every kind of tissue. On day 3.5 after fertilization, cavitation in early blastocyte will occur. Then on day 4.5, cell segregation and differentiation are clearly visible in the blastocyte here.

An epithelial layer of cells at the periphery is called as the trophectoderm and develops into extra embryonic tissue. The inner cell mass is localized at one pole of the blastocyte and this is the source of pluripotent cell lineage.

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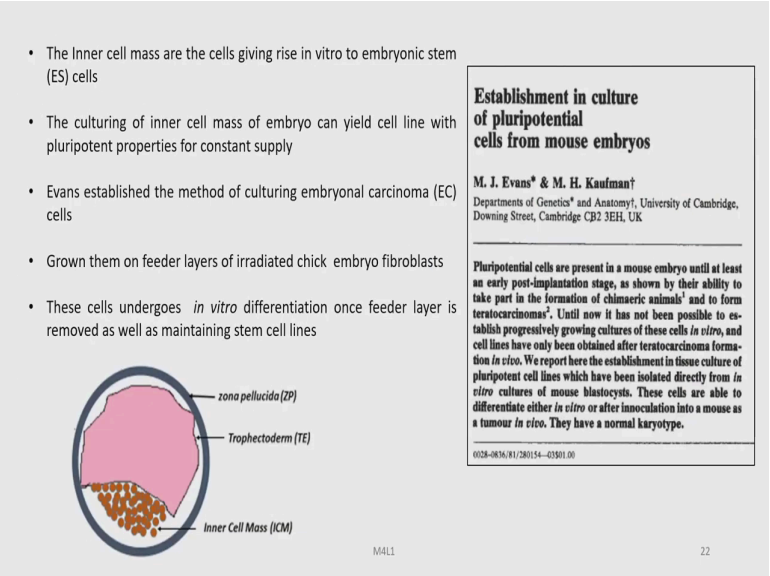

These inner cell mass are the cells which give rise to in vitro embryonic stem cells. The culturing of inner cell mass of embryo can yield cell line with pluripotent properties for constant supply.

Evans established the method of culturing embryonal carcinoma cells and he grow them on feeder layers of irradiated chick embryo fibroblast. These cells undergo in vitro differentiation once feeder layer is removed as well as maintaining the stem cell lines. And this work he published here by this title establishment in culture of pluripotent cells from mouse embryo and this is one of the remarkable work done by him.

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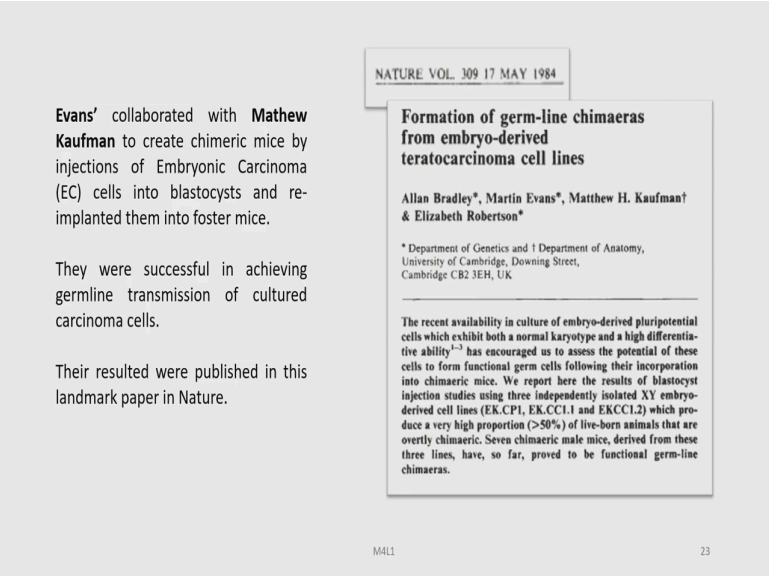

He then collaborated with Mathew Kaufman to create chimeric mice by injections of embryonic carcinoma cells into the blastocyte and re-implanted them into the foster mice. They were successful in achieving germline transmission of cultured carcinoma cells, their result were published in this landmark paper in nature which was on the formation of germline chimaeras from embryo derived teratocarcinoma cell line.

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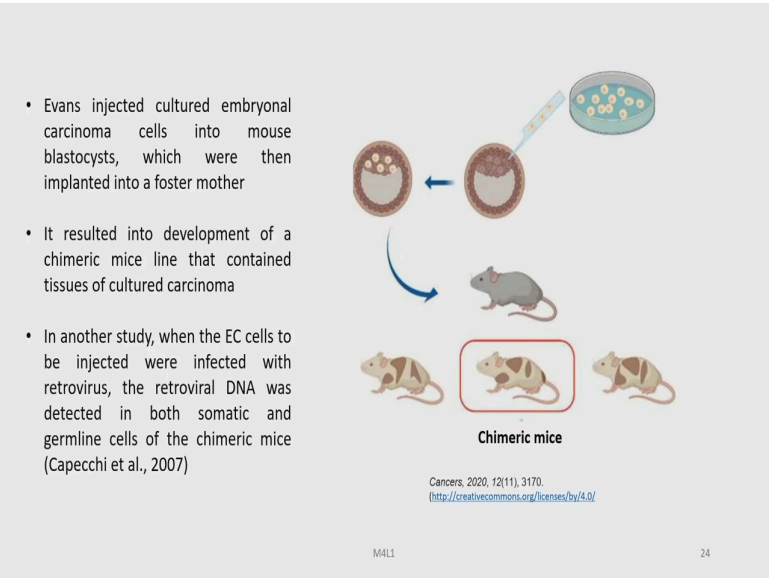

So, here you can see in this picture Evans injected the cultural embryonal carcinoma cells into the mouse blastocyte, which were then implanted into a foster mother. This resulted into development of a chimeric mice line that contain tissues of the cultured carcinoma.

In another study, when the EC cells to be injected were infected with retrovirus, the retroviral DNA was detected in both somatic and germline cells of the chimeric mice.

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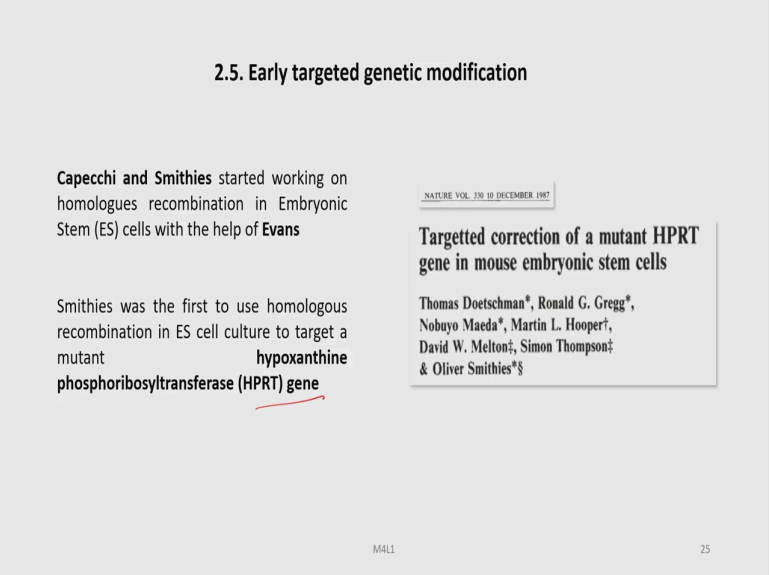

Let us now discuss about the early targeted genetic modification. Capecchi and Smithies started working on homologous recombination in embryonic stem cells with the help of Evans and they published this work in nature called Targeted correction of a mutant HPRT gene in mouse embryonic stem cells.

