Essentials in Immunology
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Module No. # 05
Lecture No. # 09
Organisation of immunoglobulin genes and
Mechanism of immunoglobulin gene rearrangement

Today's lecture is going to be on the organization of immunoglobulin genes and the mechanism of gene rearrangement. Now, the title itself will tell you that there is something rather special about these immunoglobulin genes as compared to other genes of our system. Now, what is the difference? Let us go back to looking at the B cells themselves.

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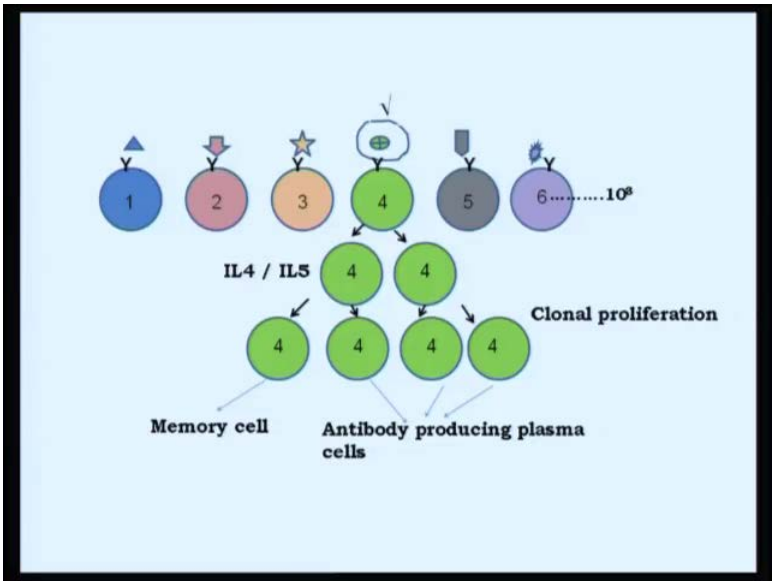

We know that we have countless number of receptors or antigen receptors which can recognize a countless number of antigens; not only antigens, let us be a little bit more specific - epitopes on antigens. It is estimated that we are capable of recognizing about a 100 million different epitopic sequences by the B cell antigen receptor. What is shown here is six different B cells. And though they have been depicted here as different colors, we know that all B cells are morphologically very similar. They have also similar receptors on the cell surface.

All of them have antigen receptors which also look very similar. We also know that each one of these cells is capable of recognizing different antigen and it is this that triggers the activation process which is what we dealt within the lecture and clonal proliferation.

We also know that such cascade of proliferation results in the generation of anti-producing plasma cells as well as a few memory cells. Now, how is it that, in this, what is represented here in this cartoon? Only one; that is cell number 4 reacts with its cognate antigen and that undergoes proliferation, whereas the others are bystanders. This can only be because of the specificity with which only clone number 4 is capable of recognizing the antigen.

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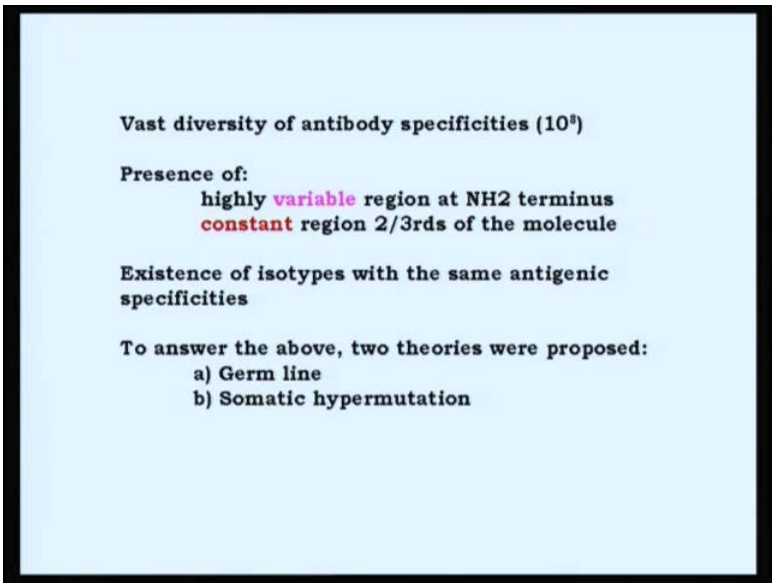

So, we have a vast diversity of antibody specificities. Like I have said earlier, we are capable of recognizing and mounting an immune response to 100 million different antigens.

