Molecular Biology

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Module - 11

Genome Editing

Lecture-47 Genome Editing (Part 1)

Hello everyone, this is Dr. Vishal Trivedi from department of bioscience and bioengineering IIT Guwahati. And what we were discussing, we were discussing about the different aspects of the molecules in the course molecular biology. So far what we have discussed, we have discussed about the cell biology, we have discussed about the biochemistry, we have discussed about the details of the different types of biomolecules and so on. And then we have also discussed how these biomolecules are interacting with each other and in that process we have also discussed about the central dogma of molecular biology, we have discussed about these application, transcription and translations. And in the previous couple of modules, we were discussing about the different types of techniques which are related to molecular biology.

So, we have discussed about the polymerase chain reactions, we have discussed about de brotting techniques. Now, in today's lecture we are going to discuss about the application of the molecular biology in context of correcting the genetic diseases. So, what you can see here is that we have the different types of genetic diseases which is been found into the human populations. So, we have the Huntington disease, we have hemophilia, we have Perkin's disease, we have colon cancers, we have so many different types of diseases which are been found into the human populations.

And how the human population or humans are you know the how the humans are managing these diseases, they are been having the different types of therapeutic options. One of the option is that you are actually going to generate the drugs and these drugs are actually going to overcome the deficiency or the other kinds of you know he is going to take care of the detoxifications and other kinds of things. So, see the mechanism of these diseases are very different. In some cases you are actually over expressing a particular factor or in some cases you are not been able to provide a crucial component and that is how you can may or may not be able to achieve the particular you cannot be able to run a particular metabolic pathway. So, either of these cases you are supposed to provide that particular factor which is responsible for the correcting these diseases.

For example, we have thalassemia, we have the muscular dystrophy, we have the cystic fibrosis, Huntington disease and so on. All these are happening because one of the crucial

factor is either been produced in a large quantity or it is not been about available for the for the human body. And the only way you can actually be able to provide this is that if you take this factor and put it into the cloning factor. So, let us see how the some of the diseases and we will take an example of few diseases how these diseases are been managed within the for the for the human welfare. So, we have the HIV infections, we have the leukemia, we have thalassemia, we have the sickle cell anemia and the Parkinson disease.

In the HIV infections we have the responsible mutated gene that is called as the CCR5 which is a chemokine receptor and CCR5 is the receptor which is responsible for the replication of the HIV virus. And the target cell for this is the CD4 positive T cells because CD4 plus positive T cells are the cells which are responsible for the immune function into the human body. And you are actually producing the CCR5 you know the genes and that is how you are using the adenovirus and as well as the messenger RNA as a delivery vehicle. And that is how you are hoping that if we do that you are been able to provide the CCR5 and that is how you can be able to you can be able to overcome the mutations and that is how you can be overcome the HIV infections. The other example is the leukemia.

So, it is a what we are discussing is the acute myeloid leukemia or AML and in that case you are actually having the mutated gene which is present in the CD 123 or T-RAC. So, T-RAC is a T cell receptor alpha chain and the target cell therapy is by the CAR T cells. So, you can be able to generate the chimeric antigen receptor T cells and these you are actually going to have the messenger RNA which are going to be delivered into a particular T cell type and that is how you are actually going to generate the chimeric cells and these chimeric cells are going to cure the leukemia. Then we have the beta cell cemia, theta cell cemia you have the BCL 11A which is called as the B cell lymphoma factor 11A and the here the target cells are CD4 HSC 7 HSCs or the hemopoietic stem cells and you are actually going to use the messenger RNA for delivering the ah the ah the clone factors. Then we have the sickle cell anemia and in the sickle cell anemia you have the ah BCL 11A and that is how you are going to have the ah CD4 positive HSC hemopoietic stem cells.