Smithies was the first to use homologous recombination in ES cell culture to target a mutant hypoxanthine phosphoribosyltransferase or HPRT gene.

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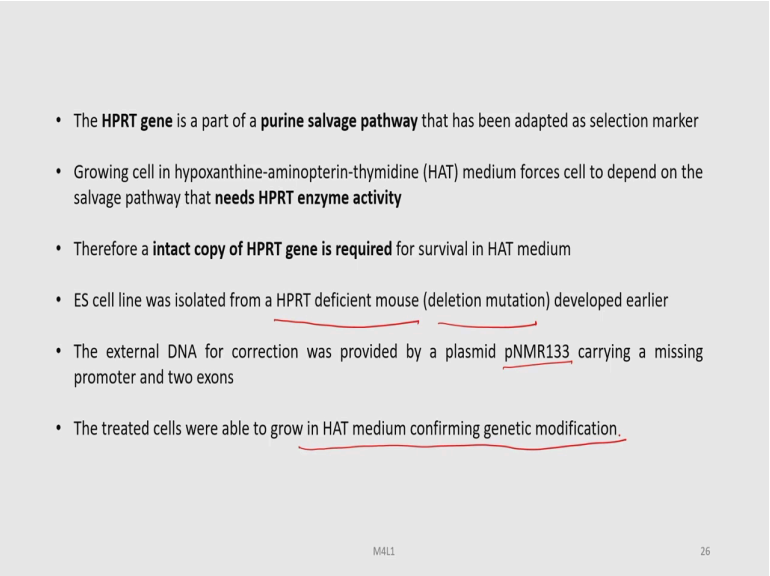
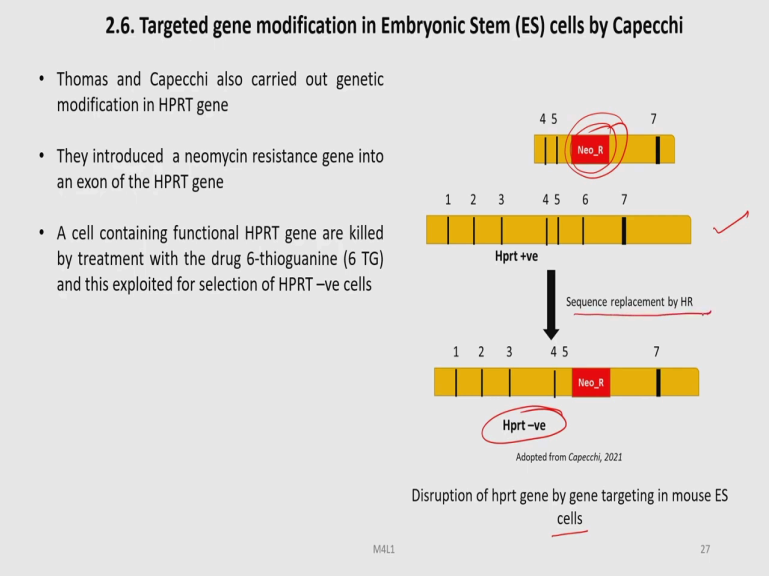


The HPRT gene is a part of a purine salvage pathway that has been adopted as selection marker. Growing cells in hypoxanthine aminopterin thymidine medium or HAT medium forces the cell to depend on the salvage pathway and needs this particular enzyme or the HPRT enzymatic activity.

Therefore an intact copy of HPRT gene is required for survival in the HAT medium, which means HPRT negative genotypes will not be able to survive in the HAT medium. Embryonic stem cell line was isolated from a HPRT deficient mouse, this was basically a deletion mutation which was developed earlier.

The external DNA for correction was provided by a plasmid pNMR133 carrying a missing promoter and two exons. The treated cells were able to grow in HAT medium confirming the genetic modification, because without that it cannot survive in the HAT medium.



Let us now discuss about targeted gene modification in embryonic stem cells as carried out by Capecchi. Thomas and Capecchi carried out the genetic modification of the HPRT gene, they introduced a neomycin resistance gene into an exon of the HPRT gene.

So, this is the neomycin resistance gene. A cell containing functional HPRT gene are killed by treatment with the drug 6 TG or 6-thioguanine and this was exploited for selection of HPRT negative cells. So, here is a disruption of the HPRT gene by gene targeting in mouse embryonic stem cells.

So, this is a functional gene or HPRT positive. So, when these neomycin r is inserted into exon of the HPRT gene, it becomes HPRT negative and this is being carried out by sequence replacement through homologous recombination.

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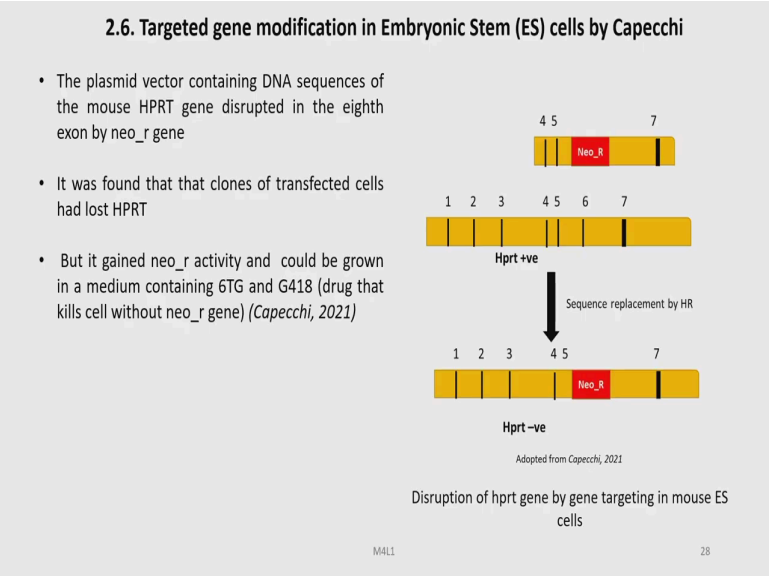

The plasmid vector containing DNA sequences of the mouse HPRT gene disrupted in the eighth exon by the neo_r gene. It was found that clones of transferred cells have lost HPRT, but it gain neo_r activity and could be grown in a medium containing 6 TG and G418 drug that kill cell without neomycin resistance gene. Let us now discuss about gene targeting versus random integration.

