Molecular Biology

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Module - 12

Applications of Molecular Biology

Lecture-49 Applications of Molecular Biology (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 within the course called molecular biology. So, so far what we have discussed, we have discussed about the different aspects of cell biology, we have discussed about the central dogma of molecular biology. So, within the central dogma of molecular biology, we discussed in detail about the replications, transcription and translations. And in addition to that, we have also discussed about the different types of molecular techniques which are being developed.

And continuing that discussion, we have also discussed about the genome editing in the previous lecture. So, now since we reached to the end of this particular course, we have decided that we should also discuss about the potentials of studying the molecular biology and how that can be helped to develop the different types of techniques and different types of products which can be very very helpful for the human society. So, if you see the molecular biology, the molecular biology is a very diversified you know field. So, it actually has a different types of tools and techniques and all those kind of avenues and that is why it has extensive applications in the different fields of the biotechnology or in general it is having application in the so much diversified field that it is very difficult to incorporate all the possible fields.

But what I have listed here is only the fields which are very very common and very very popular. So, for example, the molecular biology has a very extensive two application in the agricultural field, pisciculture, poultry, vaccines, it is been used extensively for developing the transgenic animals, different types of medicines, then it also been used for developing the genetically modified organisms and drug delivery. So, since the applications are so much diversified it is very difficult to incorporate or and discuss all these applications in a couple of lectures. So, I have decided that I will going to only focus on the three or four different aspects. So, that it will give you an idea that what will be the potential of the molecular biology and what could be done actually with the using the this particular technique.

And then with this brief discussion it could be possible that you may be able to understand

the importance of the molecular biology for the human society and it also can help you in designing and developing your own product. So, what we are going to focus we are going to focus on to the four aspects. We are going to focus on the genetic engineering, we are going to focus on the PCR based applications, we are going to focus on the how the molecular biology is been used for developing the different types of transgenic animals and how these transgenic animals are having the role in the different aspects related to the whether it is related to poultry or whether it is related to other fields. And then we also going to discuss very briefly about how the genome editing approaches can be having the applications in the diversified field and how that can be used. So, let us first start with the genetic engineering and then we are going to discuss about the transgenic animals and then we also going to discuss about the PCR based applications and then we also going to discuss about genome editing.

So, as I said in the genetic engineering this we have discussed in detail that what is the genetic engineering. The genetic engineering is that you are actually going to engineer the DNA and you are actually going to allow the development of the genetically modified organism. And this is actually the general schemes which actually going to be follow where you are actually going to isolate the particular gene from the genome. And if you recall in previous module we have discussed about how you can be able to isolate a particular gene fragment either utilizing the genomic library or the cDNA library or utilizing the polymerase chain reactions. And then once you got the gene then you are actually going to digest that with the restriction enzyme that is actually going to generate the cohesivants.

And then once the cohesivants are being generated the similar procedure you have to follow for the vector also. And then you are actually going to get the cohesivants both on the vector and as well as on your insert then you are going to put them into the ligation reactions. And the post ligation you are going to transform that or you are going to deliver this DNA into the host cells. And then you are going to screen and select the desirable clones and then ultimately these clones are ready for the future development or future applications. So, ah as far as the genetic engineering is concerned these are the tools which are required for doing the genetic engineering and these are the important procedures which are actually been important for doing the genetic engineering.

Where you are actually going to do the isolation of genomic DNA, polymerase chain reactions, restriction enzymes, ligations and all that. And once you are done with this you are actually at the end of this you are actually going to have the products right. You are actually going to develop the products or you are actually going to generate the enzymes which are actually going to have the diversified applications in the different industries. So, ah if you talk about the dairy industry right ah the dairy industry is extensively been using these products which are been developed by the genetically modified organisms. So, they are actually producing the different types of proteins or they are also producing the different types of enzymes and these enzymes are been having the extensive applications in the food industries.