Then we have the Perkin's end disease. So, in the Perkin's end disease you are actually going to have the LRRK 2 or Perkin or Pink 1 or the DGA 1 these are the some of the proteins genes which are responsible for the Perkin's end disease and here you are actually going to cure the IPCs which are called as the induced pleuroplatine stem cells or ESCs or embryonic stem cells and the mesenchymal stem cells and the virus what you are going to use is the adeno associated viruses or the adenovirus or the messenger RNA. What is the general approach for treating the genetic disorder? So, for example, if this is the patient what is actually ah you know suffering from a particular genetic disease. So, what you are going to do is you are going to collect the desired cell type for example, if we take an example of T cell. So, you have collected the T cell and suppose this patient is ah AML patient right.

So, what you are going to do is you are going to collect the the target C cells and then using

the genetic engineering tools ah whether it is the CRISPR-Cas or TALEN or all those kind of the tools you are actually going to do the ah the corrections into the genome of these cells. You are going to take these cells you are going to correct the ah the errors. So, you are going to change the mutated genes and then you are actually going to do the growing the transform cells into the in vitro patid dishes and then you are actually going to put these mutated genes ah in back into the patient and what will happen is that these transform cells which are actually going to have the correct form of the gene is going to be ah work for this particular patient and ultimately it is actually going to overcome the ah particular deficiency. Now if we want to do this right we have the ah what is the our ah main goal? Main goal is to correct the genetic information right. So, genetic information is going to be as you remember right when we were discussing about the genome the genetic information will be present in the in the form of genome right.

So, ah in this particular case what you are going to do is you are going to take the genome and within the genome also particular gene may be actually be target DNA right. So, this particular gene has and has some problem right has some mutations or it has some kind of abnormalities. So, that gene has to be corrected. Now we have the two different types of approaches what people are using one is called as the ah traditional approach or ah traditional approach and the modern approach right. So, traditional approach ah will depends on the gene sequence right and it is mostly been using the PCR based method right.

So, where you are actually going to clone a particular gene and and you are going to use that where in the modern approach you are actually going to use the ah nucleases and all other kinds of sites. So, here here you are going to use the ring finger nucleases you are going to use the CRISPR cas and so on. So, ah in this particular module what we are going to do is we are going to discuss about the traditional approach we are also going to discuss about the modern approach to understand how you can be able to correct the ah information into the genome or how you can be able to do the genome editing. Remember that ah there are ah there are excellent ah lectures available on genome editing itself. So, genome editing is a very very vast subject that is why I would only going to superficially going to introduce you to the topic and there are ah very ah good ah MOOCs courses there is a MOOCs course called genome editing itself which is available ah into the ah MOOCs platform and I would highly recommend that if you are interested into the ah ah into this particular topic you should go through with this particular ah course and it may actually give you the better understanding.

What we are trying to do here is that we are just going to tell you about what is the basis of doing the traditional approach, what is the basis of doing the modern approach and so on and so it is not going to be an extensive discussion about these ah approaches. So, let us first discuss about the traditional approach. So, traditional approach is mostly being descended on the genome sequences right. So, ah and it also depends on the one of the basic phenomena of the recombinations right. So, recombination is the core of the genetic

engineering.

Recombination is the genetic process involving the breaking and recombining of the DNA segment resulting in the creation of the new combination of the alleles. This mechanism operate at the gene level fostering the genetic diversity that mirrors the variation in the DNA sequences against the organisms. So, the recombination is one of the basic phenomena through which the ah two particular DNA are recombining with each other and that is how in that process they are sharing or they are exchanging the DNA content and that that mixing mechanism is always being used in the traditional approaches to generate the ah to generate or to correct the particular type of genetic disorders. So, as far as the genetic recombination is concerned recombination could be of the homologous recombination or the non homologous recombinations. Within the homologous recombinations you can actually be able to have the bacterial recombinations or the eukaryotic recombinations.

Whereas, in the non homologous recombinations you have the multiple approaches or multiple options right you have the CSSR, you have the within the CSSR you have the SIR type or you are actually going to have the ditarosin type. Then we have the NHEJ, we have the transpositions, then VDJ actually recombinations. In the within the transposition we have the DNA transpositions, we have the retroviruses, we have the poly A retroviruses. So, let us first start with the homologous recombinations and we will understand first the mechanism of the homologous recombinations and then we will see how the homologous recombination can be able to ah produce the genetic recombinations and how it get that in turn will actually going to change the genome. So, homologous recombinations ah homologous recombination is a genetic recombination process characterized by the exchange of the genetic information between the two closely related or identical molecule of the nucleic acid.

