Immunology
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Lecture - 14
Generation of Diversity (GOD) of Lymphocyte Antigen Receptors (Contd.,)

Welcome to today's class. So in last class we are discussing about the generation of diversity, we are going to continue again today. So hope you have already went through the book and more or less you have the idea like what is going on regarding, so like all other previous classes I will just spend a minute on the previous I mean slide that I ended up in the last class.

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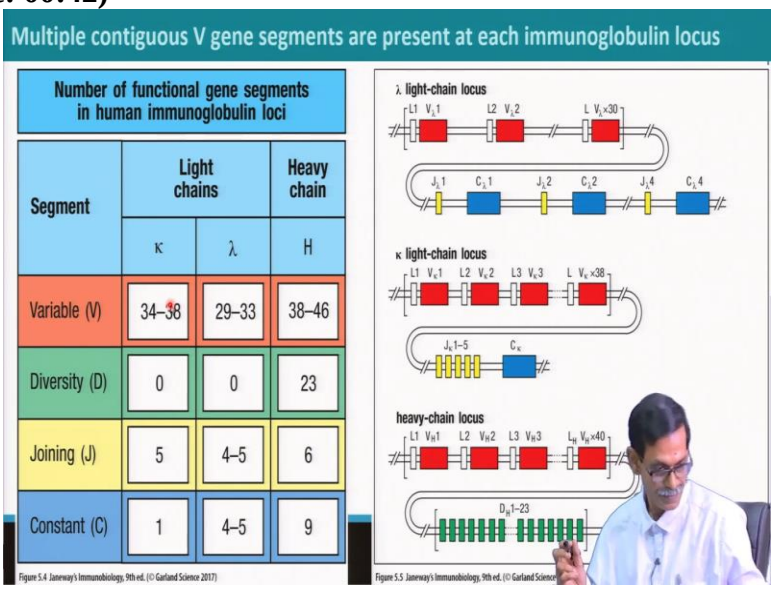

So the last class we are discussing about the number of variable region of kappa and lambda light chain and heavy chain, diversity region which is not presenting the light chain only present in heavy chain and joining segment or joining part is in a light chain and heavy chain and you know the constant domain is also varying like kappa chain has 1 lambda chain has 4 to 5 and heavy chain has 9, though there are 5 different kind of Isotypes.

There are some sub types also we will see extremely today's lecture or maybe the next lecture this lecture or the next lecture, though the constant domain is not doing any taking any part in the receptor diversity what it is there for their the effector functions of the antibody. So this part we have already discussed like the light chain, heavy chain and there how many there possible of if V and J.

So for light chain, we have our example here kappa chain we have 38 maximum and 5 joining chain, so if a randomly they mix up together or join together there will be possibly 190 different possibilities. So that kind of calculation what is going to happen and what is possible thing we calculated and even if I did some mistake in calculation or something you can calculate it is a very simple calculation.

Like for kappa chain what are the variability if I am taking the maximum number 38 then it is 38 times 5, in case of lambda chain it is 33 times 5 and in case of variable region you have to first recombination is happening between D and J which is 23 times 6 and with that DJ combination D is coming if I consider 46 is the maximum. So maximum possibility is 46 into 23 times 6. So these are the possible number of heavy chain and light chain.

Now one heavy chain can join either one kappa chain or one lambda chain in any antibody the light chain should be either kappa or lambda it is not like one is lambda and one is kappa, once it is done for a particular B cell that is fixed. So it is the B cell receptor and antibodies same that I already told you. So after activation the same receptor is synthesized differently and secreted as antibody.

So any B cell receptor if the receptor if this is the B cell and the pen is a receptor that receptor will be either kappa or lambda. So if we combined how many possible of total antibodies possible then we have to multiply this total number of heavy chain times kappa because any kappa chain can assimilate any heavy chain similarly any lambda chain can assimilate heavy any heavy chain.

So they will combine that will be the number of kappa containing antibody plus number of lambda containing antibody this is the diversity. So today we are going to see this how this V and J are going to join how suddenly the question comes automatically in mind if there is a recombination why there are 2 V segment are not joining why the 2 J are not joining why all the D’s are one after another why they are not joined.