So, you can have the enzymes which are having the extensive role in the dairy industry, you are also having the enzyme which are having the role in the brewing industry, baking industries, wine industry and as well as the meat industries. And we will not going to discuss in detail about these enzymes and how they actually going to perform the different types of functions and how they are actually going to be useful, but ah very briefly we will talk about what are the how these enzymes are ah having the application in the particular field and how you are actually going to be produce those enzymes. So, first we start with the dairy industry. So, in the dairy industry we have the 4 enzymes which are called rennet, lactase, protease and catalyst and all these are actually having the different types of applications and all these enzymes are genetically been cloned into a particular over expressing ah cell and that is how they are actually been produced on to the industrial scale and then they are actually been used into the dairy industries. So, as far as the rennet is concerned ah it is extracted from the stomach of the young calf and apart from that it also been developed by the ah recombinant DNA technology.

This contains the enzyme that cause the milk to become the cheese. So, it is actually an enzyme which converts the milk into cheese and it separates the solid curd and the liquid whey and the different animal rennets are also been used for the different cheese and most common vegetable rennet is thistel ok. So, rennet is an enzyme which is actually been used for converting the milk into the cheese and that is how they are actually going to help in ah in the in the dairy industries. Then we have the lactase. So, lactase is present in the brush border of the small intestine it is artificially extracted from the yeast and it is required for the digestion of the whole milk and it is used in the production of lactose free ah milk right.

So, you know that the milk is ah is a dairy product which contains the sugar right and the sugar component of the milk is always the lactose and you know that the lactose intolerance is a very very big issue because if you if the somebody is lactase insensitive ah then it is actually going to develop ah it this lactase will lactose is not going to be digested. Then as a result the lactose is going to be remain undigested and remain in the stomach and that is how it is actually going to cause the production of gas and bloating and all those kind of things. So, to avoid that you are actually can get the milk which is free of lactose and how you are going to do that you are actually going to ah digest the milk with the help of an enzyme which is called as lactase. So, what the lactase is going to do is it is actually going to ah you know take the lactose from the milk and it is actually going to chew up right. So, it is actually going to eat up all these lactose and it that is how it is actually going to make the milk which is free of lactose and that lactose free milk is actually having a very high economical values compared to the normal milk because that milk can be given to the patients, it can be given to a special people who are actually having the lactose sensitivity and lactose intolerance and all that.

Ah It is also been used in the production of ice cream and the sweetened flavor and the condensed milk. Then we have the catalyst. So, it is catalyst is produced from the bovine

liver and the microbial sources. It break down the hydrogen peroxide to the water and the molecular oxygen and along with the glucose oxidase it is used in the treating the food wrappers to prevent the oxidations and it is also been used to remove the traces of hydrogen peroxide in the process of cold sterilizations. So, the catalyst is very very important for detoxifying the hydrogen peroxide which is going to be residual be present into the food products because many of the food products are been sterilized with the help of the hydrogen peroxide and the hydrogen peroxide is toxic.

So, to remove the hydrogen peroxide you are just adding the catalyst and the catalyst what catalyst is going to do is it is going to convert the hydrogen peroxide into the water an oxygen. Then we have the proteases. So, proteases are the general enzyme they are actually been used in the many industries they are been used in the you know the dairy industries, the meat industries and all that. So, proteases are been widely been distributed in the biological world and they hydrolyzes the specific peptide bond to generate the paracase paracasein and the macro peptide in the production of cheese and that results in the bitter flavor to the cheese and also in a desired textures. Then let us come to the brewing industries.

So, within the brewing industry as I said you know protease is very very common with within the dairy within the different types of industrial setup. Then we can have the beta glucanase, we can have the alpha amylase and the amylo glycosidase. So, protease is protease works to provide the what with the amino acid nutrient that will be used by the yeast right. So, and protease work to break up the large proteins which enhance the head retention of the beer and reduce the haze and it fully modified the malt these enzyme have done with their work during the malting process. Then we have the beta glucanase.

So, beta glucanase represent a group of carbohydrate enzyme which break down the glycosidic bond within the beta glucan. It aids in the filtration after the mastication and the brewing. Then we have the alpha amylase. So, alpha amylase convert the starch to the aedestrine in producing the corn syrup and it stop solubilizes the carbohydrate found in the barley and other the several cues in the brewing. It decrease the time required for the mashing.

