Course Name: Basics of Crop Breeding and Plant Biotechnology

Professor Name: Dr. Joydeep Banerjee

Department Name: Agricultural and Food Engineering

Institute Name: Indian Institute of Technology Kharagpur

Week: 01

Lecture-05: Concept of Gene and Experiments on Plant Hybridization

Welcome back. So, we will continue again. So, now gradually we will elaborate the process of transcription. So, in transcription what happens, DNA to RNA formation is taken place. Ok! In case of prokaryotes the major regulation is done in the initiation part of the transcription, different factors are involved there. So, what is the major enzyme involved here? We have discussed that is RNA polymerase. Ok!

So, let's discuss about the transcription process in general. So, if you think about the prokaryotic gene expression, their naked DNA is available within the cytosol, no nucleus is available there, no chromosome is available there. Ok! So, their transcription process is relatively easier. While in case of eukaryotic gene expression, the DNA is highly coiled, the nucleosome is available there, DNA is packed within the chromosome.

So, it is much more complex. Ok! So, the initiation process relatively easier in bacterial system or prokaryotic system and it is much more complex in eukaryotic system. In eukaryotic system, especially, the packed DNA has to be unwound properly, the nucleosome should be relaxed, different histone proteins available within the nucleosome, I have mentioned about histone proteins available in the nucleosome, it should be modified. Ok! And finally, we can see, the double stranded DNA stretch will be available, that could be accessible by RNA polymerase. So, the RNA polymerase has to access the DNA, otherwise it cannot initiate the transcription process.

Now, suppose, it is a double stranded DNA, it is 5′ to 3′ and this one is 3′ to 5′ strand.

So, for transcription initiation, let's assume in this particular DNA, this is the transcription start site of a particular gene, means the first base will be transcribed from here. So, if it is the transcription start site in the upstream position, the -10, -35 regions is available in case of prokaryotic transcription or the -25, -30 elements are important in case of eukaryotic transcription. So, within this region in the minus, in between -10 to -30, let's assume in between this region, the major transcription factor binds with the RNA polymerase. So, RNA polymerase binds over here along with other different transcription factors. Ok!

In case of prokaryotes, one of the important factors that is involved in transcription is sigma factor, different sigma variants are there. While in case of eukaryotic transcription, a huge complex is involved along with the RNA polymerase, several factors are there like transcription factor 2D, 2E, 2F, different transcription factors are involved in this initiation process. Ok! But the thing is that, RNA polymerase has to bound to the upstream region along with some transcription factors, they will target those regions on the DNA available in the upstream part of the DNA. So, once this transcription factor and RNA polymerase binds, thereafter, this double stranded DNA basically will be relaxed little bit. And once this relaxation is done, thereafter the actual transcription will be started. Ok!

So, first of all, the RNA polymerase has to bind along with different transcription factors in the upstream region and after binding the double stranded DNA is relaxed, then the transcription, actual transcription is started. So, in transcription, 3 steps are there- initiation, elongation and termination, this is the initiation part, that is very important part in case of prokaryotes as well as eukaryotic system. In case of prokaryotes, it is a major check point of gene regulation, while in case of eukaryotes, along with the initiation, in other parts also some regulation could be occurred. So, now, we will discuss about the elongation as well as gradually we will move into the termination part. So, let's think that it is an unknown DNA it has.

So, let us think that it is the unknown part of a DNA, it has 5′ to 3′ end and another end

is 3′ to 5′ end, ok! Suppose, we are covering this part also means the DNA has been unknown we are just covering this part over here. So, let's assume over here, we have a few sequences like on one strand of the DNA, we are talking about DNA now, A T G C C G A this sequence is available on one strand of the DNA, in its opposite strand, the sequence will be T A C G G C T. Ok! Now, this is, we are discussing, now the elongation step of the transcription. In elongation, basically the transcription initiation occurs then from the transcription start site, different bases will be added one by one based on its complementary sequences.