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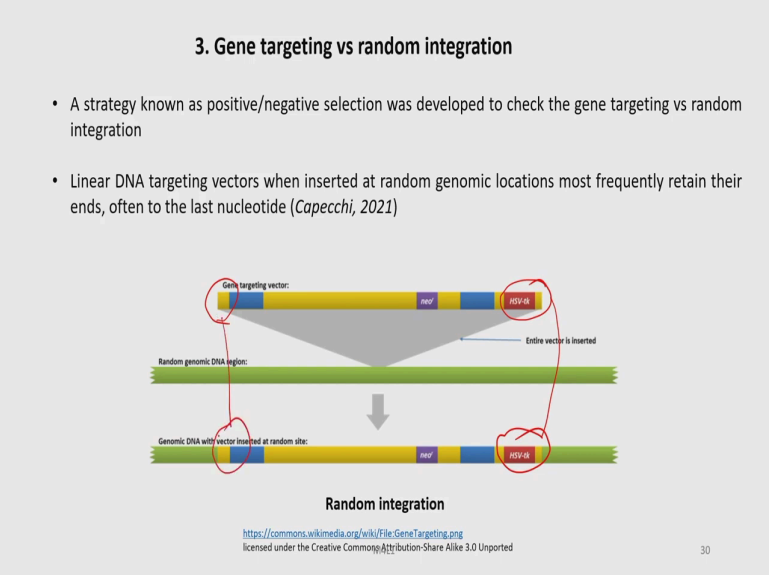

A strategy known as positive negative selection was developed to check the gene targeting versus random integration. Linear DNA targeting vectors when inserted at random genomic

locations most frequently retain their ends often to the last nucleotide. So, you can see the last nucleotides on both the ends and these have been included in this insertion.

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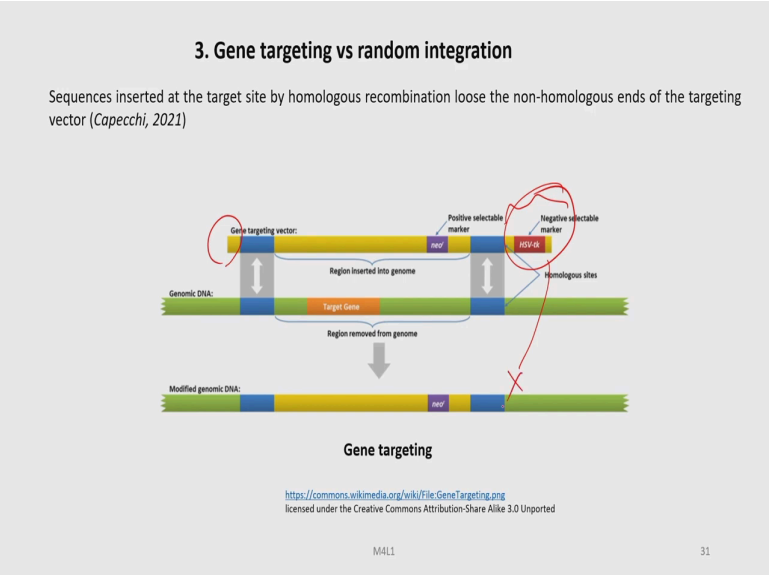

Sequences inserted at the target site by homologous recombination on the other hand, loose the non-homologous ends of the targeting vector. So, this part is the non-homologous end and these are not included in the final product.

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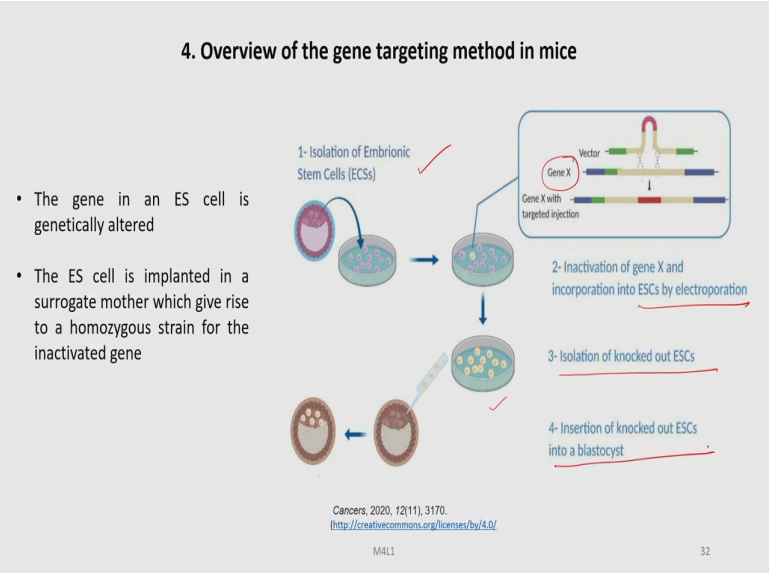

Overview of the gene targeting methods in mice. So, this is carried out in a multi-step procedure, the first step is the isolation of embryonic stem cells.

The second step is the inactivation of gene X and then incorporation into the embryonic stem cells by electroporation. The third step is the isolation of knocked out embryonic stem cells and the fourth stage is the insertion of knocked out embryonic stem cells into a blastocyte. The gene in an embryonic stem cell is genetically altered as a result of these multi stage procedures and these ES cell is implanted in a surrogate mother which gives rise to a homozygous strain for the particularly inactivated gene.

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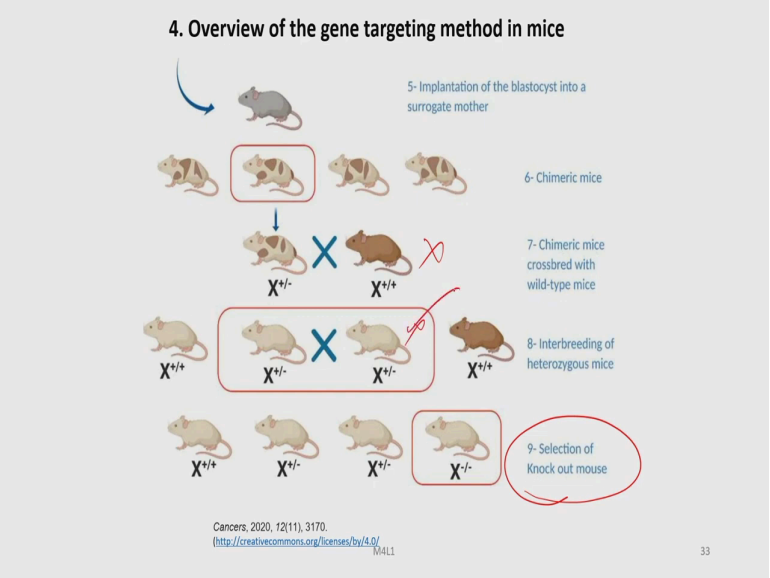

So, this is the fifth step where implantation of the blastocyte into a surrogate mother is being carried out and which produces the chimeric mice. These chimeric mice is crossbred with wild type mice and this will produce a progeny and in breeding of the heterozygous mice is carried out. And finally, at the ninth step selection of the knock-out mice will be done.

So, you can see here starting from isolating embryonic stem cells, then inactivating a gene of interest which is kind of silencing or knockout and then introducing it by electroporation. And finally, isolating the knocked out embryonic stem cell and inserting into the blastocyte and finally, implanting it in a surrogate mother, then leading to the production of chimeric mice and then crossing will wild type and then inbreeding of the heterozygous strains obtained. And finally, we get a mice population in which that particular gene X will be not available or will not be functional and this is the knockout mice with respect to the gene X.