All these technical terms like mashing and brewing and all those we are not going to discuss and neither I expect that you should know all this, but if you are interested you can read about these content and read material in somewhere else. Then we have the baking industry. So, in the baking industry you can have the maltogenic amylase, you can have the glucose oxidase and you also have the pentonases and all these are actually having the diversified function and the applications within the baking industries. So, maltogenic amylase. So, it is a floor supplements, it has anti-stalling effect, it modify starch while most of the starch starts to gelatinize.

Resulting starch granules become more flexible during the storage. Then we have the

glucose oxidase. Glucose oxidase oxidizes the glucose and produce the gluconic acid and the hydrogen peroxide. hydrogen peroxide is a strong oxidizing agent that strengthen the disulfide and non-dulciferide cross link in the glutens and it good working condition help proper functioning of the baking bakery systems. Then we also have the pentonases.

So, it exact mechanism is not yet discovered. It improves the dove mechanability yielding a more flexible easier to handle dove. The dove is more stable and give better oven spring during the baking. Then we also come to the wine industry. So, in the wine industry we have the two enzyme dot the pectinases and the beta gluconeases which have the extensive role and both of these enzymes can be produced with the help of the recombinant DNA technology.

So, pectinases prevents the pectin from forming the haze and hence to get the clear solutions. Similarly used for the extraction of color and juice from the fresh fruits, it break down the pectin and releases the methanol and high amount is hazardous. Then we also have the beta gluconeases and it accelerate all biological mechanism linked to the maturation on leaves. It reduces the maturation durations and it improve the clarification and filtration and improve the action of the ah finding regions. Then we come to the meat industry.

So, within the meat industry we have the two enzyme which is called as the proteases the papain. The proteases or the papain both are actually being used extensively in the meat tenderizations ok. So, meat tenderization actually enhances the ah economical value of the meat and also enhances the taste of the meat and that is how it actually been very very desirable and both of these enzymes are being cloned and over expressed and that is how they are being used in the industry. So, first is the proteases. So, proteases ah cleave the bond that hold the amino acid together as the enzyme break down protein which will disrupt or loosen the muscle fiber and tenderizes.

So, when you treat the meat with a ah with a ah protease it is actually going to you know break the peptide bonds and that is how it is actually going to make the meat little soft and easy to digest and it also gives the some different flavor and that is how it is actually going to be ah used in enhancing the taste of the meat and that is how it is actually been desirable to have this particular type of ah enzyme in large quantity and that is how they are being used ah they are being produced with the help of the recombinant AI technology. Then we have the papain. So, papain which is found in papaya 95 percent of the meat tenderization available in grocery stores are made from the papain it is extracted from the latex in the papaya fruits and these enzymes are purified and sold in the powder or the liquid form and that is how they are actually been used ah. Now, let us come to the ah another field where the product of the genetic engineering can be used. So, another industry is the medicinal ah medicinal world ok.

So, in the medical medical world the ah the product of the recombinant technology is been

used extensively as a drug, as a vaccine, as a adjuvants, as in the gene therapy and all those kind of things and all these are actually requiring the knowledge of the molecular biology. So, let us first discuss about the applications in the medicinal science. So, medicines are class of molecule used to correct the disturbance in the host physiology. They can be chemically in nature and used to inhibit the abrid enzyme activity from the host or pathogen. In few cases the host enzyme can be supplied as a drug formulation to drive the biological reaction.

Biotechnology has a potential to contributing into the development of the drug molecule and biotechnology means the genetic engineering right. So, with the help of the genetic engineering the combinatorial technology you can be able to clone these ah proteinaceous substances or the enzyme and then you can be able to supply. So, you can actually have the four different classes. One is the production of therapeutically important proteins, you can actually be able to do a gene therapy, you can be able to develop the vaccines and you can also be able to produce the monoclonal antibodies. And all these aspects require the extensive knowledge of molecular biology so that you can be able to manipulate the cells, you can be able to clone a particular gene and you can be able to use that for the different types of applications.