Now RNA formation is taken place in 5′ to 3′ direction. So, basically this strand of the DNA, i.e. the 3′ to 5′ strand of the DNA will be used as a template and in this way, the newly formed RNA will be gradually transcribed. So, what will be the first base over here? It will be complementary to this one, it will be A, here it will be U because no T will be available in RNA, in spite of thymine, uracil residue will be there, here it will be G, here it will be C, here it will be C and here, it will be G, because it is forming pair with this particular strand of the DNA. So, what RNA sequence we are getting? We are getting a RNA sequence of A U G C C G in this way. Ok! This is the 5′ this is the 3′ end. So, now, on this DNA if you see carefully, initially two strands were there this strand the 3′ to 5′ strand is known as the template strand because using this particular strand, the RNA synthesis is taken place. Ok!

While, if you see about this strand, the 5′ to 3′ strand, this strand is similar to the mRNA sequence, almost just one variation is there, in case of RNA no T will be available, there uracil (U) will be available. So, this strand is known as coding strand. So, in DNA, will be having coding strand or it is also known as non-template strand. While another strand which is used as template strand, that is non coding strand. Ok! So, in DNA, two strands are there, no confusion should be there.

So, in this way, the elongation is taken place and finally, we can reach to the termination part of a particular gene. So, now, we will discuss about the termination. In case of transcription termination, in case of eukaryotes ok, once the newly synthesized mRNA is

formed, then some uracil residues are available and a hairpin loop like structure is formed on the newly synthesized RNA. Once that loop like structure is formed, thereafter, the RNA polymerase movement is hindered and finally, RNA polymerase falls off from the DNA-RNA hybrid. Ok! Because if you just recap the initial part, once the transcription is in full fledge within the double stranded DNA, the RNA is being synthesized, the RNA polymerase bound over there.

So, it is a hybrid structure DNA-RNA-RNA polymerase; everything is there. So, in case of prokaryotic transcription, at the termination part, once this loop like structures are formed, hairpin loop like structures are formed, then RNA polymerase falls off and DNA-RNA hybrid is also released and finally, the mRNA or RNA could be separated. Two means are there, one is rho dependent termination; one is rho independent termination. Mostly we have discussed about rho independent, in rho dependent termination, just another protein rho factor is involved there. It basically binds somewhere on the nascent mRNA, that is rho utilization site and gradually it go to the 3′ end of the RNA and once it reaches that type of loop like structure, finally, it cause the release of RNA polymerase from the DNA-RNA hybrid.

In case of eukaryotes, the transcription termination is little bit different. In case of eukaryote, it has been found that on the RNA, few proteins bound on the RNA and once the particular gene has been transcribed totally, thereafter, also a several basis transcription is taken place means, wherever the gene is supposed to be finished, thereafter also transcription goes up to few bases. So, then two different proteins join the RNA and eventually they cleave the RNA from a particular site and after this cleavage, some modification is taken place on the RNA, later on, we will discuss those things. So, let’s discuss that what are the different RNA polymerase available in eukaryotes. In prokaryotes, only one type of RNA polymerase is there, while in case of eukaryotes, at least four to five types of RNA polymerase are there. Ok!

Major RNA polymerase in eukaryotes are RNA polymerase I, II and III. So, RNA polymerase I is responsible for large rRNA genes, ribosomal RNA genes. Ok! So,

ribosomal RNA that is involved in the formation of ribosome along with some RNP ribonuclear proteins. Then polymerase II, it basically codes most of the protein coding genes which code for mRNA messenger RNA is produced by RNA polymerase II. And RNA polymerase III, it transcribed tRNA genes that is transfer RNA genes some small nuclear RNA genes and 5S rRNA genes. Ok!

The larger rRNA that is 20, 8S or 5.8S those are basically coded by the RNA polymerase I. So, these are major 3 RNA polymerase in eukaryotes. Now in eukaryotes, the RNA polymerase IV and V has also been discovered. Ok! Those are mostly found in plants and they transcribe small interfering RNA ok, which is involved in transcriptional silencing, siRNA involved in transcriptional silencing.

So, now we will discuss about translation in brief. So, in transcription, if mRNA is produced that mRNA will undergo translation only, right? If rRNA, tRNA, snRNA is produced, it will not go into translation. So, we will discuss about these things how mRNA is available, how ribosomal subunit binds on the mRNA, how tRNA comes into the picture, how codon-anticodon pairing is taken place and how E site, P site and A site are available on the ribosome and how translation is finally done. So, let us think that we have an mRNA because from mRNA translation could be formed.

