Host-Pathogen Interaction (Immunology)
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Lecture: 55
Adaptive Immunity-Antibody Diversity

Hi so, we have learned a lot about the antibody and now you know that there are variety of antibody there are subclasses which we call it as a Iso~~SO~~ type and there are further in some cases there are further some types. And if you see this molecule it is a very interesting and elegant molecule the reason is that this molecule has a variability as well as a constant region is also there.

So, all these things are very good very good for understanding but if you look at what is the genetic basis for encoding such molecule it is a massive challenge. And this challenge was there in the field for quite a long time.

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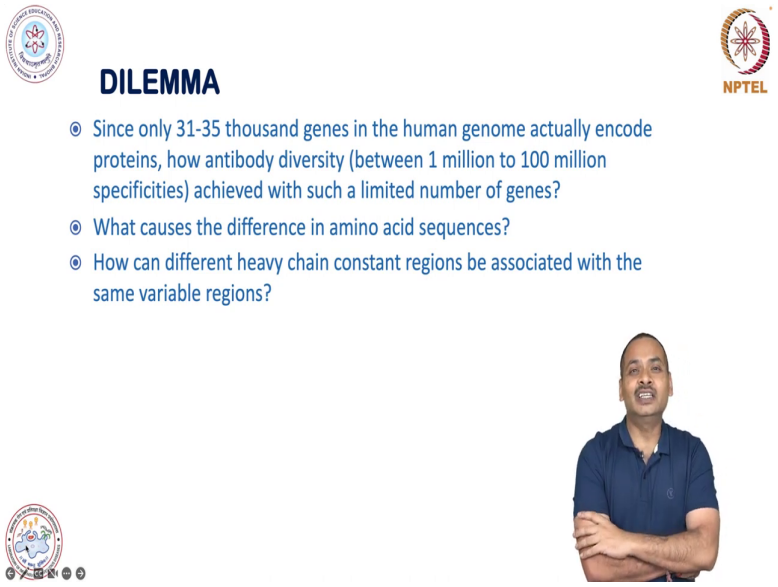

And this if you see there are few very important points are there are some dilemma before understanding this the genetic basis for these antibody production it was a quite quite difficult and still there are so, many things which are under a very active research. But the most important thing is that you know that our genome is basically encoding about approximately 30000 genes which encode protein.

So, in this scenario when the genome is encoding only 30 approximately 30000 genes in this scenario how the the antibody the diversity is generated which is in very big scale in very big order of a scale it is a million to 100 million. By this such a limited number of genes. So, this was a quite interesting and big challenge in the field of Immunology and what causes the difference in amino acid sequence.

So, here you can see that the immunoglobulin has a variable region and constant region and this is also again changing from one class to another class. So, the variable region is remains constant and there will be another constant region for example if the antibody is generated against some antigen X. So, the variable region the first variable region will be the in light chain and heavy chain.

And the constant part of these antibodies first will be IgM or it could be a in case of light chain it could be a ~~copper~~kappa or Lambda right. But this IgM having the same variable region and change to the IgG. So, this is very interesting this is very complicated to understand. And how does how can different heavy chain constant region be associated with the same variable region that what I was explaining. How this is this is genetically taking place these are very big challenge in the field.

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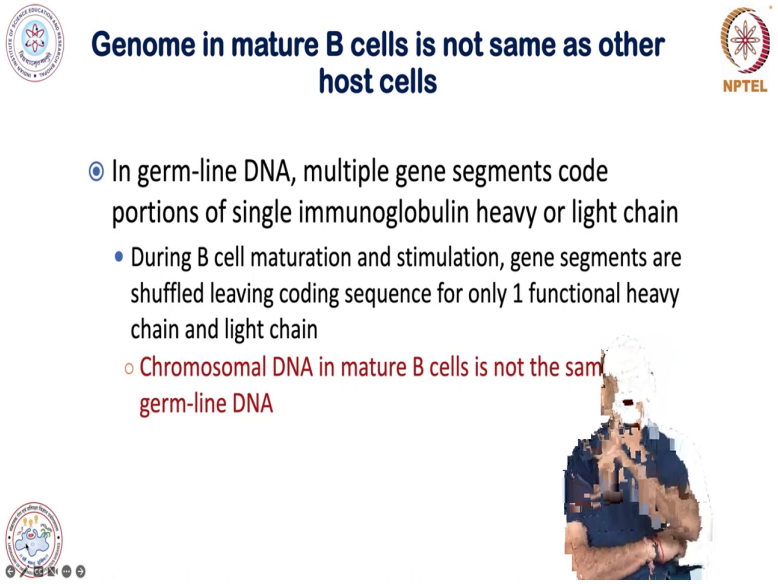

And if you consider the simple simple theory of a genetic one gene one polypeptide then this is not fitting well with this kind of observation. This kind of observation means then there will be a huge number of Gene and the cell cannot accommodate that many Gene right. So,

this was a very interesting. So, in germ line DNA multiple, so, to answer this thing the in germ line DNA .

