Immunology
Prof. Sudip Kumar Ghosh
Department of Biotechnology
Indian Institute of Technology - Kharagpur

Lecture - 15
Generation of Diversity (GOD) of Lymphocyte Antigen Receptors (Contd.,)

Welcome, to next class of generation of diversity of lymphocyte antigen receptor though your initial part is mostly the antibody or B cell receptor, so in last lecture we ended up 2 segment V and J, they are heptamer and nonamer how they are coming, how this loop form and it is deleted or if the sequence is in the reverse direction or opposite orientation, then how this recombination happened.

So, before studying this part in previous lecture, this part I will suggest to you those who are not familiar with or do not remember much the recombination you just go through the figures given in book about recombination. So, it will be very useful or helpful to understand. So, I am trying to explain like what exactly happening it is not that critical. So, the thing is, we are talking about diversity, how diversity is generated in receptors.

Initial part you know VDJ different number of multiple numbers of VDJ segments and then combination of different prime light chain and heavy chain makes the gives a 2 different component of diversity in antibody or basal receptor. But what we are discussing in last lecture also in this lecture, how these VDJ joined together, what are the proteins and how this week, it is important.

Because just that number I mean that 2 points would have been essential, but there is something which will give another diversity to understand that part if we understand a little bit detail, it is what I personally feel that to know little details, sometimes it is good to generate the interest like otherwise just to remember a few lines are very tough. So, if you remember the whole picture, what is happening is very easy to remember the things.

If you can remember the image you can remember the figures that can help you remember the whole immunology. It is not like you have to read again and you just remember the figure so you understand the logic and definitely you have to remember the correlation between the

events, then you will see that immunology is not that tough. So today I mean, this lecture, next slide.

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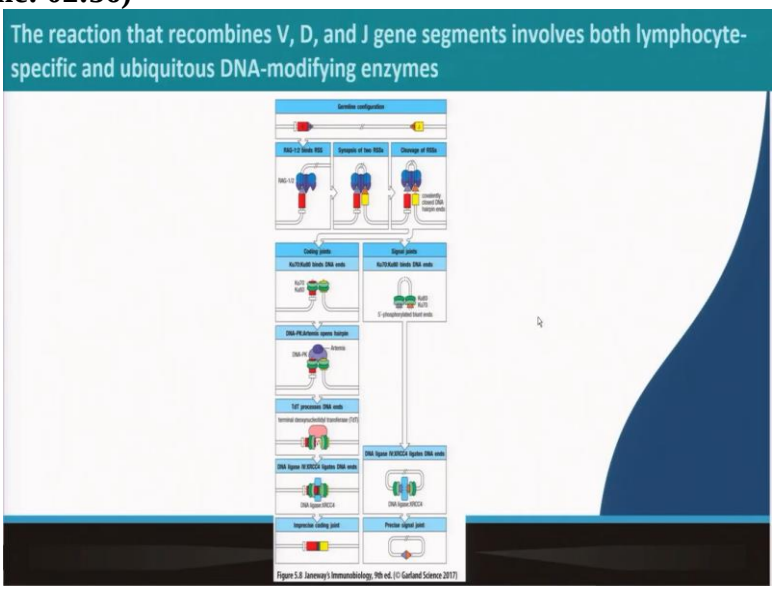

So do not be scared about seeing so many colors. And protein what it is saying that saying is basically the reaction that recombines VDJ different gene segments, it involves some very specific proteins that lymphocytes specific protein which is not expressed anywhere else or any other cells, not only that, it is also not expressed all the time in the lymphocyte I just I told in the previous class as soon as the recombination is done many protein will switch up forever and no show that no recombination happening.

But some proteins are ubiquitous DNA modifying enzyme because any recombination protein there are various kinds of DNA repair system present in our cell, which helps us to save from mutations and any kind of deletion alterations in our chromosome. So, these protein or those proteins are also involved, which are some are common which is present everywhere.

Wherever there is a DNA break, they will act wherever there is a DNA break and repair for example, the ligase we joined to DNA fragment that is common. So there is a combination of lymphocytes specific proteins and ubiquitous protein. And here I will tell some the name of the protein not all that important from lymphocyte point of view, but you will see that many of them are very common. So, if you see, let me get the laser. So, if you see this V segment of this thing, and if this J segment one is again 23 it is again make it very small and let me go to a little bigger version of that.