These molecules can be either double stranded or the single stranded and typically composed of the DNA in the cellular organism although in the viruses RNA may be also involved in the recombination process. Mostly it is involved into the repair mechanism and the meiosis of the prokaryotic and the eukaryotes. So you can understand that you have the duplicated parental chromosome. So, this is the two different types of chromosomes which are homologous or homologous chromosomes. So, you can imagine that if this is the chromosome from the male and if this is the chromosome from the female then what will happen is that these homologous recombination the homologous chromosomes are actually going to go through with the process of recombinations and they will actually going to because they are actually having a particular DNA sequence which is they are common right and because of that it is actually going to do the recombinations and as a result of this recombination there will be a genetic exchange of material between the two chromosome during the process of crossing over.

Remember that when we were discussing about the mitosis and meiosis which said that the crossing over is one of the phenomena which is responsible for the genetic diversity

because during the crossing over one chromosome may you know one part of the chromosome may go to the other chromosome and the other chromosome may also change the content right. So, it is actually been responsible for the diverg, genetic diversity and the same phenomena can be used for producing the genetic modifications. So, once these there will be a crossing over between the chromosomes you will see that the pink portion is been given to the to the blue portion and the blue is going to be given to the portion. So, this portion is actually a part of this chromosome and this portion is actually been a part of this chromosome and that is how these portion is now altered in both the chromosome and as a result what will happen is that you are actually going to have the separate features of the offspring. So, and this is just a simple chromosome this you can actually have the genetic recombinations even in this region you can have a genetic recombination in this region you can actually have the you know the.

So, depending upon what kind of wherever you have the sequence similarity it is actually going to do the crossing over. Now in a homologous recombinations with you can have the two different types of pathways within the prokaryotic and as well as the eukaryotic pathway. So, within the prokaryotes you can have the RecBCD pathway and RecF pathway whereas, in the eukaryotes you can have the DSR pathway and as well as the SDSR pathway. So, first discuss about the prokaryotic recombination homologous recombination into the prokaryotic system and then we will discuss about the homologous recombination into the eukaryotic system. So, in the mechanism of the prokaryotic homologous recombinations.

So, this is the DNA of the homologous recombination where you are actually going to have the different types of factors. So, in the first is the initiation. So, RecBCD bind to the blunt or the nearly blunt area of the double standard DNA BRCK. Then it is actually going to have the unzipping. So, RecB and RecB helicase work together to unzip the DNA.

So, this is actually going to happen here right. So, RecB and RecC is actually going to unzip the DNA which means they are actually going to have the helicase activity they will unwind the DNA. Then the RecB nucleates domain cuts the emerging single strand. So, it is actually going. So, then you are going to have the RecB which is also have a nucleus activity and it is actually going to cut one of the strands.

Then we have the chi side encounter. So, unzipping continue until the encountering a chi side and chi side is having a sequence which is called as GCT GGCGG. And then we have the chi side recognitions. So, DNA unwinding pause briefly and then resume at a reduced rate. RecBCD cut the DNA strand with chi and ultimately there will be multiple RecA proteins are loaded onto the single standard DNA with the newly generated 3 prime end and then there will be a homologous search.

So, this is the strand what is going to be available for making the homologous recombinations. And then when RecA coated nucleoprotein filament search for the similar

DNA sequence on a homologous chromosomes, the search induce stretching of the DNA duplex and then the strand invasions. So, the nucleoprotein filament moves into the homologous recipient DNA duplex forming a dual day loop and then we have the resolution options. So, if the day loop is cut further strand swapping forms a holiday functions resolution by the RuB, Abc or Xg can produce the two recombinant DNA molecules with the recipient reciprocal genetic type with the interaction the DNA molecule differ genetically. And alternatively the invading 3 prime end chi can initiate the DNA synthesis forming a replication form and this type of resolution produce only one type of non reciprocal recombinations.