There will be a multiple Gene segment which is coding portion of this immunoglobulin light chain and heavy chain and all these things are basically taking place during B cell maturation and there are different stages of maturation and then there will be a antigenous stimulation. And then this Gene segments are shuffled among all or these there are Gene segments I will show you in a short while.

And they suffler and living coding sequence and only one functional heavy chain and light chain are towards end it will be there and that will encode for the protein. We will see how it is taking place I am not going to take even very fine molecular detail it is a very complex it needs a lot of time to understand. But I will stimulate you to learn by yourself how this thing is taking place there are several good books you can you can go through that.

So, when this is taking place then one concept is basically standing wrong. So, we believe that the Genome of individual is same right in all cells but when this is happening in the in the host then the genome is not same this Gene segments are present on DNA and this there is a there is a recombination of DNA is taking place. So, therefore I can very confidently say that the chromosomal DNA in mature B cells is not same as other germline DNA.

So, in general we say nah that the DNA in all cells are same but this is an exception in lymphocytes the in mature lymphocyte the DNA is not same as other cells. If you take the liever cell and if you take the mature b cells or T cells then the genome will be different and this is the basis for the discovery of discovery of this diversity how the antibody diversity is generated.

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And there are so, many workers involved in this in finding out the genetic basis of antibody diversity but among all I am just talking about the key work of Susumo Tonegawa. He basically found out that there are the gene segments and these Gene segments Shuffle among the in the genome and then that will make a mature DNA and then this will transcribe and then translate and then that makes an antibody.

So, his work is I have seen his original paper it is a very complicated work it is a extremely hard work of his group and by a lot of experiment at that time not. So, much sophisticated Southern blot or RNA isolation methods were there but he has done all those experiment using radioactivity and then finally he established how this genetic diversity for antibodies generated and for his this great contribution he received the Nobel Prize in 1987.

So, I will not discuss about his paper I will just give you some glimpse a very short glimpse about his work only one slide.

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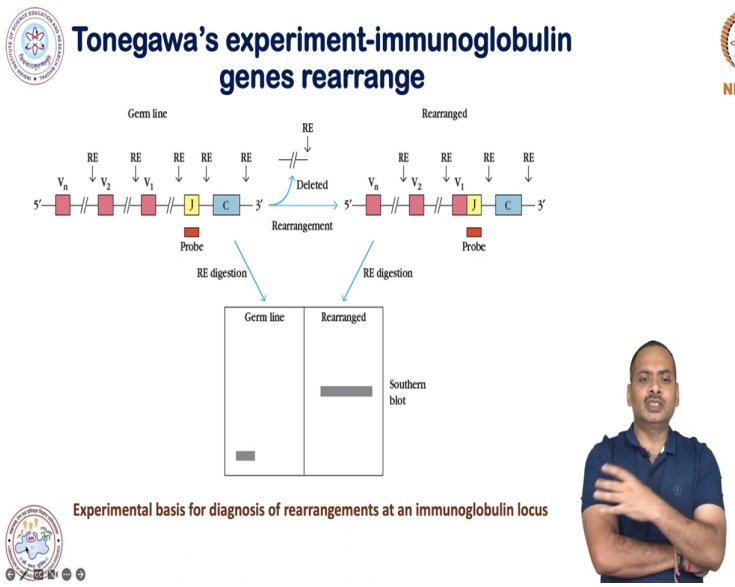

So, if this is theory initially his hypothesis was that then how he can prove it is very simple. Here he has isolated the DNA from say another cells another cells means a non-lymphocytic cells and he also isolated the DNA from lymphocytes the B cells also. And then he performed restriction enzyme digestion and he probed with here you can see that he made a probe for one sequence in this which is which is identified from mature B cells.