Intriguingly the same molecule, you know, the antigen receptor has a highly variable region at the amino terminus that the terminus with through which it recognizes the antigen at two-thirds of the same molecule, it is very constant. So, if we can go back to the previous slide, we can see that two-thirds of this receptor which is present on each of these B cell is identical. It is only the amino terminus as small fragment, which is highly variable.

The other intriguing part is that the same antigenic specificity can be conferred on different isotypes of immunoglobulin. Now, I have not yet introduced the class on isotypes of immunoglobulin molecule, but it is something that you may have studied already, that we in mammals have five different classes of immunoglobulin. Now, ideally, one would have only IgM production in the primary phase of the immune response. Now, the same IgM starts to get switched to another isotype, but the specificity of binding to the antigen remains same.

So, how is it that there is existence of different isotypes, but with the same antigenic specificity? Now, to answer the question - how is it that we are capable of recognizing such diverse antigens? How is it that immunoglobulin molecule is very variable at one region, yet very constant on the other region? To answer this, there were two theories that were proposed about 50 years ago. One was the germ line theory and the other one somatic hyper mutation. Now, the germ line theory propose that we in fact do have that many number of genes which correspond to a 100 million different specificities.

This of course, one can imagine, could not be so because the body cannot invest in such a large proportion of genes being dedicated only to the immune system; not only immune system, the B cells, in specific. So, the second theory that was put forth then was somatic hyper mutation that, this theory said that all receptors on the B cell surface are identical with the same region or sequence at the amino terminus, but it is when such a B cell encounters an antigen that there is hyper mutation in the region and there is antigen and receptor interaction.

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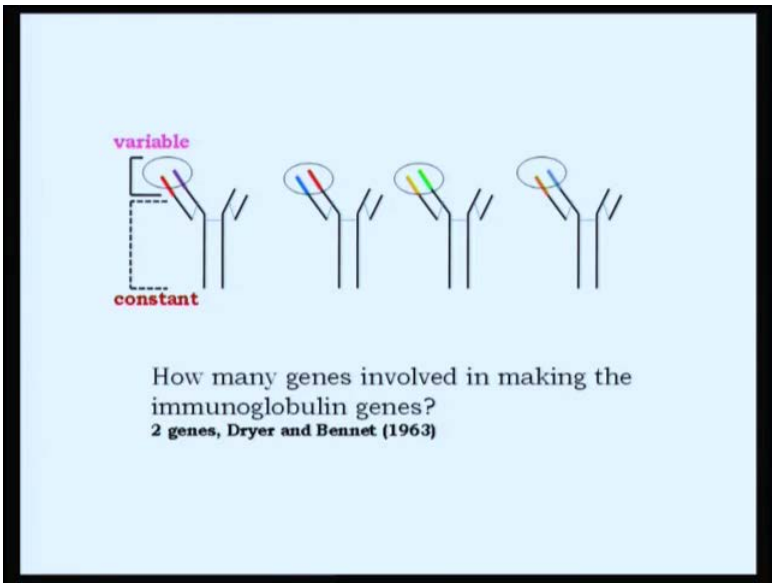

This also did not hold much water and it was in 1963 that Dryer and Bennet proposed for the first time that the immunoglobulin is coded for by two different genes.

We do know now that is not two different genes, but two different gene segments: one that corresponds to the variable region and the other gene segment to the constant. That here I have represented several molecules of immunoglobulin which are identical, as you can see, with a heavy and the light chain; two-thirds of the molecule is identical which is shown in black and the variability lies only in the amino terminus and you can see that heavy and light chain both of them have variable domains.