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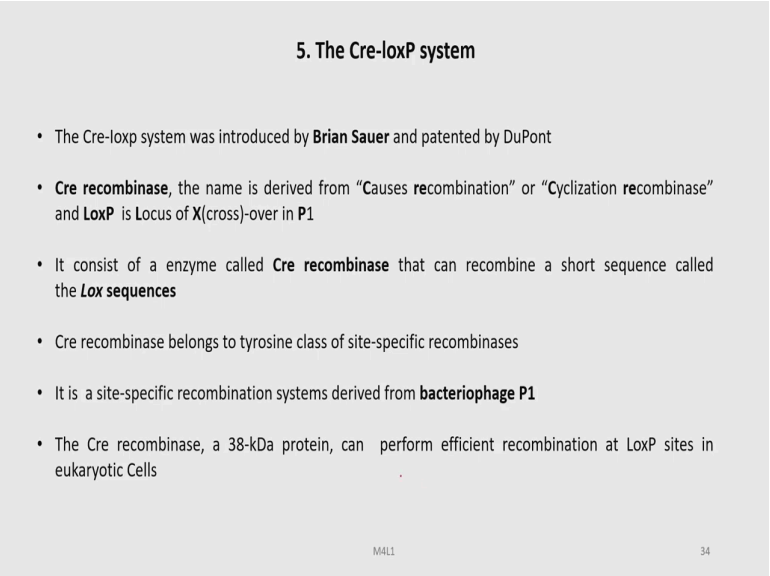

Now, there are the methods by which similar work can be carried out, let us study one of the system used in such cases, the Cre-loxP system. The Cre-loxP system was introduced by Brian Sauer and patented by the company called Dupont, the enzyme called Cre recombinase is basically derived from the word "causes recombination" or "cyclization recombinase" and loxP is locus of X which is cross over in P 1.

So, it consist of an enzyme called Cre recombinase, that can recombine short sequence is called lox sequences. The Cre recombinase belongs to tyrosine class of site specific recombinases, about which we have discussed in detail in earlier lectures, but here also we will do some brief discussion.

It is a site specific recombination system which is derived from the bacteriophage P 1, the Cre recombinase is a 38 kilodalton protein, which can perform efficient recombination at loxP sites in eukaryotic cells.

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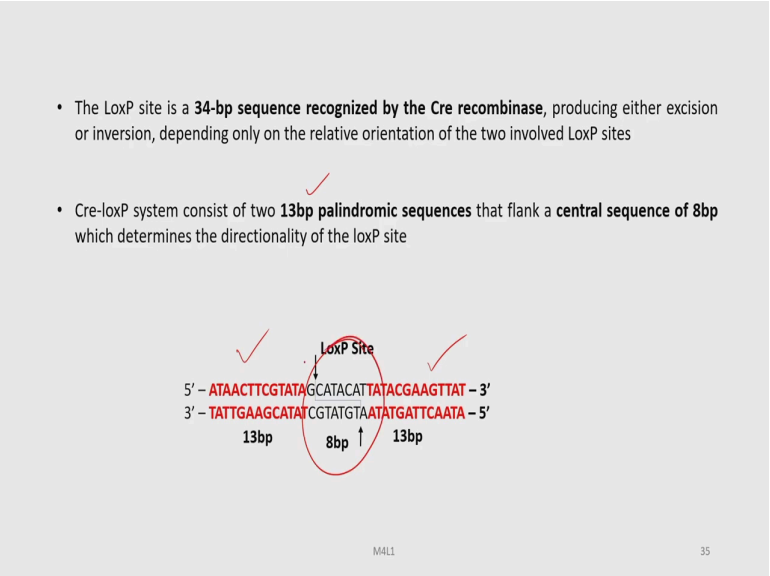

The loxP site is a 34 base pair sequence which is recognized by the Cre recombinase producing either excision or inversion depending only on the relative orientation of the two involved loxP sites. Cre-loxP system consist of two 13 base pair palindromic sequences which that flank a central sequence of 8 base pair which determines the directionality of the loxP site.

Let us study in detail the tyrosine class of site specific recombinase and what is the mechanism of recombination.

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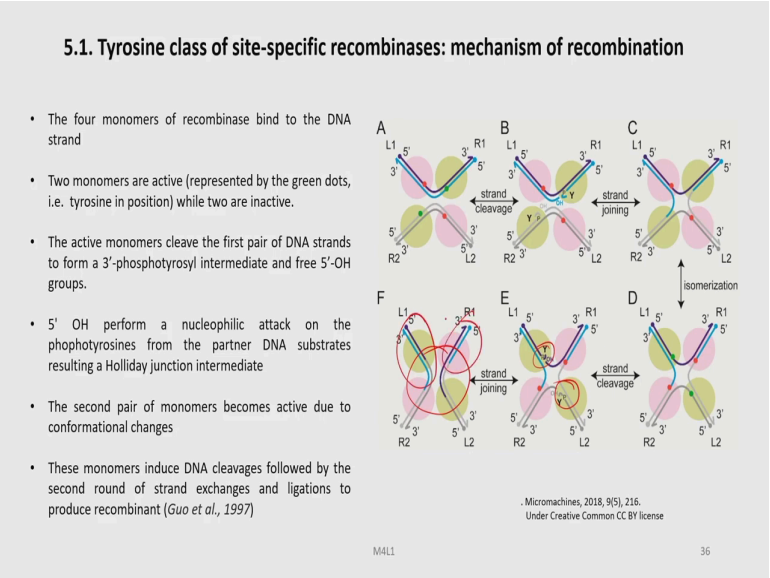

Here four monomers of recombinase bind to the DNA strand. So, these are the DNA strands and you can see here the four monomers which are binding. Two monomers are active represented by the green dots. So, this monomer and this monomer is active and the other two lying diagonally are inactive.

The active monomers cleave the first pair of DNA strands you can see here, to form three phosphotyrosyl intermediate and free 5 prime hydroxyl groups. The 5 prime hydroxyl performs a nucleophilic attack on the phosphotyrosines from the partner DNA substrates leading to formation of a Holliday junction intermediate.

As you can see over here and then this is followed by isomerization reaction which we have also studied in our earlier classes. The second pair of monomers becomes active due to the conformational changes. These monomers induce DNA cleavage followed by the second round of exchanges and ligations to produce the recombinant DNA molecule.

So now, the second cleavage has occurred here and these are the recombinant products as you can see which is the final outcome of these entire procedure.

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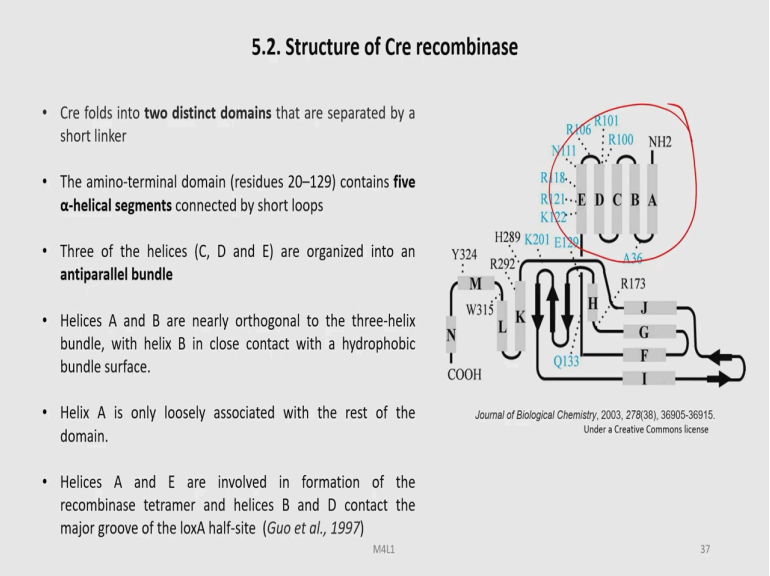

Now, let us look into the structure of the Cre recombinase. The Cre folds into two distinct domains that are separated by a short linker. The amino terminal domain from residue 20 to 129 contains five alpha helical segments which are connected by short loops. Three of the helices C, D and E are organized into an antiparallel bundle.