So, this is what exactly it is shown here right you have the RecBZ system it is actually going to unwind the DNA and then it is actually going to truncate one of the strands and then it will reach to the chi side and from the chi side the DNA is going to be Breck and then this is the Breck DNA is going to be coated with the RekA gene, RekA proteins right and then it is actually going to participate into the recombinations with the closely related sequences and as a result of this recombination it is actually going to produce a two different types of DNA with the recombinations. Then we have the another pathway which is called as RecF pathway. So, in the RecF pathway homologic recombination bacteria employ a repair mechanism for the single standard gaps in the DNA when mutation inactivate the RecBCD pathway and additional mutation disable the SCS, SCCD and XO1 nucleus the RecF pathway can also repair the double standard DNA breaks. So, in this you are going to have the initiation and so RecQ helicase unwinds the DNA then RecJ nucleus degrade the strand with the 5 prime end leaving the strand with the 3 prime end intact then we have a RekA binding. So, RekA protein bind to the strand with the 3 prime end and then RecF and RecO and RecR protein aid or stabilize RekA in this process and then there will be a strand invasions.

So, RekA nucleoprotein filament search for the homologous DNA and the exchange place within the identical or nearly identical strands in the homologous strand the strand invasions and then there will be a branch migrations. So, similar to the RecBCD pathway involves the moment of the holiday function in one direction and then there will be a resolution. So, similar to the RecB pathway holiday junctions are cleaved apart by the enzyme in a process of resolutions and both the pathways may undergo alternate non reciprocal type of resolutions. Despite differences in the protein and specific mechanism in their initial phase both the RecBCD and RecF pathway share the similarities. They both require single standard DNA with the 3 prime end and RekA protein for strand invasions additionally the pathway exhibit similarity in the phases of branch migration and the resolution of holiday functions.

So, this is the pathway of the other pathway and the mechanism of this pathway is also going to be same. And then we have the mechanism of the eukaryotic homologous recombination for repair. So, remember that the homologous recombination mechanism is being used only to repair the you know the damaged DNA because the damaged DNA

information is missing. So, you can actually be able to bring that information from the neighboring residues right. So, with the help of the neighboring residues you search for the homologous DNA and then you are actually going to copy the damaged DNA.

So, in the normal cell you are going to have the double standard DNA breaks and that is how you are going to have the activation of the homologous recombinations in the eukaryotic system. So, ATM recognizes the DSB phosphorylate H2X and facilitate the MDC binding and then we have the MRN the MRE1 plus RAD50 and NBS complex is localized to the DSB. Then we have the CT IP creates C prime overhang where its exonuclease activity and then the RPA binds to the C prime overhand. Then we have RAD51 BRAC BRCA1, BRCA2 replace RPA to form the filament of DNA to proceed to the homologous recombinations. So, this is what exactly the you have a double standard DNA breaks and that activates the ATM and then ATM is recognizing the dual standard phosphorylates H2AX and facilitate the MDC binding and ultimately it is actually going to do the the homologous directed repairing of the DNA strands.

Then we have the two different types of proposed pathway for the eukaryotic homologous recombination mediated repairing. You have the classical double standard break repair pathway or the synthesis dependent strand annealing pathway. Both of these pathways are operating in one organism to another organism. So in the classical double standard break pathway you have the three prime invade and intact homologous template, then formation of the double standard holiday functions, junctions and then junction resolution results in the crossing over or the non-crossing over. Similarly, we have the synthesis dependent strand annealing pathway and it is conservative and result oriented exclusively in the non-crossing over events.

So, the key factors and which are involved in their role in the eukaryotic homologous recombination. So, we have the MRN complex and their job is to initially stabilize the double standard breaks. Then we have the ATM or ataxia telekestia mutated it is recognizing the double standard breaks. Then we have the BRCA1 and BRCA2 which is a breast cancer associated genes and these are the checkpoint activation and the DNA repairs. Then we have a CTIP C terminal binding protein interacting proteins and it is interact with the BRCA1 and D phosphorylate the 53BP1.