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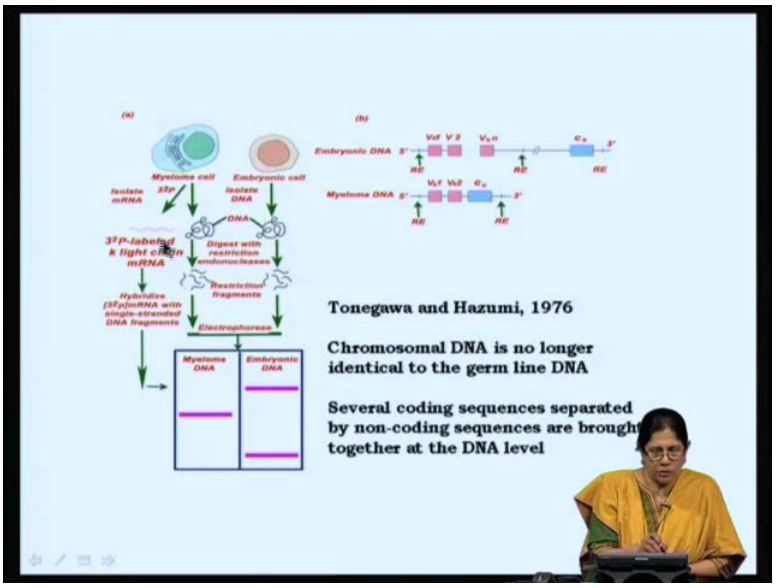

So, though dryer and Bennet proposed in 1963 that there could be two gene segments, well you know gene segments now, code for the immunoglobulin whole molecule. It was in 1976 just 13 years later that Tonegawa and Hazumi did very elegant experiments to demonstrate that indeed there are different gene segments at the DNA and there is reorganization of the immunoglobulin genes at the DNA level itself. Let us look at the experiment that they carried out.

Now, what they did was, they have taken DNA from embryonic cells which now represent the germ line. They have then taken a myeloma cell. What is a myeloma? It is a cancer of the plasma cell; plasma cell, just to make you remember that plasma cells are the end stage cell of a B cell. They produce antibodies and as you can see from the picture, plasma cell has a very well developed endoplasmic reticulum. Now, they have taken the DNA from the myeloma cell as well as DNA from the embryonic cell. The DNA was then isolated and digested with restriction endonucleases. So, that is small fragments were generated. These were then electrophoresed separately.

In parallel, m RNA was isolated from the myeloma cell. The RNA, m RNA was labeled this P32 - radioactive phosphorous, and the mRNA corresponded to the light chain of the immunoglobulin gene. The labeled mRNA was then hybridized to the fragments of the DNA taken from the myeloma cell or the embryonic cell. Now, what they saw always that in case of the myeloma cell, the hybridization happened with a single band, whereas

embryonic DNA hybridized with two different bands. What do they suggest? Very easily one can say that the DNA corresponding to the kappa like chain is not identical when you compare the myeloma cell, the end cell stage as well as the embryonic. So, there has been a change in the DNA itself when the myeloma cell is established.

Chromosomal DNA is no longer identical to the germ line DNA and now what did they observe here? That several coding sequences separated by non-coding sequences in the germ line DNA are brought together at the DNA level in the B cell. I tried to put this in the form of a diagram which says, now the embryonic DNA is much longer. And by deletion of some segments, now in this particular case, several segments including this one are deleted and the myeloma DNA in the immunoglobulin kappa chain is smaller than the embryonic DNA.

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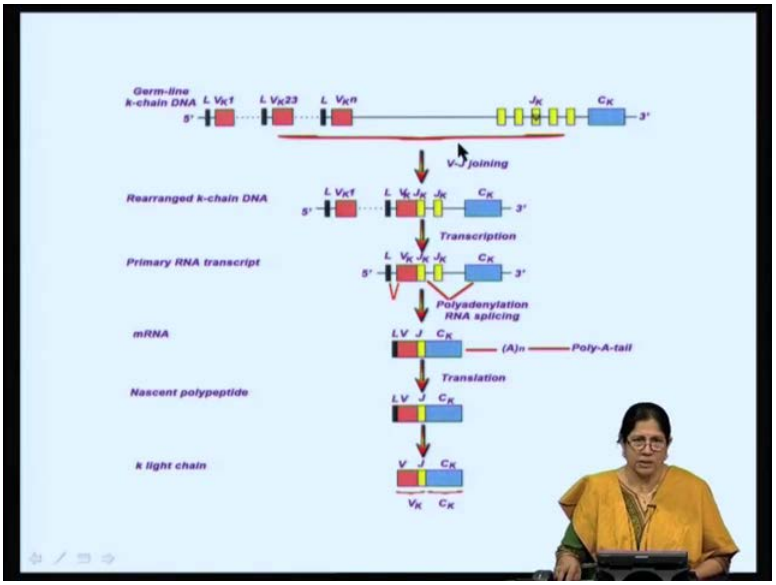