The helices A and B are nearly orthogonal to the three-helix bundle, with helix B in close contact with a hydrophobic bundle surface. Helix A is only loosely associated with the rest of these domains. Helices A and E are involved in formation of the recombinase tetramer that we just discussed and helices B and D contact the major grooves of the loxA half-site.

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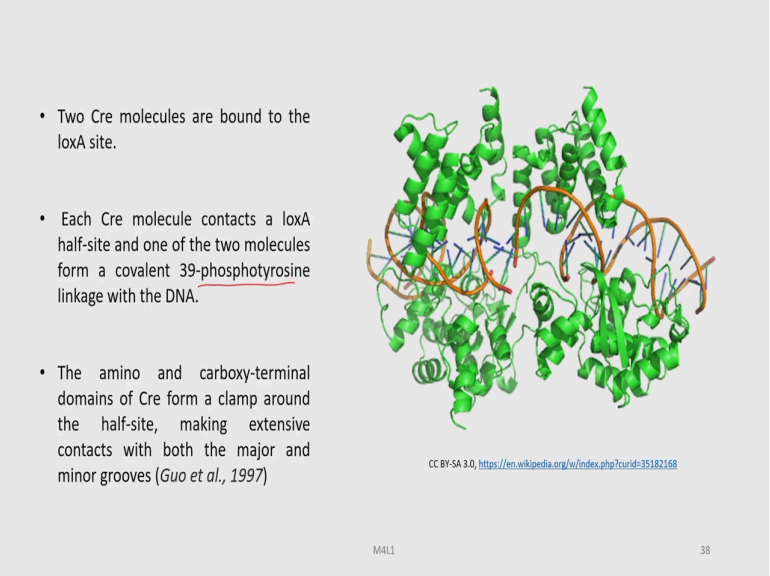

Two Cre molecules are bound to the loxA site. Each Cre molecule contacts a loxA half-site and one of the two molecules form a covalent 39-phosphotyrosine linkage with the DNA. The amino and carboxy terminal domains of Cre form a clamp around the half-site making extensive context with both the major and minor grooves.

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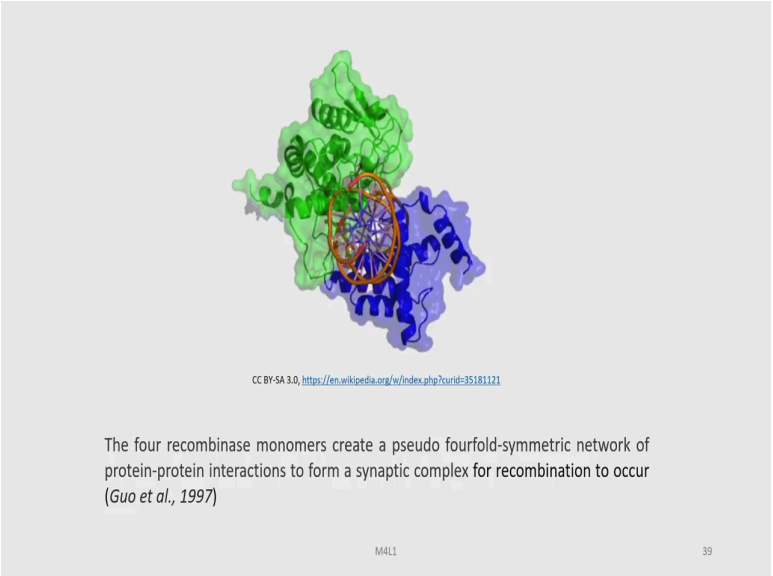

The four recombinase monomers create a pseudo fourfold symmetric network of protein-protein interactions to form a synaptic complex for recombination to occur. How does Cre recombinase can carry out editing?

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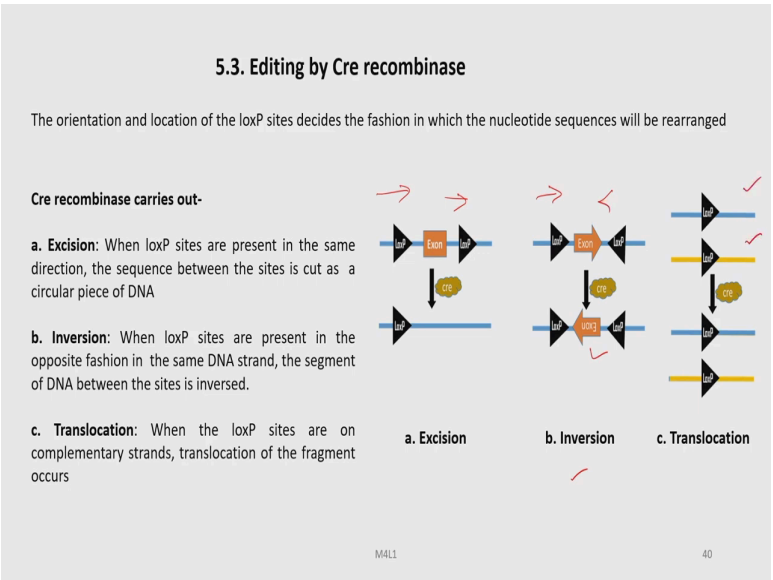

The orientation and location of the loxP sites decides the fashion in which the nucleotide sequences will be rearranged. Cre recombinase carries out excision inversion and translocation. So, in excision, when loxP sites are present in the same direction, the sequence between the site is cut as a circular piece of DNA. In inversion, when loxP sites are present in

the opposite direction in the same DNA strand the segment of DNA between the site is inversed. While in translocation, when the loxP sites are on complementary strands two different strands translocation of the fragment would occur.

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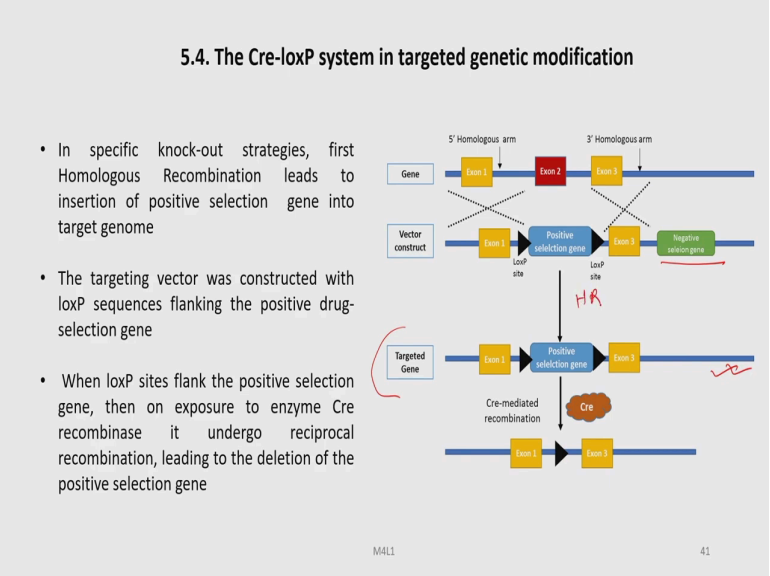

The Cre-loxP system in targeted genetic modification. Let us study how it works in such a system. Let us first examine this diagram, you have a gene which has three exons, 1 2 and 3 and it has two homologous arm, one is the 5 prime homologous arm the another is the 3 prime homologous arm. Then we have a vector construct, these vector construct has exon 1 and exon 3, but it does not have exon 2.