Similarly why J segment of heavy chains are not joined? So who regulates or control this thing or how it is regulated? Like one V will join with only one J in light chain and one D will join with only one J chain and then this DJ combination will join with only one V, why there is no multiple V segment in one antibody hyper variable or variable region and why there is no multiple D or J. So how it is regulated?

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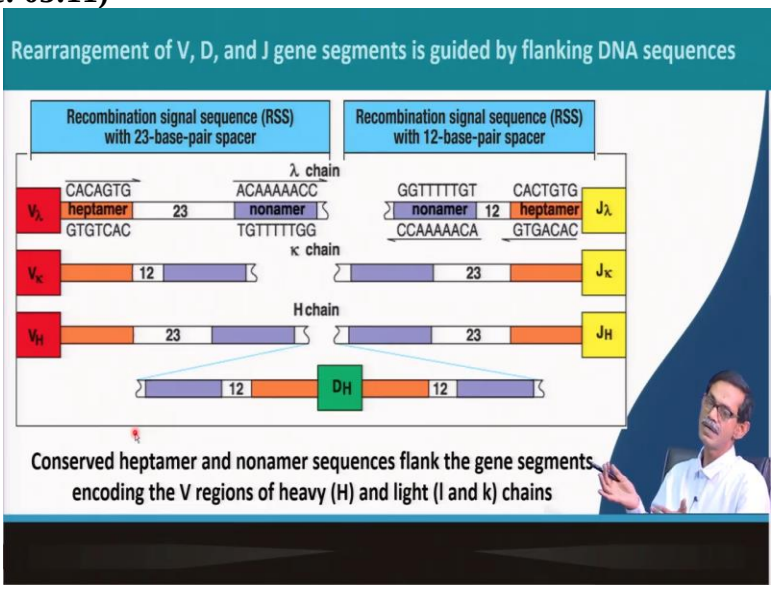

So to understand that we have to see some detail nucleotide structure of the VJ region sequence. Basically we have to see or in fact we have to see the flanking region of the V and J and D segment how it is located. So if you see carefully I mean it is known now. So if you see this is the V region so this is the V region and this is J region, so I am talking about the lambda chain. If I am talking about to a lambda chain if you see this is the lambda so this is J and this is V.

If we go say for cell consider just one V segment and one J segment and whatever we will discuss right now it will be true for any V or any J segment. So let us see all one for lambda chain. So the V lambda immediately the flanking sequence in the 3 prime end if you see carefully there is a heptamer. Heptamer means CACAGTG this is a fixed sequence, this is heptamer.

And we after that there is 23 nucleotide is 23 is the number of nucleotides 23 nucleotides which is random which is not I mean any nucleotide is possible, after this heptamer and 23. There is another repeat sequence or sequence which is nonamer that means 9 nucleotides which is

ACAAAAACC. So these nonamer sequence and it is definitely a complimentary sequence you know what should be it is.

So this nonamer and heptamer sequence is very very important for this recombination and this is called recombination signal sequence RSS. So this is faced in case of V you see heptamer 23 nucleotide nonamer again it will continue and then another V segment then another but these orientation like heptamer 23 and nonamer these orientation for lambda light chain is fixed. Similarly if you see J chain of the lambda sequence, it is again a heptamer instead of 23 here it is 12 nucleotide. So heptamer 12 nucleotide nonamer and it is in the 3 prime flanking sequence.

This is also the RSS or recombination signal sequence with 12 bass pair spacer. So if I say 12 and 23 is the spacer between heptamer and nonamer that is going to take a big role in recombination. So now if you see same way in variable region of kappa chain we have a heptamer but there is a 12 base pair spacer then a nonamer it is just reverse in J it is heptamer then 23 pair nucleotide spacer then a nonamer.

So it is just reverse in a lambda chain it is heptamer 23 nonamer and in case of kappa chain the same sequences with the J segment like heptamer 23 nonamer, it is just reverse. In same way if we see the heavy chain. The heavy chain variable region flanking sequences very similar to lambda chain that is heptamer 23 nucleotide, nonamer spacer and heptamer 23 and a nonamer. And if you see this D region which is both side is heptamer, heptamer then spacer is 12 nucleotide then a nonamer.