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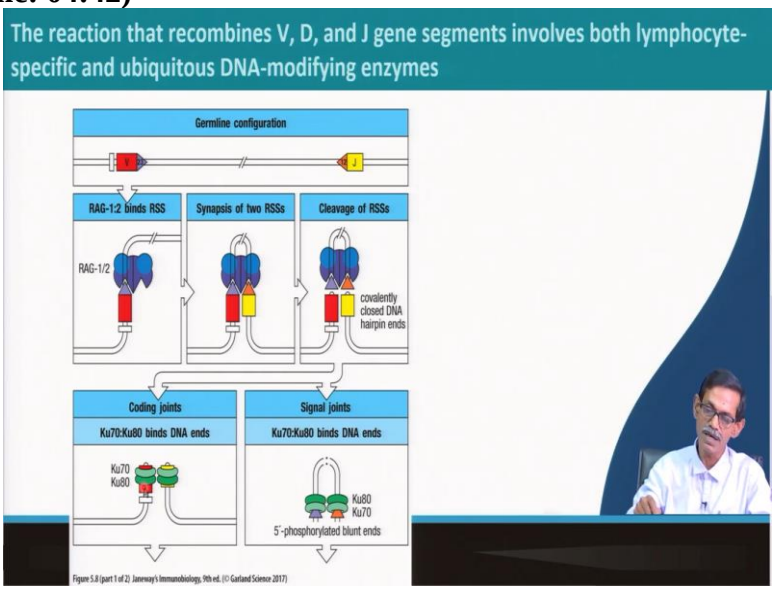

So, this V segment or you can see this 23 here, and here J is 12. So, now we will see how this J and V come together. If see first what happened there is a protein called RAG. RAG it is actually the product of recombination activating gene recombination are activating A and gene G recombination activating gene product. There is 2 different protein RAG-1 and RAG-2, this RAG-1 and RAG-2 make a complex.

That I will show you just to show you how it makes a complex. So RAG-1 and RAG-2 make a complex and these complex first bind to suppose this variable region V segment, another V segment. So this violet part is RAG-1 and the blue circle is a RAG-2 so this is a heterodimer and then they again makes basically tetramer, 2 RAG-1 and 2 RAG-2. So they first bind here then what happened? Another one binds here.

So, what just in last class I told one protein binds here another protein binds here they come together. So, what this will happen, so they will bring this V and J segment together. And V and J segment when they come together, then there is an endonucleus activity that endonucleus will cut these 2 strands. So, one cut will be at V segment and other cut will be a J segment and then they will be open.

So, this cut but you see this here, they cut the segment from the top part. So, there is a loop and the loop was cut and this covalently closed DNA hairpin ends. That is very unique kind of thing that I will discuss later in this lecture only. So, this loop go out, then what happened?

This Ku70 and Ku80. What is this Ku if you remember your recombinant class Ku is a heterodimer to get Ku70 and Ku80 and it is a heterodimer?

This Ku protein or Ku protein is doing what it is making a ring like structure over the DNA single stranded DNA and it is happening both in case of this region also, and this region also I mean in the bottom part also it is binding upper part is also binding. So, this actually it is helping DNA recombination, these protein helps to DNA dependent bring some DNA dependent protein kinase, which another protein is there, which we will see how it looks.

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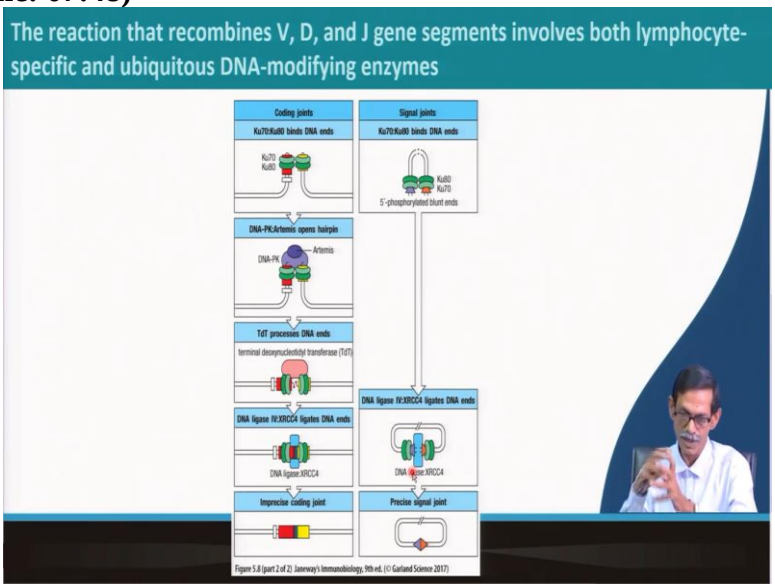

So, if this is the Ku70 Ku80 bind, Ku70 Ku80 bind which is end and this what we see here this is the covalently close DNA hairpin ends that I will see tell you a little detail later and which is very interesting and this then DNA PK this ballot big one, this DNA PK the protein kinase come, then there is another protein Artemis come together then this cutting and joining is coming when to happen then there is a TdT polymerase, which I will show this place.