In place of the exon 2, there is a positive selection gene and this is flanked by two loxP sites and there is also a negative selection gene. So, in specific knockout strategies, the first homologous recombination leads to insertion of positive selection gene into the target genome.

So, here we can see this is the targeted gene. So, this positive selection gene is now being inserted in this targeted gene. This is the first homologous recombination occurring at this stage. The targeting vector is constructed with loxP sequences flanking the positive drug selection gene which I have already explained to you. When loxP sites flank the positive selection gene, then on exposure to enzyme Cre recombinase it undergoes reciprocal recombination leading to the deletion of the positive selection gene.

So, this is the step where the positive selection gene is removed. So, overall, you can see that these genetic modification utilizing Cre and loxP system happens in a two stage procedure. In the first stage, the positive selection gene is included in the targeted site and in the second stage, the Cre recombinase deletes the positive selection gene out of this location.

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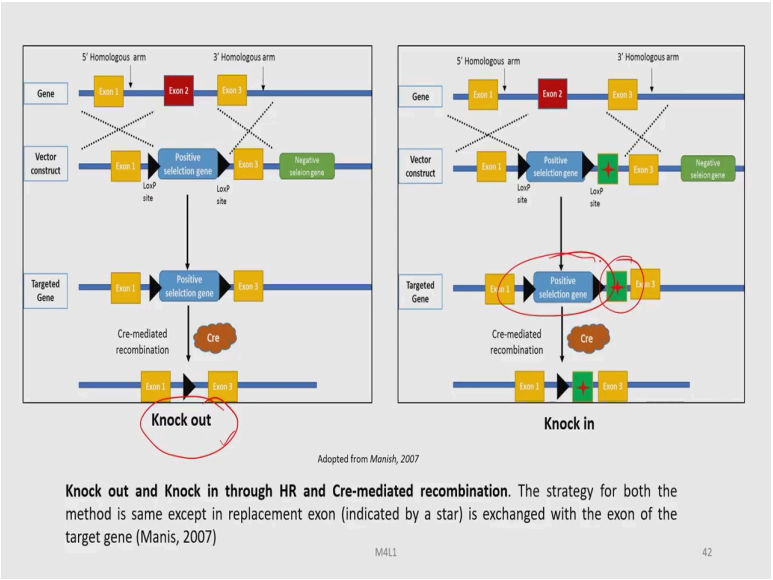

Now, let us study about the use of Cre lox system in knock-out and in knock in. So, you can see here the first system is the knock-out which we have already studied and the second picture is almost similar, but there is a knock-in or addition of a gene over here.

And this gene which is being shown in this green box as a star is included in the final product. In both the knock-out and knock-in this happens in two stages, where first the positive selection gene is included in the targeted site, in the knock-out case as well as in the knock-in case.

But in the knock-in case along with the positive selection gene, a desired gene is also included which lies beyond the loxP site as you can see over here. So, when the Cre recombinase will act on this intermediate product, it will remove the positive selection gene because it acts on the loxP sites, but it will retain the desired gene which was included along with the positive selection gene.

So, in brief the strategy for both method is same, except in replacement exon which is indicated by a star as I already told you is exchanged with the exon of the target gene.

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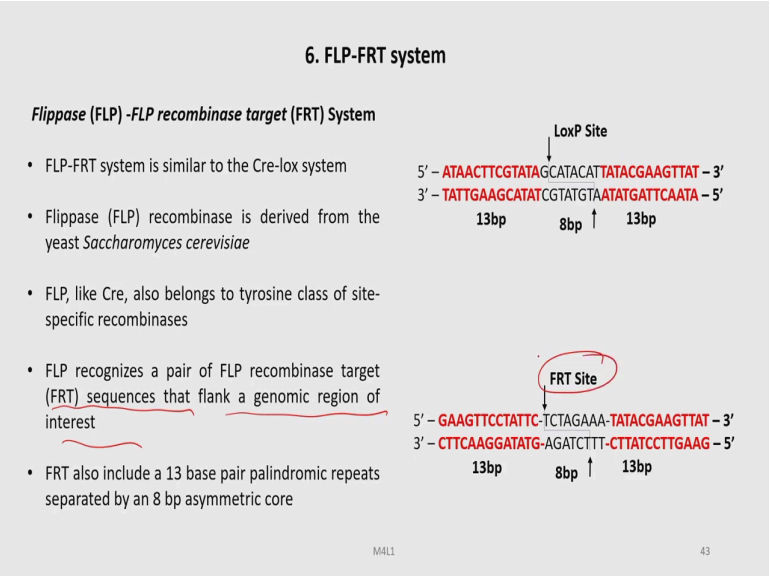

Let us now discuss about the FLP-FRT system. The flippase recombinase target system or FRT system. FLP-FRT system is similar to the Cre lox system that we just discussed. The flippase recombinase is derived from the yeast saccharomyces cerevisiae, the flippase like Cre recombinase also belongs to tyrosine class of site specific recombinases.

FLP recognizes a pair of FLP recombinase target called as the FRT sequences that flank a genomic region of interest, similar to the loxP site in the case of Cre recombinase. FRT also includes a 13 base pair palindromic sequence separated by an 8 base pair asymmetric core which is similar to the earlier case.

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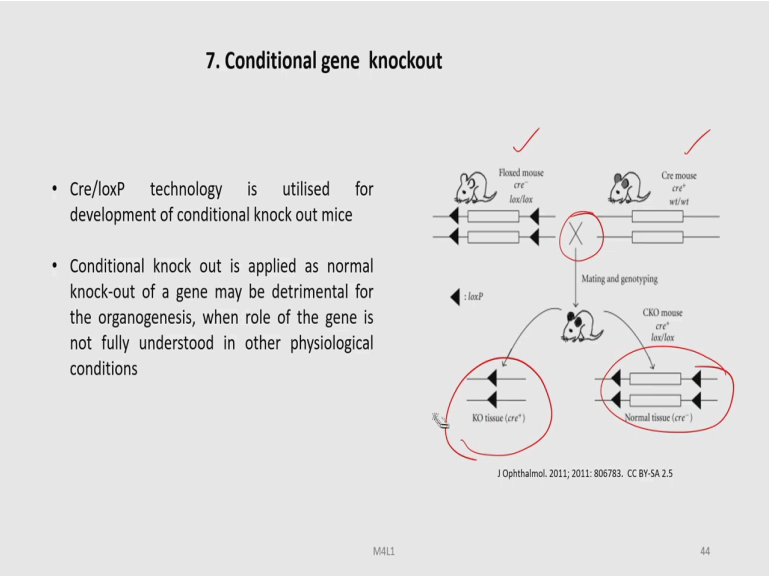

Let us now discuss about conditional gene knock-out. Cre-loxP technology is utilised for development of conditional knock-out mice. So, this is a floxed mouse and this is a Cre mouse and due to mating, we will have a knock-out species and we will have a normal a tissue species.