So, during translation, first thing on the mRNA, the ribosome should bind. A ribosome will have a smaller subunit and larger subunit. So, it is the larger subunit, it is the smaller subunit of the ribosome. Then in mRNA, different sequences will be available. Suppose over here, this sequence was available AUG and GGG. Ok!

These sequences were available thereafter CCC sequence was available, in this way different sequences are available on the mRNA. So, what happens, first of all in translation along with the ribosome, the tRNAs are also involved, that is transfer RNA. So, they basically transfer different amino acid in this translation process. So, in our system, in most of the organisms 20 different types of amino acids are available. And, different tRNAs are available that are charged with this particular amino acid.

So, one tRNA will be charged with a particular amino acid. Suppose, one tRNA could be charged with methionine, one tRNA could be charged with proline. Ok! So, that particular amino acid will be available on the tRNA. So, now what happens during translation, first ribosome binds on the mRNA. The scenario will be little bit different in prokaryotes and eukaryotes, I will mention.

So, first, briefly let me tell you how the overall translation process takes. First RNA polymerase II subunits bind to the mRNA, then one tRNA molecule comes over here which is, which is already charged with a particular amino acid. So, this amino acid will be available based on the sequence available over here. Ok! So, in mRNA these 3 nucleotides are present one by one, these 3 nucleotides are known as codon. So, codons are available on the mRNA.

While on the tRNA, which is pairing with this particular codon, few bases will be there that will be complementary to these 3 bases and this is known as the anticodon. So, in this way, codon-anticodon pairing is taken place between the tRNA and mRNA and on this tRNA, a particular amino acid is available. So, this is the initiation of translation, means translation is initiated in this way. Thereafter, once, we are moving into the elongation part, then next tRNA will come over here. It will be having maybe another amino acid based on the sequence available on its anticodon.

So, this codon-anticodon pairing will be taken place and based on that specific amino acid, charged aminoacyl tRNA will be coming over here. So, then a phosphodiester bond formation is taken place in between these 2 polypeptides in between these 2 amino acids, sorry not phosphodiester bond, polypeptide bond is formed between these 2 amino acids ok. So now, once this step is over, means this polypeptide has come over here, then what will be happening in the next part? On the mRNA, we had AUG then G, sorry, then GGG then we had CCC AAA different sequences were there.

So, once this polypeptide will be transferred, this amino acid will be transferred, then

basically the ribosome will move little bit. The ribosome will move into the next part of the mRNA and whatever was available over here, the tRNA which was available over here, that is known as the P site. This site is known as A site. So, the tRNA which was available in the P site over here, in this case, that tRNA, that empty tRNA will be have come over here. While this is the tRNA having 2 amino acids and the third tRNA and the third tRNA will come over here, its amino acid will be based on this codon-anticodon pairing.

So, the thing is that I have told that ribosome is moving in this direction across the mRNA, it has moved little bit. So, the tRNA which was initially in the P site will come into the E site, that is the exit site, no amino acid is here. So, the next step will be again polypeptide bond formation will be taken place between these 2 amino acids and this newly introduced amino acid. So, in this way, eventually a polypeptide chain will be formed on the tRNA. So, that is the process of elongation in translation.

In translation, initiation occurred, the first tRNA came then this is the elongation part, 1 tRNA is being empty, gradually it is coming to the E site in between P site is there and here A site is there, E, P, A in this way 3 sites are available on the ribosome. So, how the termination is taken place in translation? During termination, 3 codons are there, if 3 sequences are available on the mRNA, those are UAA or UAG or UGA. If these sequences are available at that time, for these 3 codons no specific tRNA is available. Basically, if these sequences are available on the mRNA, then in-spite of the availability of any incoming tRNA, a particular protein release factor will come over here, in the A site, if these sequences are available. So, once the release factor comes, then basically 2 parts of the ribosome is dissociated, the mRNA is released and eventually the polypeptides which is formed over here due to addition of different amino acids, it will be cleaved from the tRNA and thereafter, it will go to the Golgi body for subsequent folding and other functions.

So, this is in brief the translation. Now, we will discuss about the basic structure of an eukaryotic gene. So, in a eukaryotic gene, the double stranded DNA is here will be

having a promoter sequence right, thereafter the transcription start site is available. So, these things are available in eukaryotic gene. Some sequences are available before the exon 1, this sequence is known as 5′ UTR. UTR is untranslated region, sorry, untranslated region, those regions are not converted into the protein part, but on the DNA, it is available and on the newly synthesized RNA also it will be available.