I mean this reaction part is very interesting and I will tell you much more detail in molecular level what is exactly happening then DNA ligase and XRCC both are coming which is again a DNA repair protein present these Ku70, XRCC4 DNA ligase these are ubiquitous protein for cell. So, what as a result of this, this TdT functionality later, so, this cut Ku70 brings all this repairing non homologous end joining protein double strand break repair protein.

So, these are the protein up necessary for DNA recombination repair mechanism. So if you want to study these detail, you go to any molecular cell biology book and cellular molecular

biology book either way you read tell then you can find it there what exactly they are doing in recombination there is nothing new for this purpose only so I am just mentioning the name this is not I do not want to go the detail of the recombination.

And what exactly going on in this recombination but these proteins like all other regular recombination in similar process, they are also taking part here. And ultimately this makes a joining and these join if you see there, this is not only this red and yellow, this is not the red yellow there are a few more colors are here. I will tell you why these what these colors are, and in this case.

The loop part they come and the same complex happen they will cut in joining and the loop form and loop eliminated from the system not in the same cycle. Next cycle of cell division what will happen that is outside the chromosome. So, next cell division cycle, next cell cycle that will not replicate so the loop will there till that cell survive as soon as the divide next cell or daughter cell they will not be there anymore.

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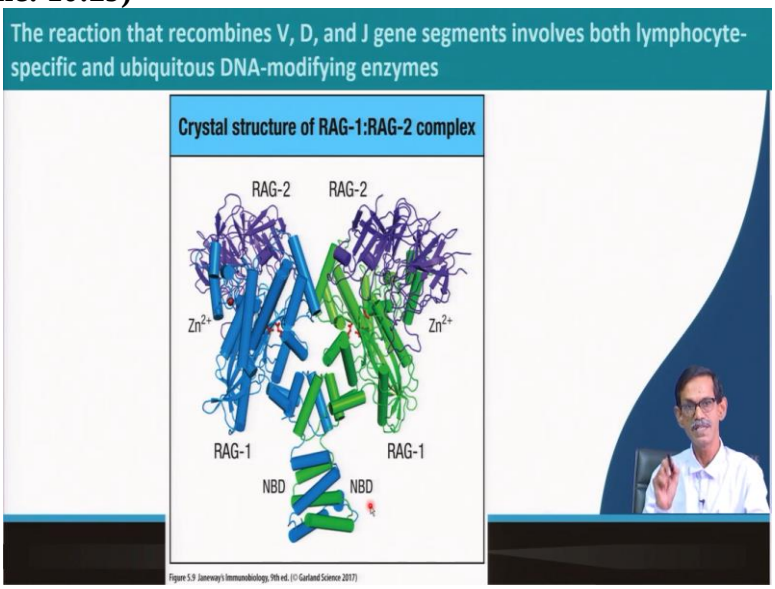

This is how this RAG-1 and RAG-2 look like this upper violet part is a RAG-2 and this is bottom for the green and red this is the RAG1 why I am telling showing you this is a crystal structure just remember. So, what they have the RAG-1 has a gene dependent endonuclease activity. This is this enzyme active part or in active part of the enzyme and this part, this is bottom tail part is NBD. NBD stands for nonamer binding domain, nonamer means that same repeat, nonamer binding domain you will see the next slide will be a little better to understand.