And then he after probing he found out that the genome is not same and that leads to the discovery of or he initiated the concept of Gene segments. So, his work is very huge it is a lot of work but I am not going to take all those things because if I will discuss those work then it will take a quite long time. So, I am straight away jumping on the antibody diversity ~~for~~ from his work and later on many immunologists followed his work and then several things are discovered.

So, this was the basically experimental basis for diagnosis of rearrangement in an immunoglobulin Locus.

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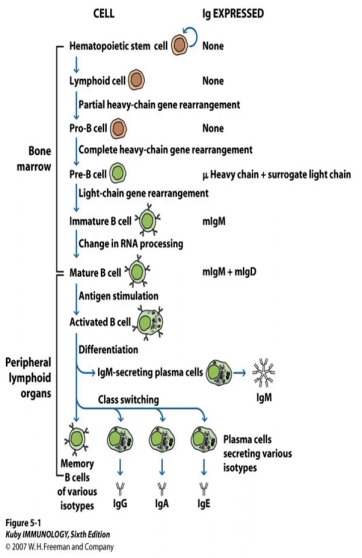

Figure 5-1
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So, here I am giving a very simple schematic for B-cell development as you know very well the B cell development is taking place in bone marrow. Once it is mature in the bone marrow and here there will be a clonal selection and clonal deletion I have explained you earlier clonal selection and clonal deletion. Once this the B cell is mature means they will be and naive they will be naive they have not seen the antigen it is an antigen independent process.

So, maturation of B cell in bone marrow is antigen independent process and once they mature they are called as a naive immunocompetent B cells and they basically see the antigen first time in peripheral lymphoid organ and then they further mature and they start secreting the antigen~~ous~~ specific antibody and later on there will be a class switching class switching means there will be a for example the B cells is producing only IgM. So, from Ig~~G~~M to IgG IgA IgE they change the classes.

So, this we call it as a class switching here you can see that most of maturation take place in the bone marrow and antigen dependent processes are taking place in peripheral lymphoid organs.

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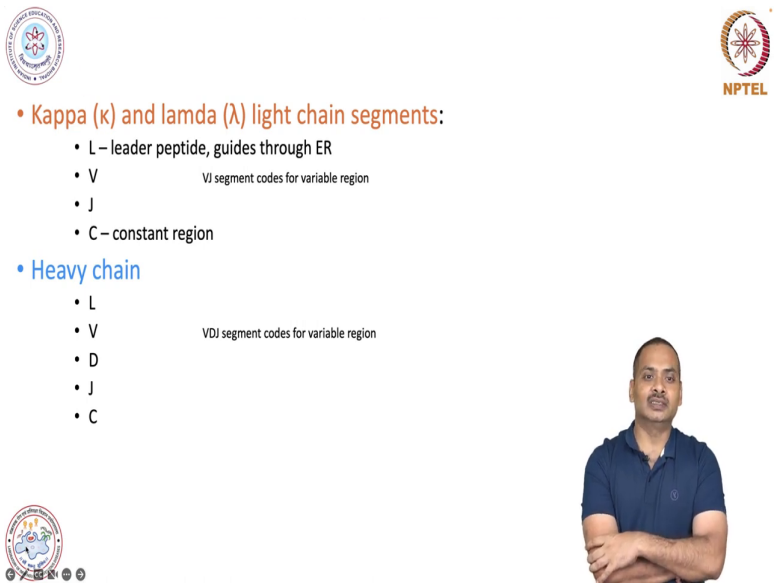

So, by the Susumo Tonagawa's work and other immunologist work they found out that for light chain there are four Gene segments. The four gene segments encode for light chain that is L. L is nothing it is a simple leader or peptide sequence which basically directed the polypeptide to a particular organelle here you can see that this is a endoplasmic reticulum. So, if you see carefully then so, there are three Gene segments which basically decide the sequence of light chain and theis sequence theis region we call it as a V J C.

V stand for variable J stand for joining and C stand for constant region. So, the whole light chain is encoded initially encoded by four Gene segment that is L V J C but after maturation of this polypeptide this will be having only sequence amino acid sequence derived from V, J and C. And in case of heavy chain there are one more Gene segment is there besides L V J and C and there is a one more additional Gene segment is there which we call it as a D.

D stand for diversity, so, L stands for leader V stand for variable D stand for diversity J stand for joining and C stands for constant. So, there is a there is a recombination taking place in both light chain and heavy chain now we will see how it is taking place.

