Molecular Biology
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Module - 06
Transcription
Lecture-29 Gene Control Mechanism (Part 2)

Hello everyone, this is Dr. Vishal Tewedi from department of biosciences and bioengineering, IIT Guwahati. And in this particular course, we are discussing about the different aspects of the molecular biology. Now, let us move on to the next operon and the next operon is the tryptophan operon. So, tryptophan operon is a part of the anabolic operon, compared to that the tryptophan operon is a catabolic operon. So, the tryptophan operon founds in the E.

coli, it is a group of genes that encodes enzyme for the synthesis. So, remember that the first operon but we have discussed is for the breakdown of the lactose it is going to break down the lactose into glucose and galactose and that is how it is going to be drive the energy from the lactose molecule whereas, here you are actually going to consume the energy. So, this is actually an anabolic pathway, it is a anabolic or negatively controlled operon it always remain on in the normal conditions and off when the tryptophan level is high. So, this is exactly reverse what we have just discussed for the lacto operon.

Tryptophan does not need to be synthesized by the E. coli bacteria when it is present in the environment. Hence, the transcription of a gene in the tryptophan operon is turned off on the other side, when the availability of tryptophan is low the operon becomes on and the genes are transcribed by synthetic enzyme for the tryptophan synthesis. TIP repressor does not always attach with DNA instead it binds and inhibit transcription only in the presence of tryptophan. Tiptophan binds to the repressor molecule and alter their structure which switches an inactive repressor into a active state thus the tryptophan act as a corepressor.

Remember that this is very important tryptophan act as a corepressor because it enhances the repression activity of the repressor. So, it is become converts the inactive repressor into a active repressor. One unique feature of the tryptophan repressor is the attenuation. So, like regulation by the TIP repressor attenuation is a mechanism for reducing the expression of a tryptophan when the level of tryptophans are high. However, rather than blocking the initiation or transcription attenuation prevent the completion of the transcription.

So, this is a very unique feature of the tryptophan operon. Now, the structure of the

tryptophan operon. So, the there are 5 structural genes TRIP E, D, C, B and A that code for the enzyme involved in the conversion of the carboxylic acid to the tryptophan. Remember that we have already discussed about the tryptophan biosynthesis when we are talking about the amino acid metabolisms. So, TRIP E actually codes for the enzyme anthonyl synthase 1, TRIP D actually codes for the enzyme anthonyl synthase 2, TRIP C it encodes for the enzyme 5-phosphoryl anthonylate isomerase and the endole 3-bristol phosphate synthase.

Then TRIP B is encodes the enzyme tryptophan synthase B subunits and TRIP A is actually going to encode for the enzyme tryptophan synthase A subunit. The controlling site in the tryptophan lies next to the TRIP E and consists of promoter and overlapping operator and a leader region or the TRIP L. So, in these are the regulatory region. So, this is the operon and this is the regulatory region, you are going to have the promoter, you are going to have the operators and you are going to have the leader region which is called a TRIP L and then you are going to have structural genes like TRIP E, TRIP D, TRIP C, B and A and they are actually going to give you a polycystronic messenger RNA. So, it is also contain a repressor regulatory gene called TRIP R.

When the tryptophan is present the TRIP R protein binds to the operator blocking the transcriptional tryptophan operon by inhibiting the RNA polymerase binding. So, this is the repressor regulatory gene TRIP R. So, when it is going to be produced it is going to bind the tryptophan and that is how it is actually going to make the active repressor and that is how it is actually going to allow the binding of the repressor to the operator region and that is how it is actually going to block the transcription of the these genes structural genes. Reactions catalyzed by the enzyme synthesis from the tryptophan operon. So, this is anyway we have discussed in detail the biosynthesis.

Ultimately from the indole you are going to have the synthesis of the tryptophan where are you are going to have the activity of enthynyl synthesis, enthalase, transferase, PRA isomerase, IGP synthase and so on. So, all these genes are actually going to be of part of the tryptophan operons. This we are not going to discuss in detail because we have already discussed these things when we were talking about the tryptophan biosynthesis. Now, tryptophan operon regulations. So, in the absence of tryptophan which means then when the bacteria actually require the synthesis of the tryptophan.