Now, this was the first report and for this, in fact Tonegawa got the Nobel Prize several years later and this was really a remarkable experiment. Now, the reason why people were not able to demonstrate this earlier is just because of lack of appropriate technology. So, as soon as technology became available, molecular biology technology has become available. It was possible for people to do experiments to demonstrate that there is rearrangement in the immunoglobulin gene in the B cell or the similar event happens also in the T cells, and you will probably hear about it in another class.

Now, after this first report of Tonegawa and Hazumi, several gene sequences of the immunoglobulin genes were revealed, and now I am going to show you typically the gene reorganization in the kappa light chain gene and then also the heavy gene.

Now, let us look at the germ line kappa chain which has not undergone rearrangement. As you can see that there are two sets of sequences here in the variable domain; one that is shown in red here which is called V K - variable kappa; there are three segments that have been shown here: V K 1, V K 2, V K 3. Now, these are separated by a set of sequences here; gene segments, rather which are known as J sequence after the J sequences is the constant region and you all might remember that kappa or the light chain is much smaller and has only one constant omega.

Now, every V gene segment is separated by introns from the other V gene segments and separated by large stretch from the J gene segments. On the 5 prime side of every V gene segment, there is a L segment or the leader peptide segment. Any one of the J can join with any one of the V. There is a slide that is going to depict later on, how many V gene segments we have. Well, how many we have and how many mice have? Most of the studies that have been carried out by immunologist have been carried out in mice because mouse is a good model and almost whatever is true of mouse is true of humans as well.

Now, like I said, there is a random process and any one of the J can join with any one of the V. So, this happens at the DNA level. I would like to keep on saying that the rearrangement is happening at the DNA level and what is shown here is V segment 2 joins with J segment 4. This is the rearranged kappa chain DNA. When transcription takes place and the primary RNA transcriptor is formed, then you have still J 5 which is in position, but which gets deleted when the mature mRNA is made. This V gene, the variable gene segment which is formed by 1 V and 1 J is what gets associated with the kappa sorry the constant kappa. Then you have the nascent polypeptide and you have the kappa light chain which is either cells of is exposed or is a part of the antibody.

You can see the absence of the leader sequence and the leader sequence is required because immunoglobulin is a secretory protein and it goes through the transport from the ribosomes to the endoplasmic reticulum to the golgi and the and then by vesicular transport to the cell surface or get secreted.

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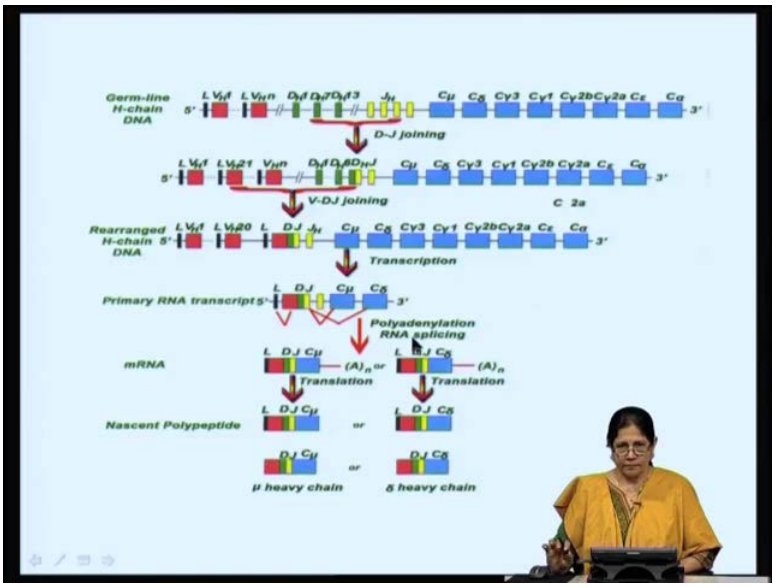

The heavy chain is more complicated as one can imagine. First of all, let us look at the constant domain. Kappa chain had only one constant domain. The heavy chain has several each one corresponding to the constant domain of the classes of immunoglobulin m V IgG 3, IgG 1, IgG 2B, IgG 2a. Then immunoglobulin e and immunoglobulin a; all of these have a very specific orientation. These are separated by the variable region of intervening sequences.