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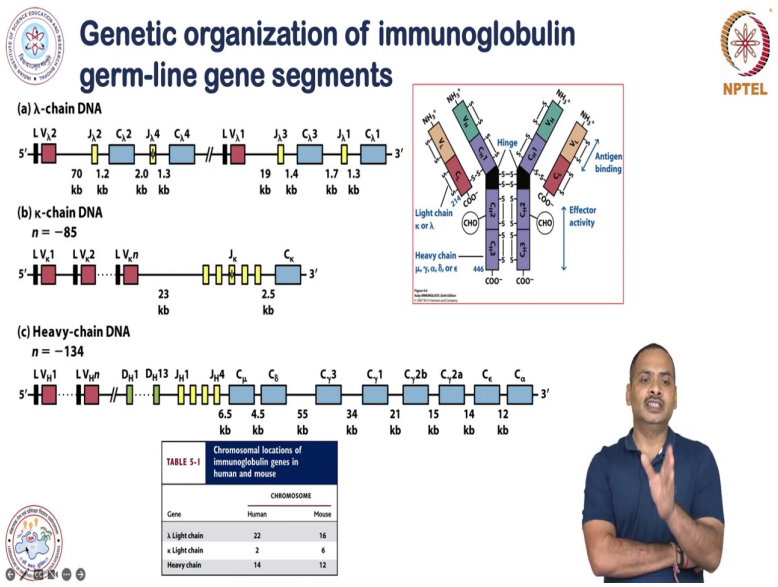

Here I am just showing you in a very simpler way the genetic organization of immunoglobulin Gene segments in germline. Here you can see here one more piece of information I would like to give that this in case of human the Lambda light chain is present on chromosome number 22 and in case of mice it is 16. And kappa light chain all these Gene segments are present on chromosome number two.

And in case of mouse it is 6 and heavy chain Gene segments are present on chromosome number 14 and in case of human and in case of mice it is 12 this is just a information you may you may need. So, here I am showing you the arrangement of these Gene segments. Here you can see that there is a V Lambda. V Lambda here you can see there are various J sequences and there is a various constant region sequences.

Here you can see the presence of these Gene segments over the DNA in case of Lambda chain this is for Kappa chain here you can again see that there are there are various V sequences and J sequences are there and there is a constant region in this slide. And in heavy chain for heavy chain again you can see there are various variable region variable region Gene segment and diversity region Gene segment and constant region.

So, here you can see that the constant region is present and here you if you if you see little carefully there is a first the constant region is encoding IgM that is why probably the first antibody which is generated is IgM this is this is present in sequence. And then there will be a Delta which is present on B cells in mature B cell and then there will be a production of IgG and this is subtype is IgG3 like that.

So, this is the arrangement of various Gene segments in case of light chain there is a variable region there is a joining region~~reason~~ and constant region. So, it is it is shown in this schematic. So, you can see that this heavy chain has a various V, D and J segment and constant region. This is the arrangement of a gene segment over the DNA.

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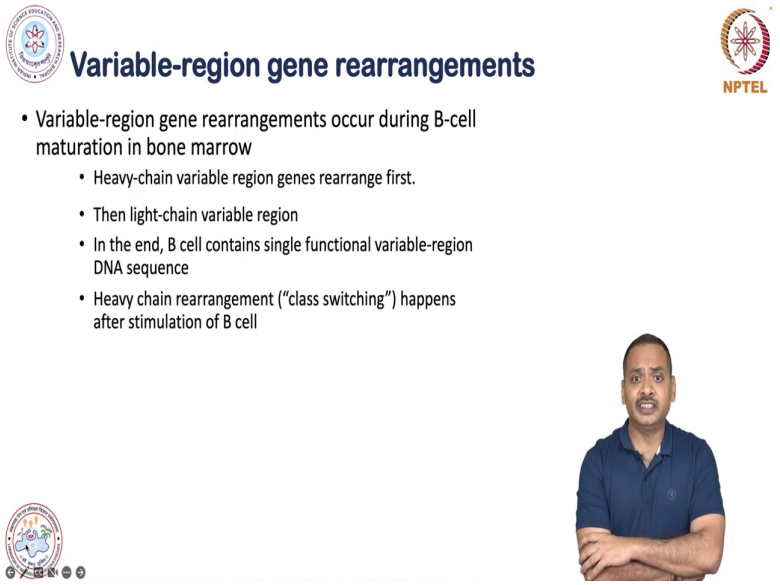

Now I will talk about the variable region Gene rearrangement. How this Gene rearrangement is taking place. So, variable region gene rearrangement occurs during B cell maturation in bone marrow. Here this is you have to noted that this is antigen independent and heavy chain variable region Gene rearrangement takes place first. Thereafter the light chain variable region Gene rearrangement is taking place.