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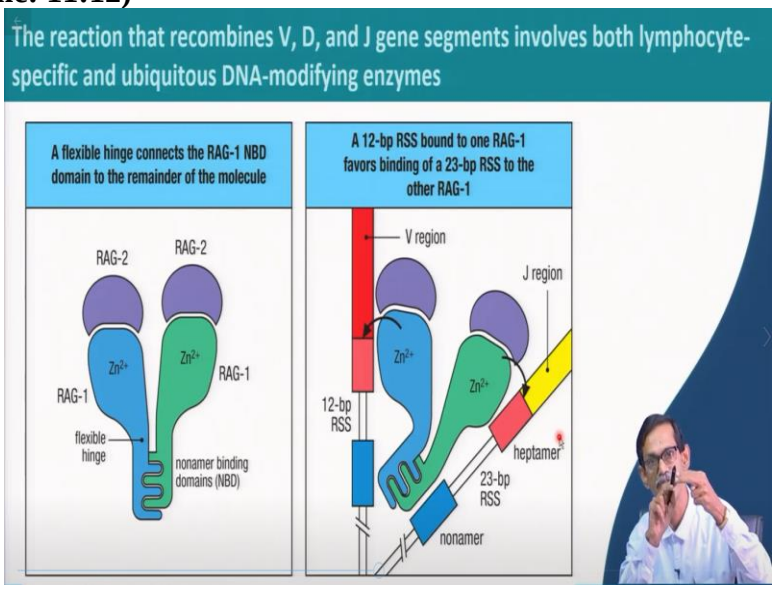

So, this is same thing the difference that was a crystal structure this is cartoon. Cartoon is always better to understand. So, this part is the nonamer binding protein which makes flexible hinge and they make a dimer here this gene dependent nucleus domain and this is RAG-2 what actively have, exactly happening these nonamer binding site binds to this nonamer, this is the nonamer sequence.

So, this is same one, DNA sequence. This is a variable region and this is the J segment J region. So, V region and J region both are heptamer, this region is heptamer and 2 heptamer spacebar one is 23 and other is 12. Everything as in before whatever we explained, so, this RAG-1 this NBD domain interact with nonamer. So, if you go back and see the orientation or the arrangement of nonamer and heptamer.

Nonamer, heptamer is just there is 1 heptamer very close to the segment that we need and then there is a nonamer so, to nearest nonamer of V segment, V segment and J segment are brought together by this NBD domain as soon as. So, there is a common domain here which brings one sequence this side and another sequence this slide. So, this is the V part and this is the J part what will happen then, because 2 DNA is very close here.

So, if there is this protein this NVD actually brings them together. So they are this V segment is very close J segment is very close, which is very close to the RAG. RAG is what have 1 is this gene dependent endonuclease what it will happen do it will cut here you see the arrow it

will cut here. So, there is no heptamer sequence anymore. So, they will cut just up to the heptamer and nonamer.

So, in that region there is a specific site they cut and that cut will taken care by that Ku protein and all these things just, we have seen after Ku binding, we saw a another protein that I told a few minutes back the TdT. TdT is terminal deoxy transferase Terminal deoxy transferase what is the beauty of that this enzyme can add a nucleotide to the 3 prime end of DNA with a template independently normally DNA polymer is what it is doing during DNA synthesis.

You need a template and depending on that template and a primer. What you need? what the DNA polymerase is doing if there is A they will add a T, if there is a G they will add a C, if there is a C they will add G that you know that complimentary DNA addition that is how DNA synthesis happened. But terminal deoxy transferase is that particular enzyme has a unique capacity that they can add nucleotide randomly without any template that means.

If you give all possible nucleotide like 4 nucleotide, like deoxy, ATP deoxy, GTP, CTP and TTP random they will add the nucleotide and there is I mean how long they will add as long as it is I mean here it is there is a limit but it I mean there is efficiency it can add nucleotide if you give only A it will continuously will add A, but it will add at the double standard DNA 3 primer only, because DNA synthesis normally happened 5 primer to 3 prime synthesis.

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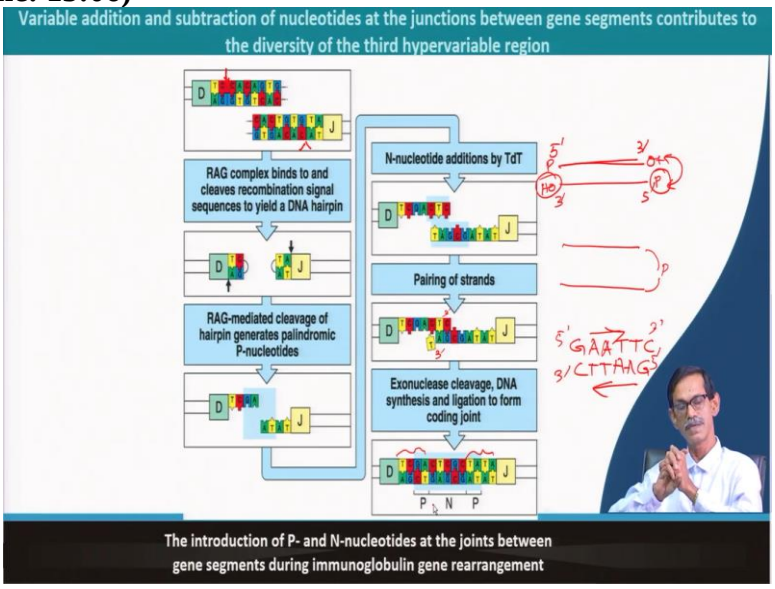