Now, what people found, you know, the light chains, of course, were easier to sequence. It was later when people started looking at the heavy chain they found that 4 amino acids found in the variable region of the heavy chain could not be counted for by sequences from the V or the J. So, when they looked closely, they found the presence of yet another segment which contributed to the hyper variable region; this is the D gene segment.

Now, any one of the J in case of the heavy chain joins with any one of the D gene segments. These do not recruit any one of the V gene set. Just like in case of the light chain, the way these gene segments are called are V H for variable heavy 1 variable heavy 2 and so on, and the number could be 300 to 1000. The D gene segments are fewer. These could be 7 to 13 and then lastly the J gene segment. Like I said a little while ago, first the joining in case of the heavy chain happens between any one of the J yellow with any one of the D green.

Once these are recruited, then they recruit any one of the V. The joining takes place at the DNA level itself, rearranged heavy chain and when this is gene is transcribed, then you have the primary RNA's transcript that associates the variable domain with the constant mu corresponding to m and/or constant delta corresponding to D.

Heavy chain reorganization is such that simultaneously the cell can produce both heavy as well as light chain corresponding to the mu or the IgM as well as the IgD.

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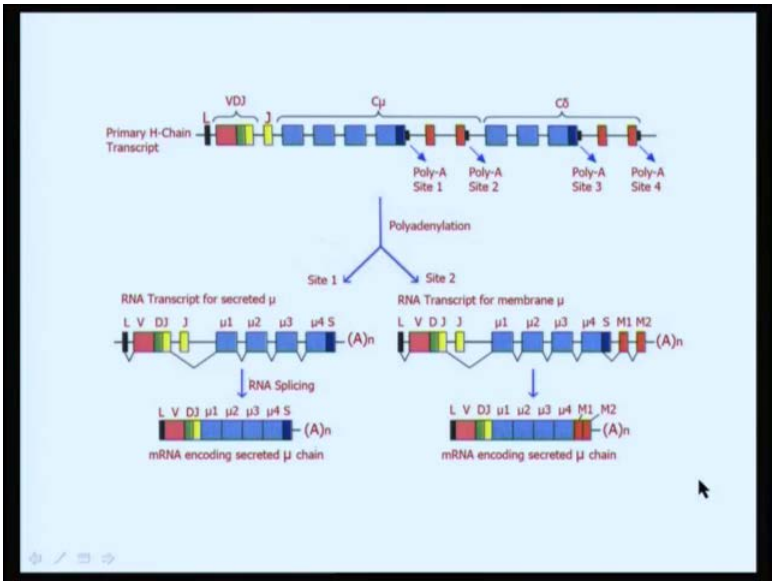

Now, how this happens is something I will come to a little later, the heavy chain gene is even more complex than what I just mentioned because what we dealt with is mostly the variable domain, but let us look at the constant domain, a little bit more closely.

I have not yet described to you the immunoglobulin structure, but I would just like to mention here that the constant domain of the immunoglobulin are made up of several constant domain called 1, 2, 3, 4 in case of IgM and 1, 2 and 3 in case of the I g D.

Each of these domains is coded for by a distinct gene segment. So, not only you have the constant domain, as such which is what was shown in the previous slide, but you also have smaller fragments thereof. Now, why is this necessary for me to explain is when we you will appreciate this when we come to the structure of the immunoglobulin.