So, let us first discuss about the production of the typically important proteins. So, a large number of genetic or metabolic diseases can be corrected by supplying the protein or the factors. Following the advancement in the biotechnology many other proteins or factors are produced in the different bacterial expression system. In an approach the gene of the enzyme or the proteinaceous factor is cloned into the appropriate plasmid to produce the recombinant clone. One of the such example is the human insulin right.

Human insulin is one of the widely ah you know supplied ah biological product right and you know that the diabetes is a very big disease right and the human insulin is a is actually been required to lower down the blood glucose and that is how it has a very huge ah market in which you can be able to use. So, earlier when the recombinant technology was not known, the human insulin is always been isolated from the animal sources and then they are actually been given to the patient and the major drawback of this particular approach is that since you are giving the insulin from the ah from the animals, they may actually have the allergic reactions or they may actually not be get accepted by the human system right and that is how in those cases in those era the insulin was creating lot of problems. So, to ah correct that particular problem what people have done is they have a when the people were you know ah recombinant known the recombinant technology how they will know how to clone the ah gene and how you can be able to use that for producing the protein, what they did is they have cloned the insulin. So, insulin is a protein of two ah chain, you have a chain, you have a b chain and both of these a and b chains are connected with the help of a disulfide leakages right. So, what you can do is you can produce the a chain, you can produce the b chain separately and then you just combine them and that is how it is you are going to get a functionally active the insulin molecule.

So, what you are going to do is you are going to accelerate the gene a, you are going to accelerate the gene b, you are going to transform that into the bacteria and that is how you are going to get the gene ah peptide a right and you are going to get the peptide b and then ultimately you are going to mix them, you are going to do the you know the you are going to change the conditions in such a way that the disulfide linkages are going to be formed and that is how you are actually going to have the functionally active insulin. So, that is what it is written here that insulin is a dimer of a chain and b chain linked by the disulfide bonds composed of the 51 amino acid with a molecular weight of 5808. A schematic representation step of given right in this particular figure in this process the gene a and b is cloned into the bacterial plasmid separately to produce the two recombinant clones. Peptide chain a and b is over expressed in the E. coli and recombined together to produce the functional insulin.

So, this is just a simple example to show you the potential of molecular biology potentials of the combinatorial technology to say that how something which was very very crucial for the human ah welfare was being done with the help of the molecular biology. Earlier people were you know getting the lot of side effects, side reactions and all those kind of things. So, apart from insulin there are so many different types of hermetically important molecules are being generated with the help of the molecular biology. So, what we have is we have the protein ah factor 8 right and the factor 9 these are the factor which are required for the black clotting. So, they are actually been required for the treatment of the hemophilia.

Then we have the tissue plasmagen activator that is also been required for the thrombosis then we have the lactoferrin. Lactoferrin is been used for treating the GI tract infections then we have the human protein C that is required for thrombosis. Then we have the alpha 1 antitrypsin that is for the inphysema then we have the fibrogen. Fibrogen is required for the wound healing right. So, in some cases what happen is that when you get the wound you are not producing the enough quantity of fibrogen right.

And you know that the fibrogen is been get converted into the fibrin and these fibrin fibers are actually covering the wound and then only it is actually going to work to you know to heal the wounds. So, in those cases you are actually going to do the you know the you are going to use a recombinant tetralogy to produce the fibrogen and that fibrogen can be used for wound healing. Then you also have the pro 540 5 4 5 4 2 and that is been used for the HIV infections. Then we have the anti anti thrombin 3 that is for correcting the thrombosis. Then we have the collagen 1 that is been required also for the tissue repair and then we also require the serum albumin that is for increasing the blood volume ok.

This is very important right because if you are running with the low in protein you your blood volume is also going to be reduced to maintain the tonicity and as well as the osmobulartie. So, if you increase the protein volume into the your blood the blood volume is also eventually going to increase. So, apart from that you can also have the recombinant

chymosin and the recombinant human growth factors and recombinant blood clotting factor 8. So, these are the some of the proteins which are required for the different types of applications. Now apart from this you can also have the you can also be able to do the gene therapy with the help of the molecular biology.