So here is the magic, how this TdT work it is a very, I mean wonderful mechanism if you I mean I will go slow. So, this is you see this is a sequence which is 1, 2, 3, 4, 5, 6 these sequence this is suppose here in previous slide we are talking about variable and J. I mean V and J region here. The example is given with D and J region but it is true for any joining any 2 segment join it is happening VJ or DJ or VD same similar thing is happening.

So, in D segment this is the last nucleotide suppose. So, D ends here and J ends here. What will happen RAG complex binds to bind and cleaves the recombination signal sequence recombination signal what is the recommendation signal? You go and check heptamer is here this is you will find now, if you this RAG signal RAG complex bind and cut where it will cut if you see this let me get the pin first.

So, RAG complex binds to the recombination signal and cleave that signal and where it will cleave you see this it is cleaving just after T and C in this case it is after this TA. Normally what happened if it cut? What is happening? If this is the DNA, if this is 5 prime and this is 3 prime and this is 5 prime end this is 3 prime end what we know are all of you know I am just repeating again.

This is 5 prime is all is there is a phosphate and 3 prime there is OH and same way this is a phosphate and this is OH. So there is a 3 prime OH and 5 prime phosphate. So normally if there is any DNA if this is cut, we can assume that this is the 3 prime and upper hand side 3 prime, there is an OH here. There is a phosphate here. That is normally it happened if you cut, but this particular and the same way as it is here also so it will be phosphate and this will be OH.

So now what is happening I will erase this part now it is happening normally that will remain as free end 5 prime phosphate and 3 prime hydroxyl end is remain free in double stranded DNA for you make a PCR or even just digested DNA with a restriction enzyme they will remain as free phosphate and free hydroxyl group, but here what is happening which is very interesting, these phosphate and hydroxyl make a link phosphodiester bond.

So, open hydroxyl and phosphate will make a phosphodiester bond. So, what will happen? We will see a hairpin loop like structure so, one DNA double stranded is there, but they are a phosphodiester bond. So that means it is a continuous thing, it is just a loop like structure

then, so, that is the beauty of RAG. So, endonucleus is cutting and making that hairpin loop at the end between the 2 strands.

Then again it will cut here you see the arrow it will cut again here. So, what will happen then if you cut here the endonucleus instead of only TC it will get a sequence TCAG. So, this end is free so, what 2GA here and AT here it is extra it was not there before it was there in other stuff but not in the same stuff. Now, if these DNA synthesized these DNA straightway synthesize because this is 3 prime.

So these DNA synthesize somehow what we will see A, G, C, and T and same way if you see what will see TATA, so, this will see and if we complete what will happen? It was originally this if you just join these 2 end if you just join these 2 end then TCTA AGAT it is supposed to be that only 4 nucleotide, but now due to this cut and hairpin loop it is become 4, 4 instead of 4 it will become 8.

So, 4 extra nucleotide came in between this V and J junction that was not there before, but it did not happen this way. It is not that straight forward that it will go and happen this I mean synthesize this sequence. It will not synthesize that way after making this sequence. So after making the hairpin and internal cut RAG mediated cleavage generate P nucleotides I will come.

What is this P nucleotide, then TdT come into picture, what I just told you TdT is an enzyme which can add nucleotide randomly and template independent way to the 3 prime end of the DNA, 3 prime hydroxyl end in the DNA? So, what was there? It was there, TCG was there and here it was TATA and TdT is going to add the nucleotide randomly and there is I mean nobody knows what nucleotide is going to come and how many number it will come.

So, it will carry on like TCGA was there and CTC then it was AGCGAT it is random it is going to add how long it will continue. So these DNA are very close. They are together very close, because all of the protein kept them together. So, it will continue this way, another sequence is going to continue this way, what will happen as this addition of nucleotide is random.

So, these random nucleotide also, if you continue because there are only 4 nucleotide, there is not much variability in that. So, once they will get a complementarity. So, while this is synthesizing, they will make a complementarity like so in upper strand CTC added and lower strand and CGA is the last. So what is happening this TA and CG so 2 sequence 2 DNA sequence, our single stranded DNA sequences progressing like this and sometimes they find the complementarity.