So, there will be low tryptophan into the environment. When there is a little tryptophan or absence of tryptophan in the cell in this condition the tryp repressor is inactive because there is no tryptophan available to bind with the repressor and activate it by the conformational change. So, inactive repressor cannot bind to the DNA that is the operator or block the transcription which allows the tryptophan to be transcribed by the

RNA polymerase. So, once there will be an absence of tryptophan which means there is a low tryptophan present then the repressor is not going to be active because it has to bind the tryptophan molecule to become active repressor and that is how it will not be able to bind to the operator and as a result the RNA polymerase will go and bind and it is actually going to do the transcription and it will actually going to produce the polycystonic messenger RNA. So, there will be high level of gene production or the transcripts from the operon.

Now in the presence of tryptophan when there will be high tryptophan the things are going to be reversed because if the tryptophan is present it will go and bind to the repressor proteins and as a result it is actually going to form the active repressor and if the active repressor is going to be present it will go and bind to the operator and that is how it will not allow the RNA polymerase to grind to the promoter and to complete the transcription and that is how there will be a low transcription in the case of the if the tryptophan is present. Then we have the tryptophan operon transcriptional attenuation. So, it is possible to obtain more strict regulation in E. coli by repressing the transcription initiation alone but translation mediated transcriptional attenuation offer the additional regulation. The attenuation site in the tryptophan operon is situated after the surfactional start site.

More transcription stop here when the tryptophan levels are high when the tryptophan levels are low or sparse thus transcription continue to produce the functional protein. So, in this case you are actually going to have the attenuation site. So, this is actually going to be attenuation site what is present. So, in the case in the high tryptophan level it is actually going to be transcribed and it is actually going to produce a messenger RNA which is for this particular protein. So, when the tryptophan concentration is low the entire operon including the ligur sequence is transcribed into a messenger RNA.

When the tryptophan concentration is high only the 140 nucleotide which is only the part of the sequence that precede the attenuator are transcribed into messenger RNA and the structural genes are not been transcribed. So, it is actually when you have the low level of tryptophan the transcription will start from here and it will go all the way up to the end. So, it is actually going to have the full length messenger RNA where you are going to have the leader sequences and as well as the RNA sequences of the structural genes. Whereas when you have a very high level of tryptophan the transcription will start from here but it will only going to end up here and you are only going to have the leader sequence of 140 nucleotide and that is how you are actually going to stop this in the transcription of these particular genes. And this is a very unique phenomena what is only happening in the tryptophan operon which is called as transcriptional attenuation.

So, what is the mechanism? So, the operation leader sequence has a 14 codon open reading frame codes for a leader peptide of 14 amino acid which with 2 tryptophan codons. The mechanism of translation mediated attenuation depend on the fact that the translation in bacteria is coupled with the transcription. So, ribosome becomes translating the 5 prime end of the messenger RNA when it is still being synthesized. Thus the translation rate can affect the structure of a growing RNA chain which determine whether the transcription can continue or not. The function of the leader sequence is to the fine tune the expression of tryptophan operon based on the availability of tryptophan inside the cell.

The 2 tryptophan codes for the leader sequence lies within the region 1 and the transcriptional translational stop codon lies between the region 1 and 2. The leader sequence contain the 4 region that is the region 1 to 4 and that can form the various base paired stem loops or the hairpin like the secondary structure. So, regions are like you have the region 1, region 2, region 3 and region 4 and region 3 is complementary to both the region 1 and region 4. And as a result what will happen is that it is actually going to form a hairpin like structure. So, it is actually behaving exactly the same as we have discussed about the intrinsic transcriptional stop site.

So, it is actually going to form a loop kind of structure and as a result it is actually going to stop the growth of the RNA polymerase. If the region 3 and 4 base pair with each other they form a loop like structure called attenuator and function as a transcriptional terminator. If pairing occurs between the region 3 and 2 then no such attenuation forms and the transcriptional continues. So, this is the exactly the site what we have just discussed that ribosome binds to the tryptophan polycystin messenger RNA that is being translated when the tryptophan levels are high and start the leader sequence translation. The translation stop codon is present between the region 1 and 2 and the 2 tryptophan codes for the leader sequence are within the region 1.