Then we have the RPA1 or application protein A that stabilizes the sub single standard DNA H2AX, H2A stone family member X and that is responsible for the recruitment and the accumulation of the DNA repair protein and then we have a RAD51, RAD51 forms a filament in the onto the DNA strands. Now, if you summarize the homologous recombinations in both the eukaryotic as well as the eukaryotic one what you will see is that the there are proteins or the processes which are involved which are common between the two. So, you have the introduction of the double standard DSB that is not present in the E. coli system, but that is present in the eukaryotic system where you have the SpO1 and HO which are going to be involved into the introduction of the double standard breaks.

Then we have the processing. So, RECBCD complex or the nucleus or helicase and nucleus system and here we have the MRX complex which is involving the RAD50, 58, 60 and nucleases. Then we have the assembly complex formation and filaments, RECBCD or RECF pathway which is going to be RAD52, RECF59 and BRCA2. Then we have the pairing and strand exchange. So, RECA which is involved into the eukaryotic system whereas, here you have the RAD51 and DMC1 exclusive for the muses.

Then we have the branch migrations. So, branch migration here you have the RECUVAB complex whereas, it is unknown into the eukaryotic system. Then we have the resolution of the holiday junctions. So, RECRUVc and here you are actually going to have the RAD51c, XRCC3 complex and WRN and the BLM. So, these are the comparative study of or comparison of the summary of the homologous recombination in the prokaryotic as well as the eukaryotic organisms.

Let us move on to the non-homologous recombinations. So, in a non-homologous recombinations, the recombination involves the physical exchange of DNA segment between the chromosome or the DNA molecule. When this exchange occur between the stretches of DNA with no extensive sequence homology, it is termed as the non-homologous recombination. Unlike homologous recombination, it does not require a double-stranded break in the DNA for the initiation. It is also relatively less precise and error prone and it is often lead to the insertion or the deletion of the nucleotide at the site of recombinations. NHEJ is a DNA repair mechanism that involves the direct ligation on the broken end and thus do not require a homologous template strength.

Whereas in the transpositions, the CSSR or VDJ recombinations and the phenomena of transposition also does not require the extensive sequence similarity between the strands of DNA involved into the recombination process. So, the non-homologous recombination does not require the sequence similarity. It actually happens abruptly and it does not require the double-stranded breaks also for initiation. So, basically the non-homologous recombination is going to be an exchange of the DNA between the you know or with no with no sequence extensive sequence similarity. There could be sequence similarity, but that would be very very minor and in these kind of cases you are actually going to have the multiple examples like transpositions, VDJ recombination which is responsible for the generation of the different types of antibodies and so on.

And in all of these examples, there will be no there will be no sequence similarity which is involved. So, we have the site specific recombinations, so which is called as the SSR. So, also called as the conservative site specific recombination or CSSR is a recombination recombination between the two defined sequence element of the DNA. This process is carried out by the protein known as recombinase which brings together the specific ends of the DNA forming the synaptic complex resolving onto the three outcomes. So, you can have the insertion, inversion and deletions and these are the recombination sites right you are

actually going to have the insertion or inversion or the deletions.

Type of recombination in the CSSR, so based on the amino acid sequence homology and the mechanism followed most recombinations can be classified into two types. It can be serine recombinase or the tyrosine recombinase. Serine recombinase cleaves all fold strand involved into the exchange whereas, the tyrosine recombinase is one strand in each site is cleaved leading to the formation of the holiday junctions. So, tyrosine recombinase and serine recombinase the difference between the tyrosine recombinase and the serine recombinase. So, recombinase it is going to be a cry whereas, the recognition site is going to be locside and it is actually going to you utilize for the circularization of the fast P1.

