Molecular Biology

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Module - 12

Applications of Molecular Biology

Lecture-52 Applications of Molecular Biology (Part 4)

Hello everyone, this is Dr. Vishal Trivedi from department of biosciences and bioengineering IIT Guwahati. Today we are going to discuss about molecular biology. We are going to discuss about the different applications of the techniques that have been involved during the process of the molecular biology. So, there are molecular biology principles which are being utilized to develop the different types of diseases or different types of applications.

These applications are enormous. There are multiple ways in which the molecular biology can be exploited. What we were discussing or what we have focused in this particular course, we have focused only on to the four aspects. What we have discussed? We have discussed about the application of the PCR in genetic engineering because the PCR is the base in which the which is being utilized to develop the genetically modified organisms or which are being basic of the developing the genetic engineering or performing the genetic engineering task.

Then we also discuss about the how the transgenic animals which are being produced after the molecular biology is being utilized and then we also discuss about the PCR based applications. In the previous lecture, we have discussed about the PCR based applications where we have discussed about the real time PCR, we have discussed about the PCR and so on. In today's lecture, we are going to discuss about the genome editing tools and their applications. So, what we have discussed? We have discussed about the genetic engineering applications or applications downstream to the genetic engineering where we have discussed about how the genetic engineering is being led to the production of the different types of proteins, enzymes and all other kinds of things and how they are being utilized for the human welfare. And they and genetic engineering is also being used for producing the different types of transgenic animals.

And these transgenic animals are actually serving the multiple purposes. They are being served as a disease model, they are being utilized for producing a particular type of protein which is actually causing the less allergic reactions and then so on. And in the previous lecture, if you recall we have discussed about the PCR based applications both in terms of the diagnostics and as well as in terms of the their contribution into the basic sciences. So,

the PCR and as well as the RT-PCR or real time PCR is extensively being used in answering the different types of questions. Now, in today's lecture, we will discuss about the genome editing and how the genome editing tools, what we have discussed in the previous module can be exploited for the deployment of or for making a genetically modified or making or how the genetically genome editing tools can be used for editing the genome and that in turns will actually be good for the human welfare.

So, let us first start with the so, we if you recall in the previous module, we have discussed about the three genome editing tools. We have discussed about the zinc finger nucleus, we have discussed about the talon and the we also discuss about the CRISPR cache. And all of these are actually having the their own positive and negative. And we are not discussing about the homologous recombinations and as well as the non homologous recombination as a tool. What we are discussing? We are discussing about the tools what are been currently been utilized or what are the tools which are you know modern tools which are being used for the genome editing.

So, we are you know first start with the zinc finger nucleus, then talon and the CRISPR cache. So, talon the major application of the talon is that it is been used for the gene knockout, gene knock ins, targeted chromosomal deletions, modification of the genome, crop improvement and the application in the HIV treatment. So, let us first discuss about the gene knockout and the knock ins. So, the knockout involves the inactivation of the gene or the gene segment resulting in the loss of gene function. This is typically achieved by introducing the random insertion or the deletion into the genome to the imprecise DNA repair process of non homologous end joining or NHEG targeted by the double stranded breaks.

Before the advent of CRISPR class IX, researchers commonly rely on the random mutagenesis methods such as ENU mutagenesis induced by the radiation or the chemical for generating the DNA changes. This approach often employed in a zebra fish towards skinning was less precise. In contrast, the knock ins operate differently by introducing the specific base pair changes with the precision into the genome. This involves the targeted introduction of a template such as the synthetic DNA fragment designed to replace an existing genome sequence. The highly accurate repair process known as the homologous directed repair HDR guides these precise changes making a departure from the random distribution characteristic of the knockout models.

The process of genetic modification often initiated with the introduction of the double stranded breaks in the DNA and efficiency of precise DNA modification can be significantly improved by generating a site specific DNA. Zinc finger nucleus is served as a powerful tool for inducing the specific DNA cleavage since the variable DNA binding domain of zinc finger nucleus can be customized to recognize a specific DNA sequence chosen by the investigator. By manipulating the outcome of the DNA repair through the different selection method, it becomes positive possible to achieve either the gene knockout or the precise insertion of

the targeted transgene or the knock ins. The mechanism of the gene knockouts and the gene knock ins or the knockout by the zinc finger nucleus involves their ability to introduce induce that the targeted double strand breaks in the DNA and exploit the cellular repair machinery. Here is a breakdown of the process.

In a gene knockout, you are going to disrupt a particular gene. In a gene knockout, the objective is to disrupt or eliminate the expression of a particular gene and zinc finger nucleus are designed to target and bind to the specific DNA sequence of the gene to be knocked out. So, you are going to do the double stranded breaks and the zinc finger nucleus induce TFN at the target gene locus activating the cells repair mechanisms. Non-homologous end joining or NHEG in the absence of template for the homologous directed repair, the cell often employed the error prone non-homologous end joining pathway to repair the double standard break. This frequently result in the small insertion or deletion at the side of the break.