And in the end B cell containing single functional variable region is there in DNA sequences and heavy chain rearrangement basically the class switching happen after the stimulation of B cells with antigen.

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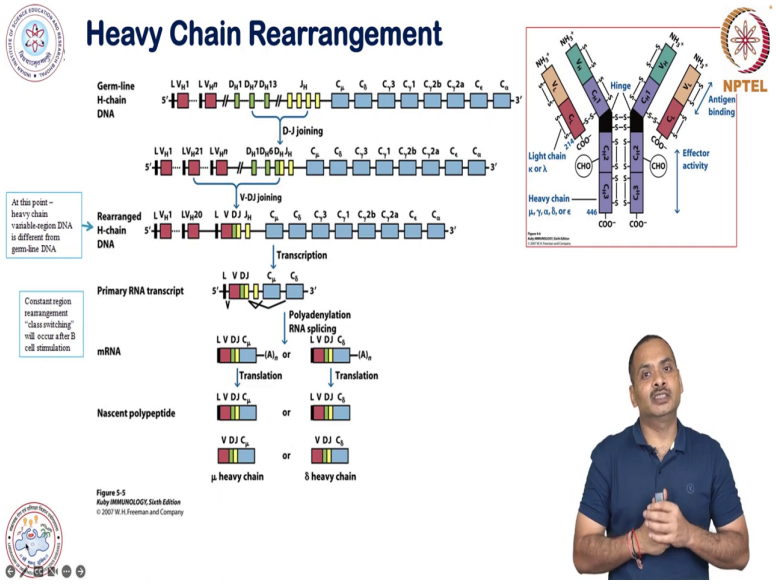

Here you can see how this gene rearrangement is taking place if you remember the previous Slide the first rearrangement taking place on heavy chain. And you have seen there are variable region there is a diversity region and there is a joining region or joining. So, these Gene segments are there. So, this Gene segments are basically present over the genome and the first rearrangement is taking place between D segment and J segment.

So, here you can see that the first DJ joining is taking place and after that there will be a V DJ recombination is taking place we join with which is V join with DJ which is already rejoined I mean. So, previously it is joint, so, finally first DJ recombination is taking place and then V DJ recombination taking place. And eventually there will be a again joining of this the rearrange VDJ will take place with the constant region Gene. Here you can see that there is a primary transcript.

So, initially there is a DJ recombination is taking place here you can see in this slide and then this V D-J joining is taking place and then this rearranged VDJ is joining with a constant region and then there will be a formation of mRNA and polypeptide and so on. So, in mature B cell both for Mu chain and for Delta chain is taking place that is why on mature B cell there will be a expression of IgM and IgD is taking place.

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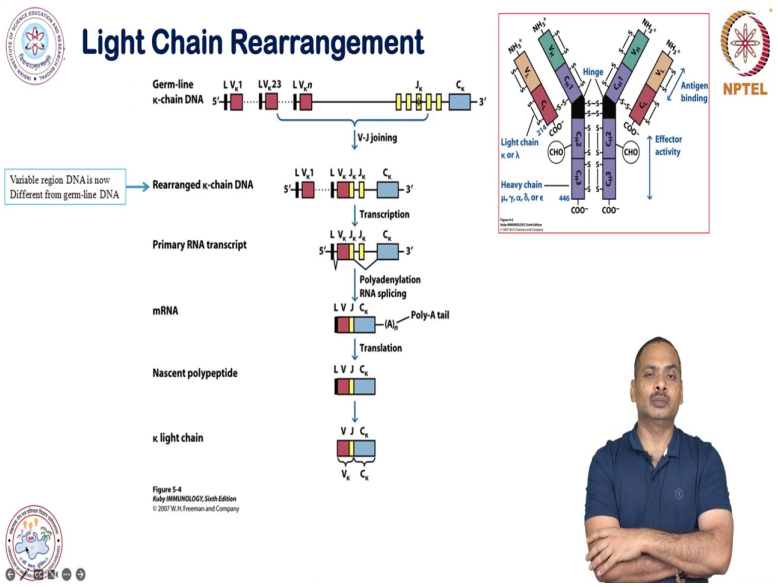

Thereafter the light chain rearrangement is taking place and this light chain rearrangement is a basically the recombination is taking place between V and J because there is no D Gene segment which is encoding for the light chain there is no D Gene segments. And the process is almost similar as previous and then the mature mRNA will be generated which is having a sequence for the leader sequence and V region J region and C region.