So what this thing is I mean this is I do not know how much I am able to confuse you but go slowly it is very simple and straightforward I can assure you will understand very easily. So if you remember your previous courses like recombination. Recombination happens with homologous recombination that means if 2 sequences are same if they come they can come like this and pair. So first recombination what you need to have I mean what there should be there in recombination DNA first should be single stranded.

And then it will come and make pair with the other for located some sequence. So here what happened these 2 repeats suppose this is double stranded, these 2 repeat becomes single stranded and then these will go on compare and make a recombination site. So they are what is normally happen one protein binds in this sequence another protein in binds this sequence they bring them together. So if this is a long DNA sequence if you consider this long trade of DNA just assume there is a long trade of DNA.

Here one sequence is the same heptamer, here one sequence is heptamer or here one sequence is nonamer, here another sequence is nonamer. So if there is a protein which binds to this nonamer and here is another protein binds to this nonamer and then if these 2 protein are very good pair and they always wants to stay together what they are going to do is they will come together, so what will happen? They will hold this nonamer sequence here, they will hold this nonamer sequence here and bring them together.

So these 2 pair that we will see what is happening. So then there is automatically a rule because why 23 and why 12, 23 means if you remember your basic DNA structure that is 11.5 actually pair turn, 10.5 pair turn. So this 10.5 nucleotide in 1 turn the current I mean if you see the old book or old version of any molecular biology or biochemistry book you may see that there are 10 base pair turn but now the calculus in is slightly modified it is 10.5 bases pair turn.

So either 10 or 10.5 does not matter much. What is happening if there is 12 nucleotides spacer that means maximum 1 turn is possible. So if DNA helix if you see that 1 turn is complete in 12 nucleotide but in 23 nucleotide if there is just almost double what is going to happen? You can see if you consider 23 nucleotide there will be 2 turn of helix in the DNA structure. So this 23 and 12 is that very important because which phase of the DNA is seeing by the protein or other part that is very important.

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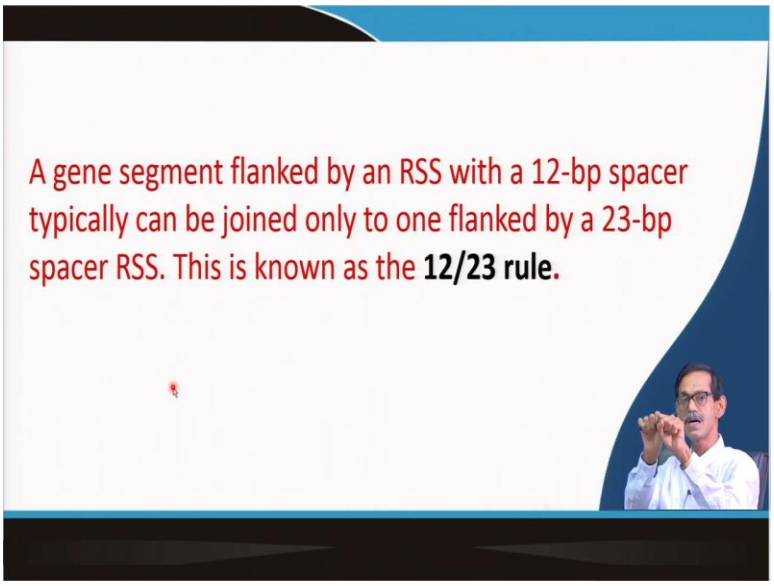

So what happened? It was found that there is a rule called twill 12 23 rule this is very important to know. So the 12 23 rule or 12 / 23 rule is a gene segment flanked by an RSS with a 12 base pair spacer typically can be joined only to one flanked by 23 base pair spacer. That means if one heptamer another heptamer joined together then one heptamer should have a 23 nucleotide spacer another heptamer should have a 12 nucleotide spacer then only they can come and join.

I am going back to a previous slide here you see these heptamer is space by 23 nucleotide spacer. Similarly if you see the J of the lambda chain it has heptamer but 12. So these heptamer can join with these heptamer because one is 23 another is 12, this is called 12 23 rule of recombination particularly played here this is discovered it is sounds by simple but if anyone is interested how it is discovered or how they figure it out you can go and find the real research article or paper it is not that simple because it is so many experiments to prove like this simple one line statement.