The conditional knock-out is applied as normal knock-out of a gene may be detrimental for the organogenesis, when role of the gene is not fully understood in other physiological conditions. For example, we cannot create knock outs of housekeeping genes.

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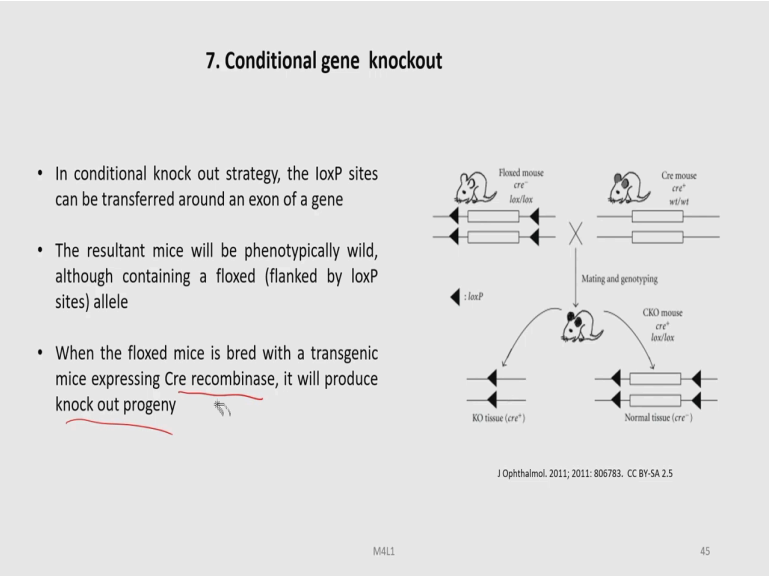

Whereas in conditional knock-out strategy, the loxP sites can be transferred around an exon of a gene. The resultant mice will be phenotypically wild although containing a floxed or flanked by loxP site allele. When the floxed mice is bred with a transgenic mice expressing Cre recombinase, it will produce the knock-out progeny.

So, we have to remember that the Cre-loxP system require both the loxP regions as well as the Cre recombinase. So, here you can see these floxed mouse is Cre negative, but loxP positive and homogeneous for loxP or it is having two sites. Whereas, this mouse is Cre positive, but there is no any loxP site in this particular mouse and when these two mouses are crossed, then in some of the cases Cre recombinase will act on the loxP sites and it will carry out the knock-out population or the knock-out progeny.

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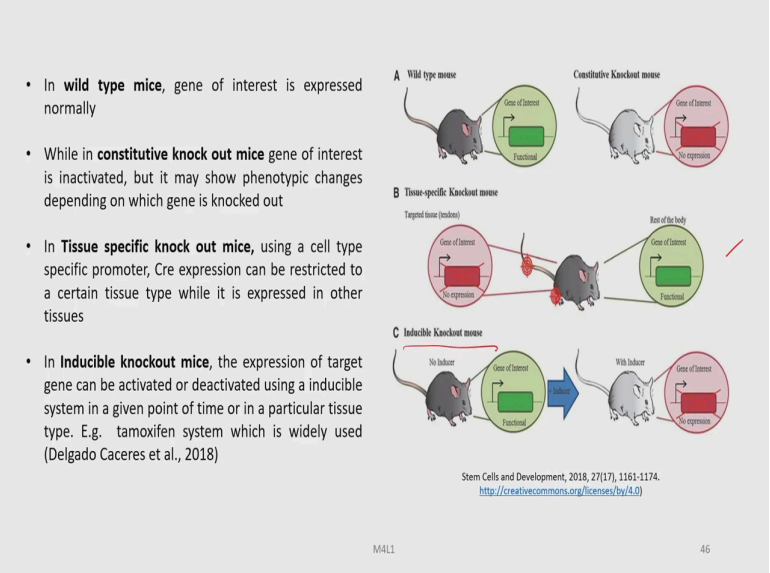

In the wild type mice, genes of interest is expressed normally while in constitutive knock-out mice gene of interest is inactivated, but it may show phenotypic changes depending on which gene is knocked out. So, there is no any expression of the particular gene which has been knocked out. So, this is called as the constitutive knock-out mice. In tissue specific knock-out mice, using a cell specific promoter Cre expression can be restricted to a certain tissue type while it is expressed in other tissues.

So, in such a mice, the genes of interest will not be expressed only in the particular tissues of interest in which the Cre expression is restricted to. But in the rest of the body, the gene of interest will be functional, such a mice is known as tissue specific knock-out mice. There is

another type of knock-out which is known as inducible knock-out mice, here the expression of target gene can be activated or deactivated externally by using an inducible system in a given point of time or in a particular tissue.

For example, we may have a tamoxifen system which is widely used to switch on and off particular genes. For example, we have a gene of interest; so in the presence of the in the absence of the inducer the gene is functional, but in the presence of the inducer the gene becomes non-functional or there is no any expression. So, these are kind of a knock-out which is known as inducible knock-out mice.

So, we have basically three knock-out system one is the constitutive knock-out system, one is the tissue specific knock-out system and another is the inducible knock-out system.

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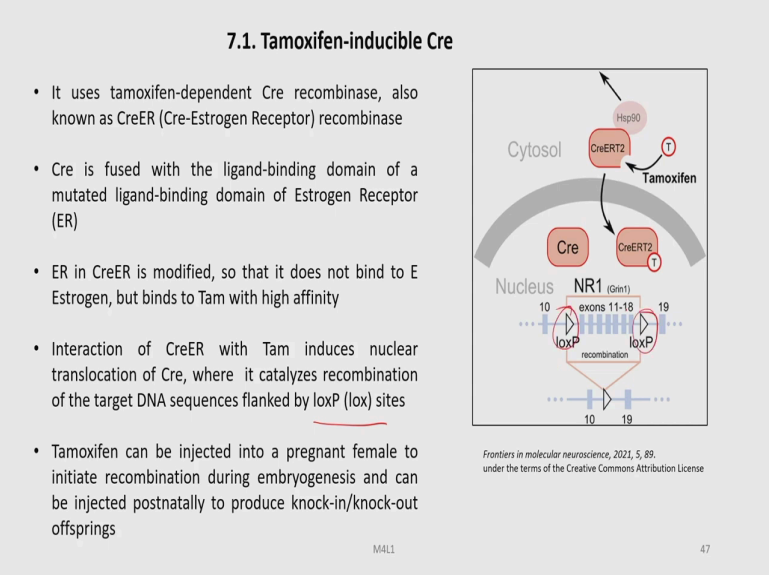

Let us now study a little bit about the third type the tamoxifen inducible Cre recombinase system. So, it uses tamoxifen dependent Cre recombinases also known as CreER, Cre estrogen receptor recombinase. Cre is fused with the ligand binding domain of a mutated ligand binding domain of estrogen receptor, the ER in CreER is modified so that it does not bind to estrogen, but it will bind to tamoxifen with high affinity.

Interaction of the CreER with tamoxifen induces nuclear translocation of the Cre where it catalyzes recombination of the target DNA sequences flanked by loxP sites. So, the moment tamoxifen binds, it will be transported or internalized and this system will start working

based on the presence of loxP sites, as already discussed in earlier slides. This tamoxifen can be injected into pregnant female to initiate recombination during embryogenesis and can be injected postnatally to produce knock-in and knock-out off springs.