So, what will happen is that you are actually going to use a zinc finger nucleus. It is actually going to cut a specific DNA sequences and then it is actually going to generate the double standard breaks and those double standard breaks are actually going to filled with the help of the exploiting the non-homologous end joining pathway. And then ultimately there will be a functional disruption. The Intel introduced during NHEA can lead to the frame sheet mutations, premature stop codon or other alteration that disrupt the normal functioning of the target gene resulting in a knockout phenotype. So, this is exactly what is going to happen that zinc finger nucleus what you have introduced is actually going to recognize a specific DNA sequence and that is how it is actually going to introduce our double standard DNA break.

And then these double standard DNA breaks are going to be filled with a random sequence And as a result of these random sequences, it is actually going to cause the frame sheet mutations, it is going to cause the deletions and other kinds of things and as a result it is actually going to make this particular gene non-functional and that is how it is actually going to have the gene knockouts. This means that particular gene is no longer be functional in this particular in this organism containing this particular genome. And similar to that we also can do the gene knock ins. So, introduction of a desired sequence to achieve the gene knock in a specific sequence is desired to be inserted into them. This can be done by a new gene or modified version of an existing gene.

Then you are going to design the zinc finger nucleus. So, zinc finger nucleus are engineered with a zinc finger DNA binding domain designed to recognize and bind to the target side of the genome where the insertion is intended. Then you are going to generate the double standard breaks. So, zinc finger induce with double standard breaks precisely at the target side and then you are going to do the homologous directed repair mechanisms and as a result the homologous sequence are actually going to replaced the this double standard break DNA and that is how you are actually going to introduce the new gene into

the organism or by the genome. So, what you are going to do is first step is same that you are actually going to use the zinc finger nucleus it is going to break cause the double standard break and after that you are actually going to put a donor DNA right.

This donor DNA is going to have the homologous arm right and this homologous arm is actually going to go with the homologous recombination and as a result this particular DNA is actually going to be introduced or going to be inserted into the genome and that is how it is actually going to be resulted into the gene knock ins which means you have actually introduced a new gene into the organisms or the genome. Now you if since these two processes can be possible that you can actually be able to remove a particular gene you can be able to introduce a particular gene you can also be able to do the mutagenesis right. So, then you can actually be able to do the insertion of a mutated gene you can actually be able to do other kinds of things. So, that also can be a application that you actually can use the zinc finger nucleases for the targeted mutagenesis. So, in that case what you are going to do is you are going to just remove a particular nucleotide or a codon and then you can actually be able to put your specific codon what you want right.

Then you can also use the modification of the genome with the help of the zinc finger nucleus. So, just what we have discussed so far right you can actually be able to do the knock in or knock out and all those kind of thing and as a result you can be able to even modify a genome using the zinc finger nucleus. This is one of the example what is showing right there is a pig right and it actually has the normal cell and what you can do is these you can just isolate these normal cells which actually contains you know the wild type genome. So, I will say the wild type genome right and all the cells are actually containing the wild type genome right what you can do is you can utilizing the zinc finger nucleus you can actually be able to introduce or target some of the cells and you can actually be able to generate the for example, the mutated genome right. So, you can actually be able to make the further genome and then you can actually just isolate these cells and then you can just introduce these cell back into the pig and what you can see here is this pig does not have a skin pattern, but this pig is actually showing a skin pattern because you have introduced a gene which will actually cause the patterning onto the skin and that is how it is actually been cause the production of the transgenic pig actually right.

You might have seen that I have I have discussed different types of transgenic animals and this is the more or less the smarter way of generating the transgenic animal. Similarly you can actually be able to do many things like you can actually be able to do the multiple ways in which you can be able to generate the transgenic animals. You can also be able to use the deletion of a chromosomal sequences in the human cell using the zinc finger nucleus and this is what it is actually going to say that you are actually going to target a particular DNA sequence and that is how you can be able to remove the particular sequence and you can actually be able to put the new sequence and that is how you can be able to generate deletion and as well as the addition into the human cell using the zinc finger nucleus. Since you can be able to do that you can actually be able to use that for crop improvement. So

advancement of the horticulture crop with desired trait in the contemporary and future agilators rely significantly on the genetic engineering.