So if this is true that means no 23 23 base pair spacer containing heptamer will not join, similarly heptamer with 12 nucleotides spacer will not join him what I mean if 2 segments have heptamer both side with 12 and 12 spacer which you can see in D H, you see the D H part, in D H part what happened there is a heptamer spacer both side but both are there are heptamer in the both side 5 prime and 3 prime side of the D H segment and both have 12 nucleotide spacer.

So if this is the case if there are 100’s of D H they will never recombine because 12 12 will not recombined similarly 23 23 will not recombine. So if you see and go detail analysis of the

sequence you see you will see all the V lambda are flank by heptamer with 23 23 in both sides, so 2 23 will not recombine 2 12 will not recombine. So only 12 and 23 will recombine and that is because this turn and other protein that turn part you just draw a sequence and try to understand which side of the draw a sequence with CSCAGTG.

And then try to play with that. So study the recombination from previous classes from genetics or molecular biology or cell biology recombination mechanism you read and then you believe my word like one turn is 10 nucleotide or 10.5 nucleotide then 23 and write a sequence and then see which turn it is coming facing lock. So these 12 23 rule actually this is happening and that is why the rule made. So no 12 will recombine, no 23 you will recombine at no 2 12 will recombine no 2 23 spacer containing segments will recombine.

And that makes like if all D H has 12 12 spacer I am repeating it several way because so that you will remember that 12 12 spacer D H, so how many D H we have? We have see 1 to 23 there are many D H and all D H which is not given yet if you see if you zoom it and then you see what is detail? The detail is that every D there is a heptamer 12 nucleotide nonamer than another 12 nucleotide or another 23 nucleotide so that is how it will go.

So every D H is flank by heptamer with 12 nucleotide spacer and if we if the 12 23 rule is or right and it is right so no D no 2 D will recombine. Similarly all J in heavy chain is to flank by 23 base pair spacer, so no J will recombine. So one is 12 another is 23. So it is only possible that one D and one J can recombined. Now the good thing is if you see one D and one J can recombine first one D and one J recombine.

So if this recombination happen what will happen? So J and then D what is there in D 12? What is there in V what is the number of spacer 23 so again these 23 and these 12 can recombine. So these 12 23 recombined then finally what we will see with these J will come here D will come here so J and D will be adjacent then there will be a 12 nucleotide spacer containing heptamer and V H has 23 nucleotide special containing heptamer these 2 will recombine and make again 12 23 or satisfy the 12 23 rule.

So this is the reason why this due to D segment or 2J segment and 2V segment are not joined. So this is normally happened in living system or in biological science they are every possibility to have some abnormalities or some deviation of the this kind of rule but this is normally happen that these VDJ recombination follow that 12 23 rule. So what is happening?.

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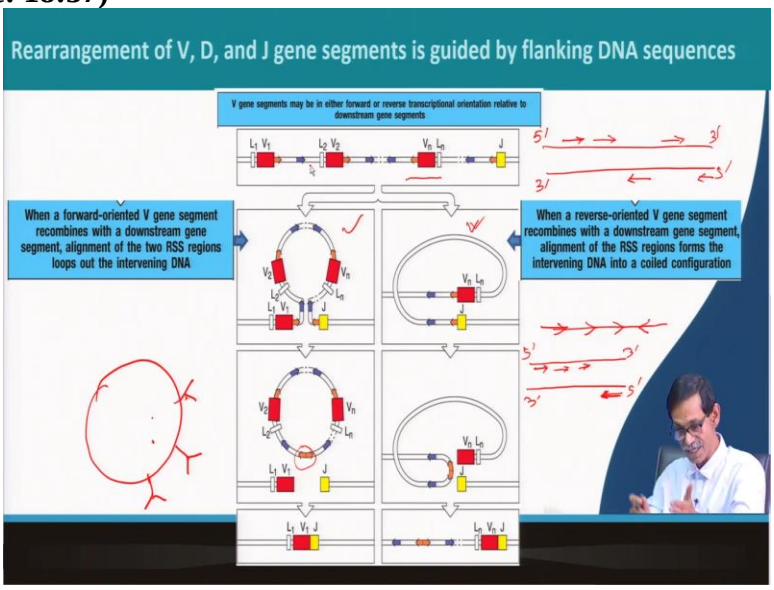

So now I am coming how this thing is happening. So if you see this is the same sequence what we have seen before it is what this is orientation say this is one segment V one of say lambda so V 1 and V 2 and V 3, V 4, V 5 and then V n which is around 38 in case of lambda. So V 1, V 2, V 3 and you see this all this heptamer nonamer are having the same color code but not in that detail like the previous slide.