The ribosome follow the messenger RNA closely during translation and creates the leader peptide. This peptide the moving ribosome complete the translation of the leader peptide and pause at the stop codon blocking chain blocking region 2. At this point the ribosome prevents the region 2 from interacting with the sequence 3. So, the base pair with the region 4 to form a 3 4 stem loop which serve as a transcriptional terminator and as a result tryptophan prevents the tryptophan operon from continuing to be transcribed. So, this is what exactly happened when you have the high level of transcription tryptophan.

So, there will be a 2 tryptophan coding region what if been present and they are actually going to allow the formation of a loop like structure and this loop will actually going to

stop the progression of the RNA polymerase and as a result it is actually going to stop the RNA polymerase and stop the transcription. So, this is exactly what we have discussed. So, if this tryptophan is in short supply then the ribosome will pause at the 2 tryptophan codon contained within the sequence 1. This leaves the sequence 2 free to the base pair with sequence 3 to form the 2 3 structure also called as anti terminator. So, the 3 4 structure cannot form and transcription continues to the end of the tryptophan operon.

So, when the tryptophan levels are low there will be a base pairing of 2 and 3 and this 2 and 3 are called as the anti terminator because it will not be able to find a strong loop structure and that is how the transcription will continue and that is how it is actually going to have the synthesis of the messenger RNA for the tryptophan synthesis. Now let us move on to the third operon and the third operon is called as the arabinose operon or the ara operon. So, the 5 carbon sugar L-arabinose must be breakdown by a operon known as L-arabinose operon also known as the ara or the ara-B bad operon in the E.coli. The 3 structural genes ara-B, ara-A and ara-D are found in the L-arabinose operon code for the 3 metabolic enzyme needed for the breakdown of L-arabinose.

These genes generate the enzyme called arabinose or the ribulokinase, ara-A which is called an isomerase and ara-D which is called an epimerase which catalyze the conversion of the L-arabinose into the D-xylose 5 phosphate and intermediate in the pentose phosphate pathway. So L-arabinose is also a part of the catabolic pathway and it is going to follow exactly the same mechanism what we have just discussed about the lac operon. So, a single transcript and messenger RNA is produced from the transcription of all structural gene in the L-arabinose operon. The catabolite activator protein or the cap cyclic AMP complex which is produced by the regulatory gene ara-C regulate the expression of L-arabinose operon as a whole. The proteins that codes ara-C control the expression of arabid by acting as both activator when the arabinose is present and a repressor when the arabinose is absent.

ara-C is sensitive to the level of arabinose at high ara-C level the ara-C protein not only regulate the expression of arabid but also control its own expression. So, these are the metabolic pathway of L-arabinose by the action of 3 enzymes. So, when you have the L-arabinose it is going to act by the L-arabinose isomerase and that is going to convert the L-arabinose into L-Ribulose and then L-Ribulose is going to act by the L-Ribulose kinase and that is how it is going to form the L-arabinose 5 phosphate and L-arabinose 5 phosphate is going to be isomerized by the L-arabinose epimerase and that is how it is going to produce the D-xylose 5 phosphate. So, what is the structure of the arabinose operon? So, you are going to have the regulatory genes like ara-C you are going to have the promoter which is called as para-C and then you are going to have the different types

of structural genes like ara-B, ara-A and ara-D apart from that you are going to have the some of the regulatory proteins and all that. So, this is the region of the L-arabinose operon where the ara-B is going to be produced by for a ribulose kinase, ara-A is going to form the isomerase and then ara-D is going to form the epimerase.

L-arabinose operon is consists of three structural gene and the regulatory region with the region with the operator region called ara-O, ara-O1 and O2 and the initiation region that is called as ara-I1 and I2. So, these are the region right the structural genes are ara-B, ara-A and ara-D and this is also there is also a cap binding site where the cyclic cap and cyclic MP complex bind to and facilitate the catabolic repression and result in the positive regulation of ara-B when the cells lack the glucose. The regulatory gene ara-C is located upstream of the L-arabinose operon and encodes the ara-B responsive regulatory protein ara-C both ara-C and ara-B have a specific promoter where RNA polymerase bind and initiate the transcription ara-B and ara-C are transcribed in opposite direction from the ara-B promoters and ara-C promoter respectively. Now ara-Bnose operon regulations in addition to being under the control of cap cyclic AMP activator the ara-Bnose system is also positively or negatively regulated by the binding of ara-C proteins. So, ara-C perform as a homodimer and interact with the operator and initial range region of initiation range of the ara-Bnose operon to control the transcription of ara-B ara-B a DNA binding domain and a dimerization domain make up such ara-C monomers 2 domain.