Now, because you also have now a complexity with regard to the antigen receptor being different from the immunoglobulin being secreted in case of the receptor, the carboxyl

terminus should have a region which is hydrophobic, so that this gets attached to the membrane. This is in case of the naïve and the memory B cells. Once the cells become plasma cells, then the same domain, the variable domain and the constant domain should now associate with a small fragment in the carboxyl terminus which is which corresponds to the secretary part which is hydrophilic. So, there are two polyadenylation site 3, prime site of the last domain of the mu which is the fourth one and third in case of the constant domain delta. It is the way the polyadenylation occurs that you will have either mRNA coding for the secreted u chain or mRNA coding for the sorry there is a mistake here; that should be membrane bound. It should be membrane bound.

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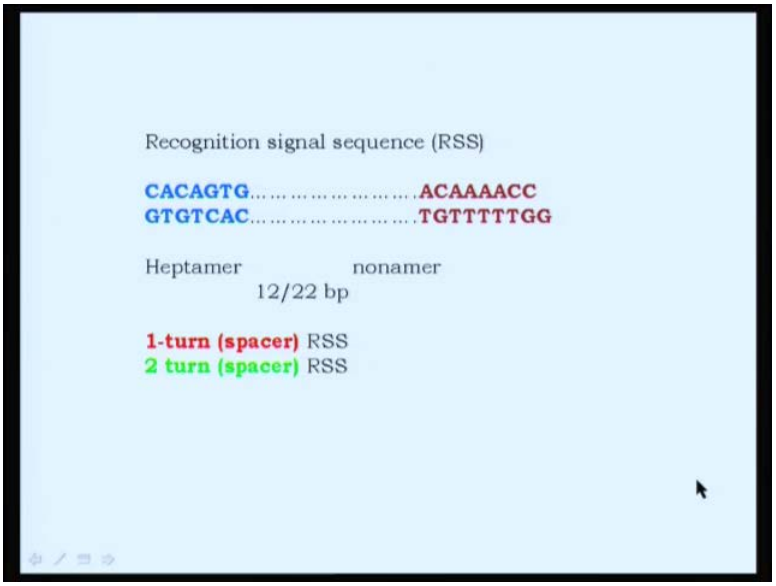

Now, the question comes; how is it that we have any one of the J joining with any one of the V? Now, remember that the immunoglobulin gene is very almost identical where the gene the immunoglobulin recognizes antigen a or antigen B. Therefore, the number of amino acids also needs to be more or less constant. We also know that though the variable region is hyper variable, the constant domain is more or less the same. Therefore, the immunoglobulin gene cannot accommodate more than one J segment or one V segment or in case of the heavy chain anyone V D and J.

There has to be a very well regulated event that c is that its only one of J that is recruited to one of V in case of the light chain gene, that this happens because in the presence of

recognition signal sequences which are present on the 3 prime site and 5 prime site of the J and V gene segments.

Let us look at what are these recognition signal sequence; in short, they are known as RSS. Now, RSS is typically made of first a palindrome sequence CACAGTG; this is, you can see very easily, a palindrome which is separated by unknown nonamer which is a T rich. Now, this heptamer or the palindrome is separated by the nonamer by either 12 base pairs or 22 base pairs. Now, when these two are separated by 12 base pairs, it is known as 1 turn RSS, 1 turn recognition signal sequence are when separated by 22, 2 turn RSS.

Now, it is a rule that the segment with 1 turn RSS joins with 2 turn RSS. Therefore, now all the V gene segments on the 3 prime site have, let us say, heptamer nonamer separated by twelve base pairs; so, 1 turn RSS and all the J gene segments have on the 5 prime 2 turn RSS. Now, like I said, it is a rule - when joining takes place, always a 1 turn RSS comes together with 2 turn RSS.

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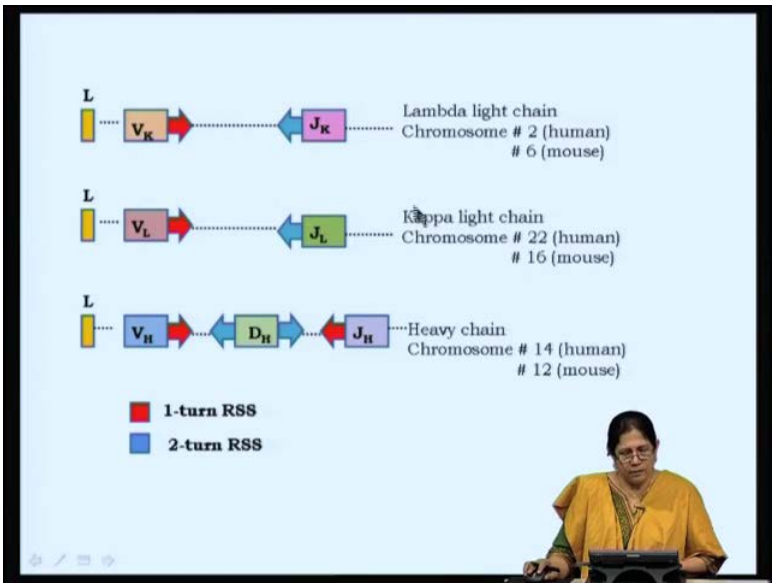