So what is happening? This heptamer the V region is immediate flanked by the heptamer then a spacer then nonamer then spacer then again another variable region then heptamer then spacer then nonamer gradually go on and there is a J which has the signal of this heptamer nonamer is reverse, reverse sequence, now 2 possibility if I consider that this V 1 and this J will join by recombination.

What is happening? One step for like V 1 has this direction of the heptamer sequence and J 1 has this direction of the heptamer sequence you see in this figure this is all forward what is happening? in this figure you see this nonamer, nonamer 2 violet sequence come together and

then these 2 arrow came here. So these regions nonamer, nonamer region will come and that I told it is not coming by their themselves.

There is one protein or a complex protein, protein or protein complex which hold them. So what is happening? One is binding in this region in this nonamer one protein is binding this nonamer and other protein is binding in this nonamer they come together. So whole sequence comes together what is happening as a result all the internal sequence between or in sequence in between this V and J they become a very big loop. Clear very big loop for V 2 to V n.

That means if there are 38, one is joining that means 39 we segment our in this loop with all this sequence then what happened then they are a very combination and recombination you know that there is a DNA card then join that will come later. So what is happening they come together then there is a nucleus which will cut the DNA then there will be joining or some synthesis or repair, ligase all this thing will come.

So what is happening? These 2 sequences will come together and these sequences will make a very big loop and this V and J will join. So what is happening actually so big length of DNA assume that there is a big trade see you make a being here then make a tie maybe given knot here. So DNA 2 sequence here bring them together you make a knot, so there will be a very big loop then if you cut it here what will happen? This one piece will come I mean one was here another was here.

So this will come and the loop part will go out the same or similar thing is happening. So here so the big loop is going and VJ join, one thing we have to remember most mostly or most of the cases are almost all the B cells and it is happening in T cells also. Once recombination is done like one VJ joined in light chain and heavy chain VDJ joining is complete. So the recombination machinery which is one of the one or several very important proteins which involved in this process.

Or the recombination process which bring them together cut it and join it and do that do this job is not expressing, so recombination machinery in B cell and T cell disappear when heavy chain

light chain recombination is happening in the T cell and in case of T lymphocyte alpha and beta chain rearrangement is happening these machinery or recombination are not expressing anymore and at the same as a result what is happening? No further recombination is happening.

So once recombination is successfully done and it makes a receptor either B cell receptor or a T cell receptor no more recombination is happening. It is normally this is the case row, one B cell is one done so one say suppose in this case what happened this is very simple and straightforward what is like V 1 is joining with J. So rest of the V is deleting out anyway. So there is no scope of other recommendation recombination.

But this is not the always case it is possible that V 3 can join with V J. So V 3 to V n will be V 4 to V n will be eliminated but V 1, V 2 will still exist in the cell or the in the chromosome but even after that it is not going to recombine anymore even if I say the last one the V n is recombined with J. So what will happen? In between some very little portion of chromosome will be deleted or rest V 1 to V n - 1 in this case it is say in case of lambda it is 37.

So V 1 to V n - 1 is still going to be in the chromosome but even after that no recombination is going to happen. So once recombination is successfully done why I am telling successful? That any recombination can lead some nonframe or misframe or deletion, addition kind of mutation. So the protein may not be perfect or some friendship can happen then we say that it is not a fruitful recombination.

So fruitful recombination if it is there no further recombination is going to happen but this is one way that it can happen it make a big loop they eliminate, so they will look completely out from the chromosome forever. But in this case. This is not very, it looks a little difficult but it is not that difficult. It is particularly purposefully shown here in this way if you see this slide what you see is V 1, V 2 all arrows are in this direction.

In case of V n arrows in this direction what does it mean? It means that these V n segment is in the other strand of the DNA, it is other strand of the DNA because in chromosome protein or gene may be in one direction and sometimes you the arrow is in different direction. It is not that

gene will start from 3 prime to 5 prime because the convention of DNA is what the way we read or we study the gene and everything we always say 5 prime to 3 prime.