And finally the polypeptide will synthesize you know all those things how the polypeptide is synthesis taking place you have studied the molecular biology.

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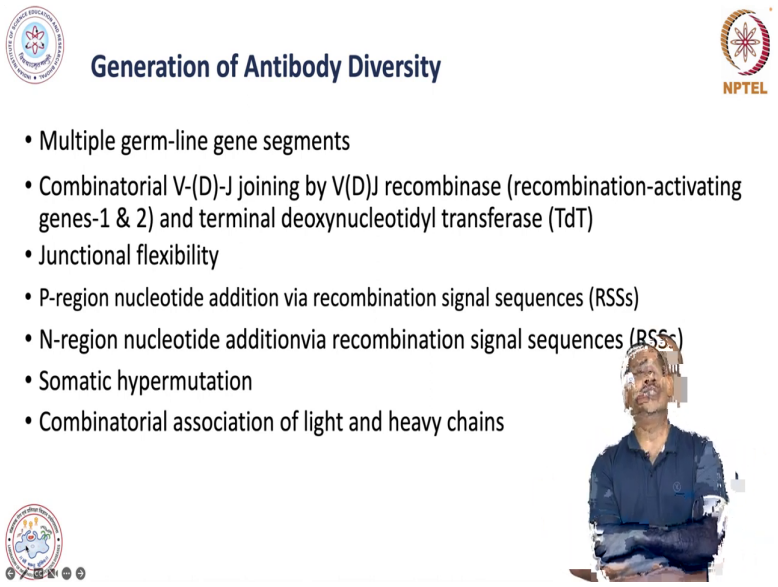

So, all these things eventually result to the generation of antibody diversity. So, but in addition there are so, many things which is taking place in formation of antibody. How this generation of antibody diversity is taking place. The way I have explained you this is only

one way but there are some more ways by which diversity specificity and high affinity generation of antibody all these things are taking place.

The first one is multiple Gene germ line Gene segments are there that is of course giving the diversity as I have explained you in previous slide. There will be a combitorialputerial VDJ joining by VDJ. So, there is a one some very important enzymes are there which is triggering this recombination generally recombination is not a very common phenomenon it is a it is a very rare phenomena.

And the enzyme for this recombination is present in these lymphocytes and these the enzyme is VDJ recombinanaset basically it is consists of two major enzyme the two major enzyme were discovered that is recombination activating Gene in short form we also call it as a RAG1 and RAG2. If you make the knockout of this rag one or red two then these mice will be immunocompromised.

This is very important to enzyme and this is needed for this Gene rearrangement and in addition there is a one more enzyme this is known as terminal deoxy Newton nucleotidal transferase. So, all these enzymes are basically playing a very important role in recombination and of these Gene segments. There is a junctional flexibility. So, I will tell you briefly about the junctional flexibility in subsequent slide what is the junctional flexibility.

Basically, if you know that this codons are triplet codon and if there is a some addition and removal of these codons then the whole frame will change or it may change the particular amino acid. So, all those things are also there when the antibody diversity is generated. There is a some specific phenomena is taking place that is P region nucleotide addition and this is basically taking place via some unique sequences.

There is a recombination signal sequences this recombination signal sequences ensures that there will be a node 2 V and there will be no two V region or Gene segments will be present in mature transcript or during DNA rearrangement there will be no 2 J or there will be no wrong recombination is taking place this is ensured by this RSS sequences. I am not going to tell the detail if I had a long time then I could explain all those phenomena at the molecular level.

It is it is a very interesting. There is a N region nucleotide addition which is also taking place via this RSS sequence then there is a somatic hyper mutation if you remember the antibody structure which I have shown you in previous session I have shown you some blue strips over the light chain and heavy chain not blue it is a black colour strips and I told you this is a hyper variable region or CDR complementarity determining region.