So, production and supply of recombinant protein is a temporary solution for a treatment of a disease condition. In another approach the human expression system is used to produce the proteinaceous factor after inserting the recombinant clone into the human cell or inside the human body. And DNA is packed into the appropriate DNA delivery system like you can use the viral system, you can use the liposomes, you can make all those kind of things. To deliver the gene into the human cell to correct a mutated gene or encode a therapeutic protein drug to provide the treatment. So, gene therapy is also very very common and very very popular method through which you can be able to correct the problem at the molecular level right.

Now what you have seen that when we are genetically you know producing the proteins you are actually supplying only the protein part, but that protein is having the half life right. So, that protein get disappear after some time, but in this case what we are doing is we are putting the DNA. So, once we are putting the DNA into the cell, DNA will keep producing the protein for a long longer period of time. And if it is a permanent transfection, if it is a permanent integration then it actually going to also change the cells and then the cure is going to be permanent. So, there are two different types of gene therapy you can have the somatic gene therapy or you can have the germline gene therapy.

So, somatic gene therapy is for those cells which are like somatic cells like for example, muscles, liver, pancreas or those kind of thing. And then you can have germline gene therapy in this the therapeutic approach the germline cells like the sperm or egg are transformed by the introduction of required gene to produce the protein or correct the mutated gene. So, this is actually going to be done in the sperm as well as the egg. So, that the offspring the corrections are not going to be done in the that current gene current generation, but it also going to be done into the future generations. And in that case if this particular correction is going to be done for the several generations.

Whereas, in the case of somatic it is only going to be for that individual where you are doing the gene therapy. Whereas, for the germline gene therapy it is also for the incoming generation as well. So, the technical problem what is associated with the gene therapy is that it is short lived because as I said you know you are going to insert the DNA into the cell and recombinant DNA may express may not express or it may actually get rejected by the system. Then we also have the immune reactions because you are injecting the virus containing gene and all that.

So, it is also can have the viral infections viral reactions. Then since you are using the viral vectors which can cause the you know the immune reactions adverse immune reactions

and toxicity. And then it also can disturb the human physiology because it also the gene in to get into a wrong place in the genome it may cause the functional defects. And this is what we have discussed when we were discussing about the homologous and non homologous recombination as an approach to for the genome editing right. So, this is what one of the approach where you are actually going to put the flanking sequences as a homologous recombination homologous sequences. And then utilizing these flanking sequences the gene of your gene what is present on to your vector is going to be inserted into the vector into the into the genome of that particular cell.

But if any of these events are go wrong then the gene may get integrated into a off target sites and that actually is going to cause the problem into the host physiology. Because then it is actually this see it is not going to correct the problem, but it is also going to make the additional problem because the it may replace some of the gene which was working correctly. Now, apart from this you can also be able to use the recombinant technology for producing the vaccines. So, vaccine is been given to develop the immunity against a disease in a human or the vertebrate animals. Vaccines are of different types like the dead or attenuated organisms or the protein derived from them.

There are different strategies to enhance the immunological responses to give the long lasting protection against a disease with the minimum adverse effects. There are four different types of vaccines you can have the killed vaccine, you can have attenuated vaccine, you can have a toxoid, you can have a subunit vaccine and you can also have the conjugate. So, all of these approaches are requiring the one or other tools of molecular biology for developing the vaccine. You might have seen the COVID vaccine how the people have utilized the molecular biology tools to develop the COVID vaccine in a small very short span of time and that is the potential of the molecular biology. So, this is what we have discussed in the in the genetic engineering.

So, within the genetic engineering you are actually going to produce the proteins or you are actually going to produce a recombinant DNA and that can be used for generating the different types of plasmids or different types of products. And that different types of product can be used for treating the different types of diseases or it can be used for correcting the particular type of errors into the metabolic reactions. So, with this I would like to conclude my lecture here in our subsequent lecture we are going to discuss some more applications of the molecular biology. Thank you.