So suddenly one gene is direction this way suppose this is the chromosome another gene is direction this way, another is direction this way but another gene may be this direction what does this mean? So that means if this is the double stranded DNA if this is 5 prime and this is 3 prime 5 prime 3 prime then this means these gene is this direction and this strand and other genes this direction means the genome this gene sequence or the sense sequences these, it is not that it is reverse it is same way other direction. So all genes are 5 prime to 3 prime.

So these 5 prime to 3 prime in this case, this 5 prime to 3 prime this particular one if and this J 1 is also in the direction. So if they want to come in this way like 2 opposite facing like this direction. So we have to make this structure. So this structure like 2 heptamer like this direction going outwards arrow it is outwards then the loop cannot be like straight forward loop, it will be a loop kind of difficult to think like how it is manage.

But ultimately what will happen? They will form this structure they will join but in this case what will happen? It will not eliminate from the chromosome. It will not be eliminated from the chromosomes and this whole structure like whole structure means whole sequence of whatever suppose this is V n that means V 1 to V n – 1, if it is a lambda that means 37 V segment will be present in chromosome but not like previous one.

So here also same thing is happening ultimately what we see? We see L n V n and J here also L n L 1 V 1 J. So it can happen with a anyone. So if the orientation of the gene or the if the V 1 segment is here so if suddenly one gene is I mean say for example I am drawing this again. So this is the double stranded DNA 5 prime, 3 prime, 5 prime, 3 prime so one gene this, this then it may be this, I do not know exactly how this all this orientation I do not remember like all V how it is located.

It may be like this. So some V may be this direction some V maybe this direction. So whatever the direction the if it is in the same direction like in the opposite direction is J like this form like

this direction is V and J is this they need to go this way. And if it is other strand it will go this way clear. So this is the 2 thing and here one thing I do not know whether I have mentioned it, what is this L 1? I never mentioned L 1. L 1, L 2, L n.

So what this means L is the leader sequence I hope you know what is a leader sequence or signal peptide because any protein goes to I mean all the proteins are synthesized in cytoplasm then some protein goes to do back to nucleus, some protein goes to golgi, some protein to mitochondria, somewhere in the membrane. So all the receptor proteins are going a surface receptor proteins are going to the plasma membrane.

So these targeting like when protein synthesized in cytoplasm how they I mean how the cell will know where to go because that signal peptide or leader peptide actually the information is written, there are some machinery how it will go but that machinery can understand said they will see the pool it is just kind of bar coding you know that if you go to airport you will see that all the baggage different I mean say suppose in airport you go to Air India counter, you see in the queue to some people are going say from Calcutta.

From Calcutta some people are going to Delhi, some people are going to Bombay, Mumbai, some people are going to Guwahati, Bengaluru. So all are in the queue and their baggage is going one after another. But they will all collected in one place and then someone is there to short them out this bag is for Bangalore, this bag is for Mumbai, this bag is for New Delhi, how they figure it out? Because there is a tag that tag is the leader peptide even after that wall proteins are synthesized in the cytoplasm.

So there is a tag that is a signal peptide that signal peptide is recognized. And depending on that whether it is a mitochondrial signal or it is a nuclear signal or it is a membrane signal or secretary signal everything is written in this leader sequence and that leader sequence direct the protein to its place or the proper location. Similarly all this receptor whatever the B cell receptor that is supposed to go to where that is supposed to go to if this is the cell, so all proteins synthesized here they will go to membrane and they will display like this.

So how they will go to membrane this leader peptide. So this L is leader and which is a part of V segment. Which is not there and this is ultimately we will see what is happening because in antibody also this leader peptide is there. But the leader peptide is located in the N terminal domain of the protein most of the time leader or signal sequence most of the time it is present in the N terminal, it is not 100 percent cases.

It is valid or true because there are some internal signal also particularly the nuclear localization signal many times it is present in between the protein or end of the protein C terminal. So this leader peptide are sequence which is present so leader peptide is present for everyone it is not that it is one so every V segment is, every V segment have this has this leader peptide before their sequence start this will be processed in eventually. So this is the, for this lecture this is where we will finish and we will see exactly more detail in next lecture what is happening and how these come together. So see you in the next lecture. Bye for today

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