So, over there is a some finer mutation is taking place in order to make a high Affinity antibody and this is basically triggered through this somatic hypermutation. There will be a combitorial~~puterial~~ association of light chain and heavy chain and overall this will give a huge diversity whatever the question people had earlier how this there will be a huge diversity with a limited genome.

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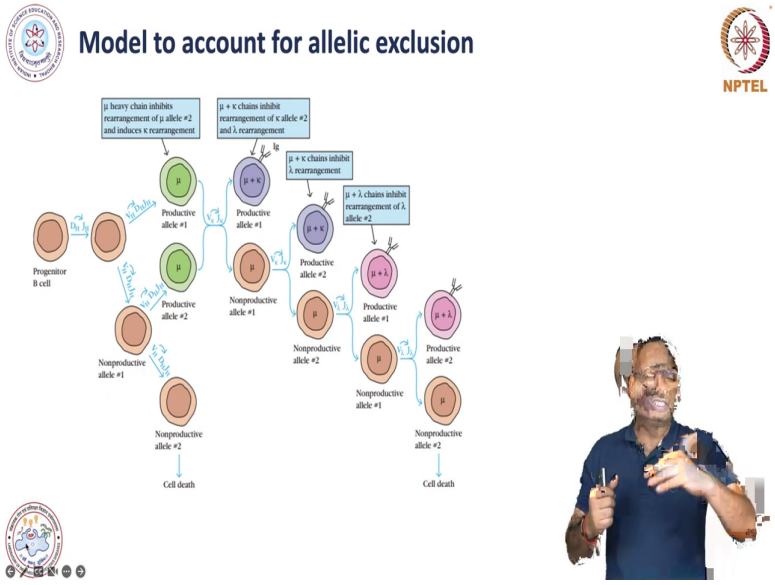

In addition, the there you know that so, far I have discussed whatever I have discussed you did not consider the ploidy of cell, generally our cells are diploid~~deployed~~. So, how it is ensured that only what is going on in if it is the diploid~~deployed~~ case. So, for you my you might not thought about that there are two sets of Gene. So, basically it is taken care by these very interesting phenomena which we call it as allelic exclusion. So, first the heavy here you can see that there will be this these Gene segments are coming from paternal side as well as maternal side.

So, first it is I will show you this is schematic here you can see that progenerator B cells. So, there will be a D J recombination taking place in one of the allele and if it is successful then it

will move to the another but if it is unsuccessful then since the cell has another copy. So, over there this DJ recombination is taking place. So, in that way this whole thing is taking place.

And if once this mature or proper transcript will be generated for like chain and heavy chain then that will the another set will be not used until both sets are exhausted. So, this is a model to account for allelic exclusion.

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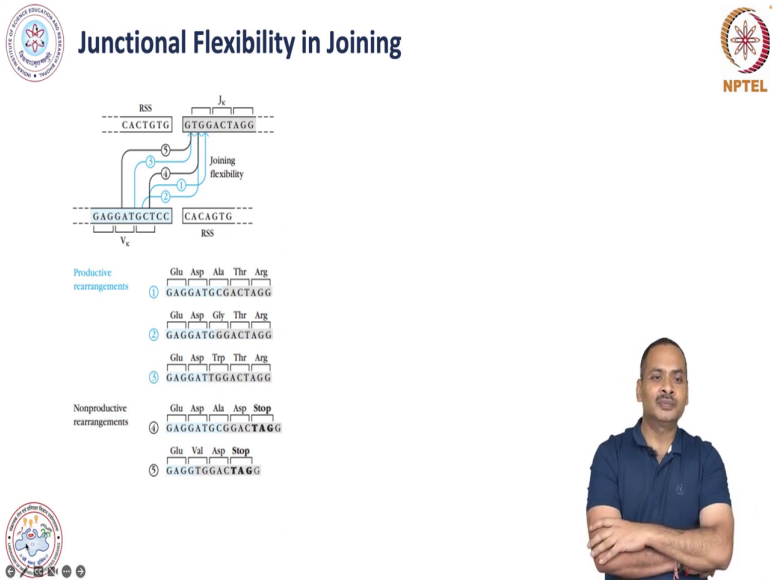

There is a junctional flexibility I have told you in previous slide what is the junctional flexibility. Here you can see that there is a diff different possibility how this once this Gene the DNA will break this can combine with the various bases on another Gene segment. And that may result sometime it may result to the productive or sometime it may result to the non-productive here you can see in first three cases there is a productive but another two cases it is non-productive.