Reverse genetics has played a pivotal role in cropping improving the crop genetic quality by elucidating the gene functions and that is how you are actually going to use the multiple approaches. You can actually be able to use the zinc finger nucleases you can actually be able to use the targeted delivery of that particular sequence and that is how you can actually be able to use the agrobacterium mediated DNA transfer and so on and that is how you can be able to make the genetically modified crops and that will actually going to improve the crop yield and other kinds of things. Then another crucial application of the zinc finger nucleus is the in the plant biotechnology is the development of the stacked transgenic traits. Currently the gene stacking process involves the breeding stacks of the randomly inserted event requiring the independent sorting characterization and intercession into the elite germplasm. This method is both time consuming and expensive.

Zinc finger nucleus offer a solution by precisely integrating a new transgene into a previously integrated trade land pad facilitating the creation of the transgenic trade stacks. In this way the herbicide resistance traits were stacked using the precise precision gene targeting through the gene finger nucleus. So, this is what it is actually going to say that you are actually going to target a particular sequence into the gene and that is how you are actually going to generate or put the particular gene and that is how you can actually be able to make the herbicide resistance crops and so on. Then we can also be able to use the chromosome with the NMC targets and so on and this is more or less they all are actually going to have the similar kind of application where you are going to use the cut of the particular genome then you are going to introduce the new DNA and so on and you can actually be able to use the zinc finger nucleases for that particular applications. Now, let us move on to the next tool and the next tool is the TALENs.

So, application of the TALEN. So, customized zinc finger nucleus and TALENs have revolutionized the genetic research by efficiently introducing the target state alteration in the various organisms and cell type. And the application wise the TALEN and the zinc finger nucleus are doing exactly the same except that the tools are different. Zinc finger nucleases are little difficult to perform compared to the TALEN, but the overall mechanisms remains the same. You are actually going to have you can be able to do all the things what you are doing with the zinc finger nucleus with the help of the TALEN. So, in the help of the TALEN also you are actually going to produce the like for example, you can actually be able to produce the disease resistance rice.

So, this is an example of how you can be able to use the reduction of the disease resistance rice right. And here what we have they have done is they have just used the TALEN to introduce a double standard break and then they have introduced a new gene which is called as OS1193 gene and that is how they are actually been able to produce the disease resistance rice. Similarly, you can be able to use the mouse editing as well as with the help

of the TALEN based gene editing. So, the Y chromosome which is a unique structure and specific gene contain crucial for the male sex determination and fertility presents challenge for the conventional gene targeting in the mouse embryonic stem cells. So, previous attempt using the traditional studies have been largely unsuccessful in generating mutation in the Y link gene, but with the help of the TALEN you can be able to even generate the mutations into the Y chromosome.

And then you can actually be able to use the TALEN based knockout as well as the knock-ins right. So, you can actually be able to use the TALENs offer a versatile and accessible platform for modifying the livestock genomes. And in contrast with zinc finger nuclease the TALEN overcome the technical challenges and the budget constraint. So, that is only the differences between the TALEN and the zinc finger because zinc finger is slightly costly and it is more challenging to perform compared to that the TALENs are easy to perform and they are actually more cost effective. So, then TALEN activity in the bovine embryo.

So, these are also going to be one of the application area where the TALENs are being used for you know developing the different types of embryos and all the kinds of things. Then TALENs are also being used for genome editing in protecting the you know the cells or protecting the organism from the HIV infections. So, HIV primarily utilizes the coreceptor like CCR5 and CXCR4 along with the CD4 for cell entry. So, individual with a homozygous CCR5 delta 32 deletion exhibits resistance to the HIV. So, if you have a CCR5 which does not contain the 32 right which does not contain the 32 region then it is actually going to resistance for HIV.

So, it is like CCR4 silencing or anti CCR antibodies and small molecule inhibitors have shown efficacy in inhibiting the HIV infection. So, genome editing approach initially focused on the CCR coreceptor using the zinc finger nuclease progress to the human trial with the phase 1 clinical trial showing promises. So, the CCR4 concern about off target effect and cytotoxicity remain with the zinc finger. TALEN offer advantage in terms of minimal cytotoxicity and off target compared to the other genome reading technology. So, with the help of that you can be able to target the CCR5, you can be able to target the CXCR4 and so on and that is how you can be able to develop an organism which is HIV resistance.

TALEN have emerged as a powerful genome editing tool with a wide range of lecture. One notable application is the modification of a model organism where TALEN enable the efficient introduction of the targeted alterations. So, there are enormous applications of these genome editing tools and we cannot you know and there are so many different avenues which are still been exploring by the scientist. So, since the applications are endless and the potential of these tools are enormous it is difficult to encompass those into the single lecture ok. So, what we have discussed? We have discussed about the application of the zinc finger nuclease, the mechanism of how the zinc finger nuclease are causing the knockout or the knock ins and then we also discuss about the application of the TALENs.

So, with this I would like to conclude my lecture here and in subsequent lecture we may discuss some more aspects related to application of the molecular biology. So, with this I would like to conclude my lecture here. Thank you. Thank you.