We have the lambda integrase which is going to be at P and B sites and it is the integration of the fast lambda. Similarly, we have XRD CD resolvase complex which is going to be diff in nucleoid or the serine plasmids. It is the resolution of the dimers into the E. coli. Similarly, for the serine recombinase we have the H-in invertase which is a HX site and the inversion of the promoter in the salmola and then we have TA3 and Y sigma resolvase which is a rest site and it is going to be resolution during the replicative transpositions.

Mechanism of the serine recombinase. So, one molecule of recombinase catalyze the cleavage of a single strand thus a total of four molecule of recombinase is required for the recombination of the recombination process. The three hydroxyl group of DNA at the three prime act as a nucleophile to attack the recombinase DNA complex at the five prime to generate the free recombinase and recombine DNA. Slippery hydrophobic part of the top and bottom half of the recombinase dimer rotate by the 180 degrees and that is how this portion will go here and this portion will go here. So, all the four strands are first going to be break by the serine recombinase. So, you are going to actually going to have the four strands and four strands are actually going to have the one strand will going to have the three prime OH the other one will have the five prime OH and so on and then there will be a you know the dimer will rotate.

So, in that case this portion will rotate on this side this portion will rotate on this side and that is how it is actually going to have the recombination and that is how they are actually going to have the exchange of material between the two strands. Then there will be electrostatic interaction stabilized initial and the rotated state of the recombinase and ultimately there will be a exchange of DNA material between the all the four strands. Then we have the mechanism of the tyrosine recombinase. So, recombinase cleaves one strand at one each side of the recombination. So, they are going to cleave this particular side and this is going to cleave the another side.

So, you are going to have the two strands which are actually been participating into the recombination process. The cut strand is exchanged between the DNA molecule. So, this portion will go into this and this portion will go into this and that is how this will now be a part of this molecule and this will going to be part of this molecule. Then we have the

formation of the holiday junctions and then cut at the second strand In each side strand exchange occur and the resolution of the holiday junction.

So, once you are going to have the cut on one strand. So, you are going to have the cut on this strand and then this strand and then there will be an exchange between the cut text and cut extent will exchange between the two strands and then there will be a formation of holiday junctions and then you are going to have the cut at the other strands right. And then there will be an exchange of the genetic material and that is how you are going to have the recombination. So, you see that here you have the blue and then you are going to have the yellow which is coming from this strand and then you are actually going to have the red instead of the blue right in this particular strand. Similarly for the lower strands those strand is actually going to have the blue and it is going to also have the red on the other side.

Similarly for this one this one has the red on both sides. So, it is going to have red on both sides, but on this side it is actually going to have the instead of red you are going to have the blue and that is how you both the strands are now going to have the new DNA molecules or recombined DNA molecules. What is the biological significance of CSSR? So, it is actually been involved into the DNA repairs, it is also involved in the gene regulations, it is involved in the genome rearrangements, it is also involved in the horizontal gene transfers, development and so in a DNA repair it allows the precise cut and replacement of the damaged DNA strands making the genome integrity in equalize. Whereas in the gene regulations the phase lambda uses a CSR mechanism to switch between the lytic and the lysogenic cycles. Then the genome rearrangement the CSSR causes the gene rearrangement leading to the various combinations like the inversion insertion or deletion of the gene sequence. For instance, switching in the flagella component of flagella in salmola is mediated to the CSSR mechanism.

Then we have a horizontal gene transfer. So, bacteria often use this mechanism to achieve the new traits from the other bacteria or mobile genetic element leading to the bacterial adaptation and evolutions. Then we have the development. So, many organism uses the site specific combination method to regulate the tissue specific gene expression and cell differentiation during the development. Then we have also have a tracking of cell lineage during the development of drosophila was done using the FLP FRT system. And then CSR is widely used to manipulate and engineer genomes of organism like the ablation of the gene function induction of gene expression at a specific time during development and etcetera.

Then we have another example which is called as VDJ recombination. So, VDJ recombination is responsible for producing of production of the antibodies. So, it is a specialized set of DNA recombination mechanism that impart the enormous diversity in the B cell and T cell receptors. This recombination process occur between the specific site onto the V, D and J segment in the gene for the generation of immunoglobulin and the TCRs. These recombination sites are called recombination signal sequences or RSS.