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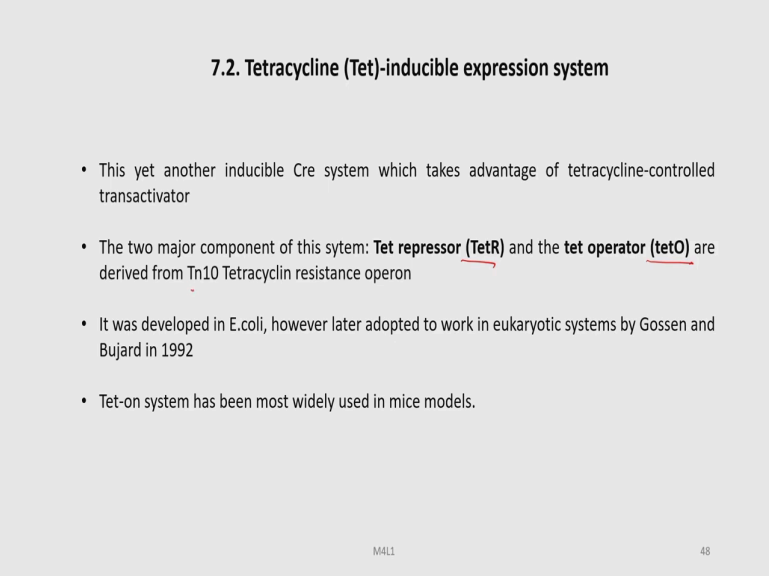

Similar to tamoxifen, we also have tetracycline inducible expression systems or Tet inducible expression systems, it works similarly and this is just another inducible Cre system which takes advantage of the tetracycline controlled transactivator.

The two major components of this system are the Tet repressor TetR and the tet operator and these are derived from Tn10 tetracycline resistance operon this was developed originally in e.coli. However, it has been adopted to work in eukaryotic systems by Gossen and Bujard in 1992. Tet-on systems has been most widely used in the mice model.

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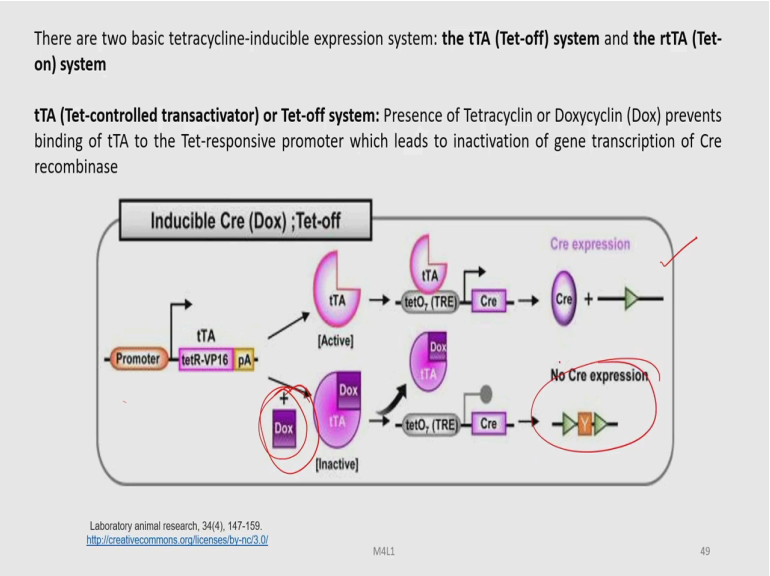

So, we now understand there are two basic tetracycline inducible expression system. The tTA or the Tet-off system and the rtTA or Tet-on system. Let us discuss about the tTA system first, this is a Tet control transactivator or tet off system. The presence of tetracycline or doxycycline prevents the binding of tTa to the Tet responsive promoter which leads to inactivation of gene transcription of Cre recombinase.

So, this is the promoter over here, as you can see and the presence of the either tetracycline or doxycycline will prevent binding of the tTa to the Tet responsive promoter and here there is an inactivation of the transcription of the Cre recombinase. So, there is no any Cre expression in the lower case. Whereas, in the upper case upper panel you can see there is no any kind of such interaction, due to which the Cre expression is working.

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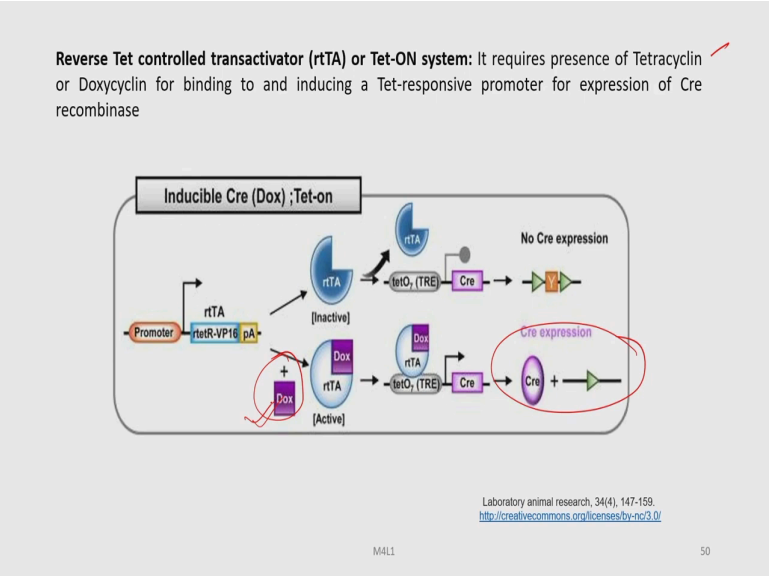

And reversely the Tet control transactivator or Tet on system requires the presence of tetracycline or doxorubicin for binding to an inducing Tet responsive promoter for expression of Cre recombinase. So, in this case the opposite happens. In the first case in the presence of doxorubicin there is no expression of Cre, but in the second case you can see the expression of Cre takes place only in the presence of doxorubicin.

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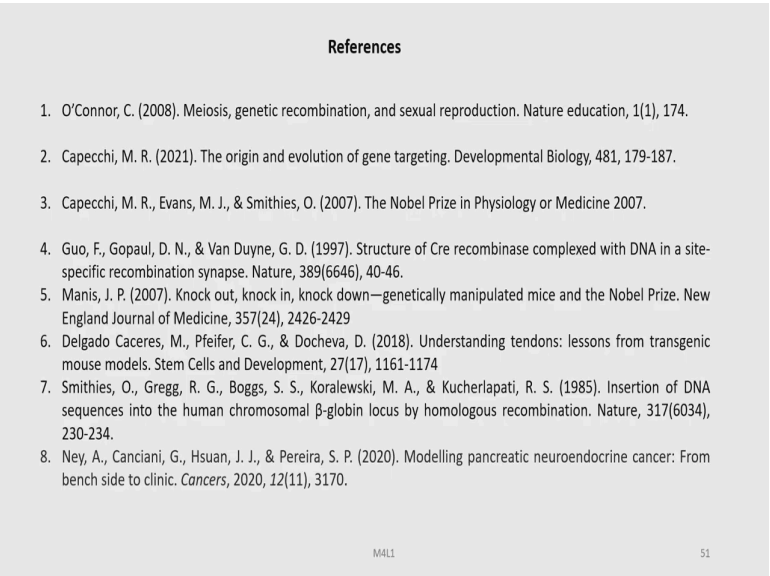

So, there are some of these references from which this lecture was prepared.

Thank you for your kind attention.