Then in most of the eukaryotic gene we can see different exons- exon 1, exon 2, exon 3, those exons are basically coding region of the DNA within eukaryotic gene, the coding as well as non-coding parts are there. The exons are coding region of the DNA, while the introns do not code for any amino acids or any particular codon. Then at the end of the most of the eukaryotic gene, we can see, we can see 3′ UTR also, 3′ untranslated region. So, let's see if the transcription is taken place, what type of transcript will be observed? First of all, after transcription we will be getting pre mRNA.

In the pre mRNA, we will be having this 5′ UTR, then exon 1, exon 2, exon 3 in between intron, intron and at last 3′ UTR. So, these things are available in the pre mRNA. We are discussing the eukaryotic gene and its transcription. Thereafter some processing is taken place, one of the important processing is splicing. During the splicing, the intron parts are excised out and only the exon parts they join together. Ok!

In this way, the splicing is taken place, because exons are only the protein coding part. So, thereafter, few other processing is taken place in eukaryotic mRNA, that is adenylation means 3′ polyadenylations, means poly A groups are added in the 3′ end and 5′ capping is taken place. So, once, we are discussing about the termination in eukaryotic gene, then we have told that some of the proteins cleave the newly synthesized RNA. So, thereafter, basically this poly A adenylation is taken place, different A residues are attached. So, then, once the translation is taken place from this matured mRNA, the translation is taken place and during translation from this coding part only, where only the exons are available, different amino acids are synthesized based on the translation, I have mentioned before.

So, in this way amino acid 1, 2, 3, it will form and a particular amino acid structure or a protein structure will be generated. So, a few things, I would like to tell that this type of processing like 5′ capping, 3′ poly adenylation and splicing, those are available in eukaryotes only. If you see in prokaryotes, they are transcription and translation taken place together, within a cytosol DNA is there, double stranded DNA, therefrom, RNA is formed and protein is formed. While in case of eukaryotes, within a nucleus, DNA is available, their transcription is taken place, thereafter, the matured mRNA transfer into the cytoplasm and the translation is taken place within the cytoplasm. So, now we can discuss on experiments on plant hybridization.

So, this is the rice flower, we know that rice is a self-pollinated crop, we have seen the experiment done by Mendel. So, can we plan any experiment on rice, let's see. So, this is the lemma, and this is the palea part of a rice flower. It has the anther, that is the male reproductive tissue, and the stigma is also there, rice is a self-pollinated crop and this is the pedicel, on which a flower sits on the particular plant. Now, these are the different reproductive parts of the rice flower in detail.

So, this is the male reproductive part, here anther is there you can see, and filament is there, in anther you can see some pollen grains are also available. In the female reproductive part, we have stigma, we have style and we have the ovary. So, now we will discuss another flower, that is also self-pollinated in nature and it is lentil flower. So, lentil flower, the flower structure is almost similar to pea. In pea plant in Mendel's experiment, you have seen different types of petals, right! Here also, different types of petals are there like standard, keel, boat, those type of petals are available.

So, these are the different parts of the lentil flower. So, these are the reproductive parts of the lentil flower. If you recall in Mendel's work, we had the 9 +1 anther in the pea flower, in lentil also the anther distribution is like that, the stamen are arranged in 9 + 1. Here in this picture, it is clearer, these are the different anthers 9+1, while this is the female reproductive part this is the stigma. So, here it is clearer, the 9+1 distribution of anthers in lentil and the stigma is also visible. In normally what happens, as it is a self-

pollinated crop, here you can see on the stigma, the pollen grains can sit and it can cause the fertilization within a closed flower, this the fertilization could be taken place.

So, if you have to plan some experiment if you have to plan to do plant hybridization, then first, we need to remove the anthers, otherwise self-fertilization will be occurred in rice or lentil, this type of crops. So, now, I will show you a small video where you can see how the emasculation is done in rice and how pollination could be done to get some hybrid plants. So, suppose, we have these two rice genotypes, one is having seed color of straw, and one is having some black color seeds. So, we are trying to make some attempt to cross these two genotypes and the process will be shown later on. Thank you. . . . . .
. .