There are two different types of RSS, 12 base pair RSS and 23 base pair RSS. The combination cannot occur between the same site of the RSS. So, this is a 23 base pair RSS and this is a 12 base pair RSS. And this is the T cell receptor. So, what you can see here is that the V, D and J are recombining with the different types of modules. So, you have the different variation of the V component, you have different components of the J component and so on.

And that is how they are actually recombining with each other to give you the different types of the T cell receptor. So, this is one of the T cell receptor, this is another T cell receptor and so on. Similarly, in the B cell receptor, B cell also will have the different types of V, different types of J and different types of the D components. So, DJ rearranged DNA joined and that is also you will see that VDJ is giving you the different types of antibodies and different types of the antigen binding sites present into the antibody molecules.

Now, another example is the NHEJ. So, non-homologous enjoining. So, NHEJ is a mechanism for repairing the rebel standard DNA breaks in DNA. Unlike homologous directed repairs which require a homologous template, NHEJ directly ligates a broken end without the need of a template. This makes the NHEJ an efficient process that can operate both in dividing and non-dividing cell. The term non-homologous enjoining was introduced by the Morie and Haber in 1996. NHEJ is often guided by the short homologous DNA sequences known as the micro homologous.

These sequences are typically found in a single standard overhang at the end of the double standard break. When the overhangs are compatible, NHEJ can accurately repair the breaks. However, if the overhangs are not compatible, imprecise repair may occur leading to the loss of the nucleotide. Inaccurate NHEJ repair can result in the loss of genetic material and may lead to the translocation and telomere fusion. These events are considered hallmarks of the tumor cell highlighting the importance of the proper NHEJ function in maintaining the genomic stability.

NHEJ is wide strap and existed in nearly all biological system. In mammalian cells, it is the predominant pathway for the double standard break pathway repair. However, in the budding yeast like the saccharomyces cerevisiae, homologous recombinations tend to the dominate under the common laboratory conditions. When NHEJ is inactive, double standard breaks may repaired by alternate more error prone pathway like the microhomology mediated enjoining. In MMEJ, end resection reveals the short micro homologous on either side of the break guiding the repair. Unlike classical NHEJ, MMEJ often result in the deletion of the DNA sequence between the micro homologies.

What is the mechanism of the NHEJ? So, you have a double standard breaks and then this double standard breaks are actually going to have the binding of the Ku7080. So, recognition of DNA end by the Ku7080 heterodimers. So, they will go and bind to the DNA

breaking sites and then they will recruit the DNA PKCs then nucleus such as artemis stream the incompatible strands and then the XRCC4 DNA ligase ligate the complete seal of the break and then the DNA double standard breaks are going to be repaired. Key players in the NHEJ and their mutation related diseases. So, you are going to have the different types of diseases which are been found if there will be a mutation of the important components.

You can have the Ku70, DNA PKCs, artemisis, DNA pol and the ligase and XRCC4 and XLF. So, there if there will be mutation of XR70 it will actually be responsible for SID and lymphoma and reduce sensitivity. If there will be mutation in the ligases then it is going to be for immunodeficiency and reduce growth and developmental issues, microcephaly and malignancy. Then if there will be a problem of XLF and other kinds of things then it is going to have the embryonic liability, syndrome, immunodeficiency, developmental delay and microcephaly. Then we will just move on to the next example and the next example is called as the transposons.

So, transposons are also been called as the jumping genes right. So, transposons are segment of DNA that then can move from one locus to a relatively nonspecific site in the genome. Movement of transposon can occur without the duplication of the element accordingly they are classified as the class 1 or replicative DNA or the type 2 which is non replicative DNA. DNA transposons carry inverted repeat sequences at their end as recombination site flanking recombination recombinase protein called transposon which bring out the process of recombination. Transposons retrotransposons contains LDR and the gene for integrase and reverse transcriptase activities. So, there are different types of transposons you can have the class 1 transposon which is called as copy and paste whereas, the class 2 transposons which are called as the cut and paste.