So, as soon as they find complementarity it become double standard. It was like this growing and as soon as they find complementarity they become double standard. As soon as it did become double standard TdT cannot work. Just terminal deoxy transferase can add nucleotide only to the single standard, so, TdT stop working. So, what is happening and what happened?

So, in this case, so, this is 3 prime and this is also 3 prime if there is any extra that you will fall apart because they cannot match. So, then there is a DNA polymerase that DNA polymerase which was we are talking about that DNA polymerase will continue to synthesize so what will happen is very simple I am not going to draw here because it is already drawn here.

So, if you see it was up to only CTC and then they pair between this AG and TC. This was pure during synthesis, then rest of the part will be synthesized this direction, and this direction. So, they will fill up the gap by DNA polymerase and after DNA polymerase fill up the gap definitely there are ligase that ligase will join this end this is very common mechanism.

Every time it is happening it is not very particular about this lymphocyte recombination inside the limb, this T and B lymphocyte but it is continuously it is happening in any site anytime DNA repair DNA breakage excision repairs, deletion repair. So, there are so many varieties of DNA repair variety of enzymes are there to repair the DNA. So these missionary is not new, but what is interesting part of this, what is the result of this?

This result is, it was only 2 before when it was started with D and J. So, 2 nucleotide. Now, you see how many it become it now 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 that means, there are 11 so, there are 2 to 4. Now, there are 13 that means 9 nucleotide was added in between,

in that 9 nucleotide, if you see that this part that TCGA, TCGA, TCGA. So, in this part and in this TCGA and this sequence and TATA.

This is palindromic, palindrome you know by normally we say the like, if there is some word that which we can read either side will same thing like madam either side you read it is madam there are many such word it but in DNA palindromic means that GAATTC is equal one side GATTC. So, if you make if you write GAATTC and the complement GAATTC, so, both direction both the standard units and GATTC, GATTC.

So, that is 5 prime to 3 prime and 5 prime to 3 prime. So, if you see these, this is called palindrome in DNA. So, these sequence you will see that TCGA, TCGA. So, this part become palindromic, because it is eventually should come because if you see this how it happened? The origin is very clear. This palindromic part is called P nucleotides. In palindrome also, there are 2 nucleotide extra.

So, this P nucleotide this is called palindrome. Because this is a palindromic sequence both sides it is a palindrome, but in between the 6 nucleotide, 5 nucleotide that in between this GACCG which is added by this TdT, which is random. So these randomness can make any combination of 5 or 6 nucleotide or 7 nucleotide that is also not in control. So, it depends again, it is a random process.

So, these 5 nucleotides, which is randomly added in between D and J. This is called a nucleotide. So, now you imagine, there are some fixed D sequence 1 to 23 and there are some J sequence. They can join that gives you suppose there are 10D and 5J, how many combination is possible if they are randomly there any one of them can join 10 and 5, 50. But if these join, make some nucleotide change which can contribute the number of amino acids.

Maybe 1, 2, 3 that number of amino acids can increase because of the addition of the nucleotide. So, that variety is not any more easily calculate, you can do like just by multiplication. So, this additions of P and N nucleotide or NP nucleotide in between the junction like DJ or VDJ. So, in case of heavy chain, there were 2 such cases, DJ one will happen and one BD recombination 2 places to add the N and P nucleotides.

In case of light chain only in the CDR3 because the J segment and D segment is are continuing the CDR3 region only. So, in light chain there will be only one place where these PN nucleotide addition is going to happen in case of heavy chain, there are 2 sites were in NP nucleotide addition will happen and that will very unlikely that each case or each time this addition is very similar or identical as it is completely normal.

And this N and P nucleotide addition gives a huge amount of diversity and which was this is the case like if you see the germline theory and somatic theory. Then what we see that definitely we are inheriting some genes or gene segment from our parents like VDJ part is inherited from our parent's combination is ours, we do not know what is going to happen. And this N and P nucleotide addition is continuously happening

Every time the recombination has happened during the synthesis and the maturation during the synthesis of B cell receptor or T cell receptor that is totally random. And this NP nucleotide addition gives a huge diversity which was not known and not existed before. And this is the third reason of antibody diversity or their nuclear I mean lymphocyte receptor diversity. So, we will have very, one more points left for that receptor diversity of B cell or B lymphocytes. And that we will try our next lecture we will discuss that. See you there.