So this is the DNA binding domain so this is ara-Bnose binding site and this is the structure of ara-C monomer and there will be a dimerization. So, you are going to have the ara-Bnose binding site and you are going to have the DNA binding site. The binding of ara-Bnose is carried out by the dimerization domain upon binding to ara-Bnose ara-C undergo and the question will shift and adopt 2 different confirmations the binding of the allosteric inducer ara-Bnose is also only factor that affect the confirmation. When the concentration of ara-C rises to high ara-C can potentially adverse auto regulate its own expression by attacking dimer ara-C to the operator region and ara-C production is in between. So, now you have the negative regulation of the arabid.

So, cell do not require the arabid product to metabolize ara-Bnose when it is not present. So, in the absence of ara-Bnose you are going to have the negative regulation. So dimeric ara-C therefore function as a repressor 1 monomer binds to the ara-B genes operator while the other monomer bind to the remote DNA half site called Arab A. DNA loop is created as a result the Arab ad promoter cannot be bound by the RNA polymerase while in this operation orientation as a result structurally Arabid transcription is blocked. So, this is what exactly going to happen you are going to have the operators which are actually going to dimerize and that is how you are going to have the binding of the

operators onto the Arab 2 and Arab 2 onto the DNA and that is why it is actually going to form a loop like structure and in this loop like structure the RNA polymerase will not be able to bind.

Then you have the positive regulation of Arabid. So, both in the presence of Arabonose and the lack of glucose the Arabonode operator is activated for expression. So, when you have the low glucose you are going to have the ADP followed by AMP followed by production of cyclic AMP and that is how you are going to have the cyclic AMP cap proteins complex formation and that complex is going to bind the cap region of the DNA and on the other hand when the Arabonose is present Arabonose will go and bind to the operators and that is how it will not allow the interaction of the operators to form the loop like structure like this and that is how there will be a transcription of the structural genes from the operons. So, ARAC and CAP cooperate and act as a activator when Arabonose is present. So, when glucose is absent a high level of cap protein cyclic AMP complex bind to the cap region side binding of cyclic AMP is responsible for opening up the DNA loop between the era 1 and era 2 O2 increasing the binding affinity of RAC protein for era I2 and thereby promoting the RNA polymerase to bind to the Arabate promoter to switch on the expression of the Arabate required for Arabonose metabolism.

So, this is all what we have discussed in relation to the regulation of the transcriptional regulation through the operons. Now, what we have discussed we have discussed that the operons are being functional mostly into the prokaryotic structure, but the operons are also present into the eukaryotic structures and in a typical operon what you have is you have a promoter then you followed by the operators and followed by the structural genes and these operons or can be positively been regulated or the negatively been regulated or they can be inducible or the repressible. So, in this context we have discussed about the 2 operons from the catabolic reactions and 1 operon from the anabolic reactions. So, in the lac operon it is a catabolic operon where you are going to have the mostly the operon is going to be present as the negatively regulated operon. So, there will be no production of the proteins and there will be no production of enzymes, but when there will be an absence of glucose and there will be a presence of lactose then the bacteria will actually going to have the transcription of these genes and because the repressor is going to be bind by the lactose and that is how it is actually going to relieve the inhibition and that is how there will be a transcription of the gene and that gene is actually going to act on to the lactose molecule and that is how the lactose is going to be get converted into glucose and galactose and that is how that glucose will be utilized by the glycolysis to produce energy.

Apart from that we have also discussed about the tryptophan operon and we have also discussed about the arabdron subchloron. So, this is all about the discussion about the

operons and how the operons are actually regulating the transcriptional activity within the prokaryotic system. So, with this I would like to conclude my lecture here in a subsequent lecture we are going to discuss some more aspects related to molecular biology. Thank you.