Because that results to the incorporation of a stop codon and polypeptide cannot be fully synthesized. So, this is a non-productive. So, if this is the situation if the cell will face this situation then the cell will move to the another set of Gene another allele. So, in that way this allelic exclusion work.

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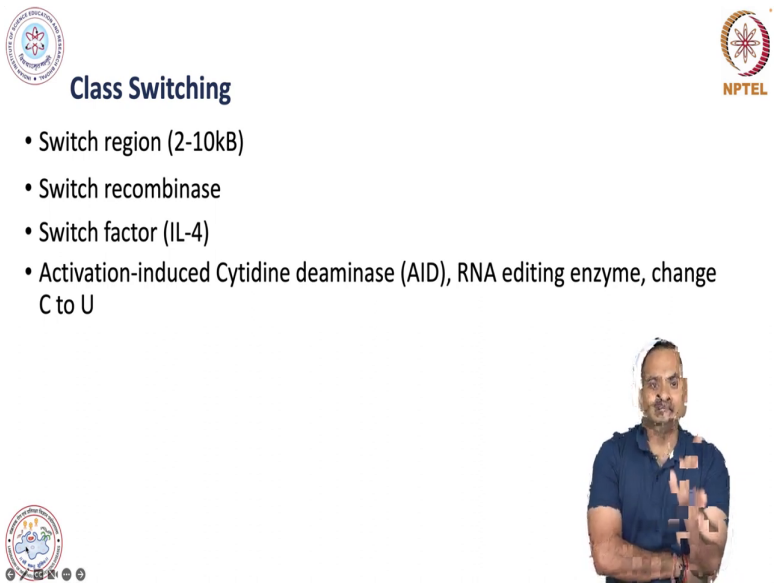

Now there is a one more question last question which is how the class switching is taking place. So, there are some still it is under quite active investigation but still we people are trying to find out the how the class switching is taking place. But we have some evidences which helps in understanding the class switching. There is a some sequence present in in upstream to the constant region Gene segment which we call it as a switch region.

And it is consists of about 2 to 10 kilo base pair there is a switch recombiness there are enzymes and in addition there are some factors like IL4 which is produced by TH2 cells they also help in class switching. And finally there is a discovery of one enzyme which is a playing very important role in class switching that is ~~activation induced cyted in dmnase~~ Activation-induced cytidine deaminase we call it as a AID. It is simply RNA and editing enzyme which changes C to U.

C to U and that if you make the knockout of these mice then there is a no class switching in antibody. So, these are the things which trigger the class switching class switching you understand from IgM to IgG type.

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Ab diversity – Multiple gene-line segments AND combination of those segments

TABLE 5-2 Combinatorial antibody diversity in humans and mice

Multiple germ-line segments	Heavy chain	LIGHT CHAINS κ	LIGHT CHAINS λ
	ESTIMATED NUMBER OF SEGMENTS IN HUMANS*		
V	48	41	34
D	23	0	0
J	6	5	5
Combinatorial V-D-J and V-J joining (possible number of combinations)	48 × 23 × 6 = 6624	41 × 5 = 205	34 × 5 = 170
Possible combinatorial associations of heavy and light chains†		6624 × (205 + 170) = 2.48 × 10⁶	+

Here overall how the antibody diversity is generated you can understand. So, this is in case of human. So, there are in case of human there are 48 V sequences are there for heavy chain and 41 V sequences are there for Kappa and 34 for Lambda. D sequences is present only in heavy chain, so, this is 23 it is not present on light chain I have explained you. And J sequences are about 6.

In case of heavy chain and for light chain light chain Kappa chain will be 5 and Lambda will be 6.. So, if you if you do simple mathematics you can find out that and there is a we can make a huge number of an antibody about 2.5 million types and here please remember that you have not included the somatic hypermutation J joining and all those things junctional flexibility and all those things.

So, if you add up all those things. So, you can the cell can generate a numerous diversity it is it is difficult to count. So, this is the genetic basis of antibody diversity this is a very important topic or very important in important Discovery in the in the antibody or in fact the Immunology. And similar diversity is also taking place in case of T cells which I will discuss in next session and I will take very short session about the T cells. And then we will finish this week, thank you.