So, in the class 1 you are actually going to have the LTR transposons retrotransposons and the non LTR retrotransposons. So, when they will going to do a transcription they are going to produce the messenger RNA and then there will be a reverse transcriptase to produce a double standard DNA and then these double standard DNA are actually going to integrate into the target sites. Similarly for the non LTR retrotransposons there will be a transcription it is going to produce the messenger RNA then there will be target primed reverse transcriptase and it is actually going to integrate into the target DNA. Similarly for the class 2 cut and paste you are going to have the DNA transposons which is going to be excised from the other DNA and then it is actually going to integrate as such into the target DNA. There are examples of transposons you have the bacterial replicative transposon which is called as TN 3 and Faz mu then you have a non replicative transposons like IS element and simple transposons.

So, IS 1, IS 2, IS 50, IS 10, TN 1, TN 7, TN 501 then we have a composite transposon which is called as TN 5, TN 9, and TN 10 then we have a eukaryotic elements like HAT family members and so on. Then we have a virus like transposons like tie element of the yeast and the copia of the drosophila then we have a poly A retrotransposons. So, that is the line and

sign in the mammals or the alu in the humans. What is the outcome of the transposions? So, altered gene expression through insertion within the gene for example, the ISI element can insert itself nearly 18 inch promoter sequence.

IS element can cause the polar mutations and the reg dependent recombinations. Site specific combination between the transposable element in the same molecule can cause the rearrangements such as the deletion, inversion etcetera and then IS 1 element can cause the deletions and TN 3 and resolve ways can cause the resolve ways reaction towards the deletion and the mutation in general through the insertions. Now these are the some of the approaches which are been very very common or which are been found when the you are using the traditional method of generating the genetic recombinations. So, you can have the homologous recombination, you have a non homologous recombination and so on. Now when you are going to use these methods and you are trying to generate the genetic modifications, you are actually going to face the lot of limitations and you are going to have the lot of issues. What are these issues? These issues are that there will be a lack of precision, there will be a low efficiency, there will be a time consuming and the limited edition scopes and the off target effects.

These are very very serious effects right. You are going to have the lack of precision which means if suppose you use the non homologous recombination methods and so non homologous recombination methods are going to be random. They can be even to one side or they could be another side. Even for the homologous recombination also you require the very precise you know the flanking sequences then only you can be able to edit the particular gene into the genome. But what happen is that even if all these depends on to the sequence similarity. If the sequence similarity is unique then it may actually be able to give you the very precise removal of the mutated genes.

But if the there will be a any you know off targets right if there will be any kind of similarity between the sequences then it may actually replace the another gene or it may actually give you the side effects. Similarly, all these depends on to the integration of the your externally supplied DNA into the system and you can actually be able to use the different types of DNA delivery method to deliver the DNA into the site of actions. That process is very very inefficient and on the other hand once the DNA will enter then it actually should go through with the recombination process and then and during this journey the DNA should not get any kind of DNA's and other kinds of attacks and though also that is why the efficiency of this recombination is also going to be very very low. Third is this all requires the genetic cassettes to be prepared then you are actually going to transform that and so it is actually very very time consuming. And the fourth is because you are dependent on the you know the flanking sequences or you are dependent on the cellular machinery the scope is very very limited.

And on the other hand the first part is that majority of the time when you are you know transforming that cells with the these externally supplied DNA they may actually get

integrate into the into the off target sites and because of that it may not give you the desirable results. So these are the some of the challenges what people are facing when they are using the traditional method for the genome editing. So what we have discussed we have discussed about the homologous recombination we have discussed about the non homologous methods within the homologous recombination we have discussed about the methods in the porochariotic as well as eukaryotic system and within the non homologous recombination we have taken an examples of transpositions we have discussed about the VDJ recombination and so on. So with this I would like to conclude my lecture here in our subsequent lecture we are going to discuss about the modern approach of the genome editing. Thank you. .