Depicted here, slightly better for you to understand; you have the leader peptide sequence which now has the close to it on the 3 prime side V gene segment.

Now, on the 3 prime site of the V gene segment you have 1 turn RSS, whereas on the 5 prime side of the J gene segment, you have 2 turn RSS. Now, 1 red and 1 blue, only

these 2 can come together and join. This is the lambda light chain and this is the kappa light chain. Now, both of these are very similar to each other except I would like to mention here and which we will discover later kappa light chain has a little bit more variability than the lambda light chain.

In mouse, in fact 95 percent of the immunoglobulin gene associates with kappa light chain. Lambda and kappa light chain genes are present on different chromosomes; chromosome 2 in human, 6 in mouse, has the lambda light chain; kappa light chain is present on chromosome 22 in human, 16 in mouse and the heavy light chain gene are present on chromosome 14 in human and 12 in mouse.

Now, what are just discussed with you was dealing with the kappa light chain that you have 1 RSS and 2 RSS which join together. Now, what was in question is in case of the heavy gene, you have 3 genes segments; you have the V, D and J.

So, how does 1 turn RSS and 2 turn RSS now contribute to allowing one of the J joining with any one of the D and any one of the V? So, when people sequence these genes closely and examined, found that the D gene segment has RSS on both sides and the RSS are identical. Therefore, what I have shown here is the RSS in blue which would mean D has 2 turn RSS on both sides; that is 3 prime side as well as 5 prime side.

Now, the V and the J gene segments have on the 3 prime and the 5 prime respectively 1 turn RSS. Now, it is known; it has been proved that first any one of the J on the heavy chain joins with any one of the D, and once this is complete, then recruitment of any one of the V takes place.

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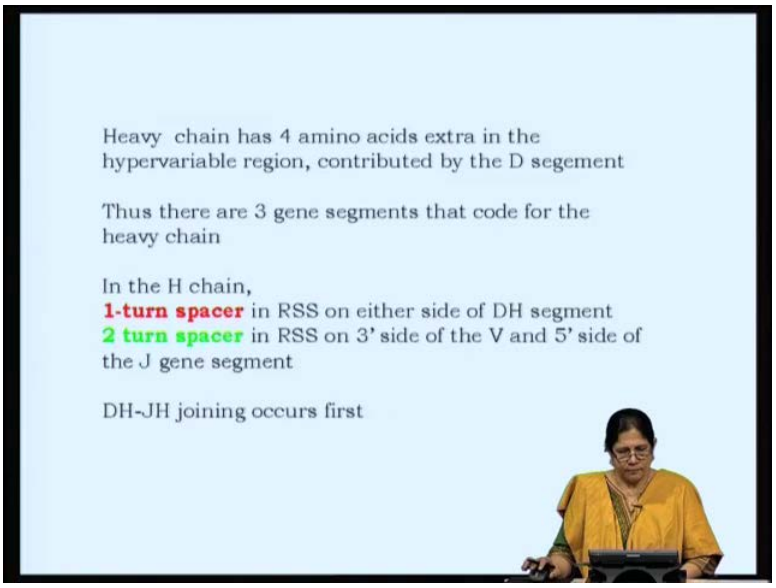

So just to tell you again since heavy chain has four amino acids extra in the hyper variable region, people looked for another set of gene segments and found that it is the D segment; D for diversity. This is therefore, this therefore, makes the heavy chain gene slightly more complex than the light chain. And we know by several studies later that the heavy chain contributes much more than the light chain in antigen binding.

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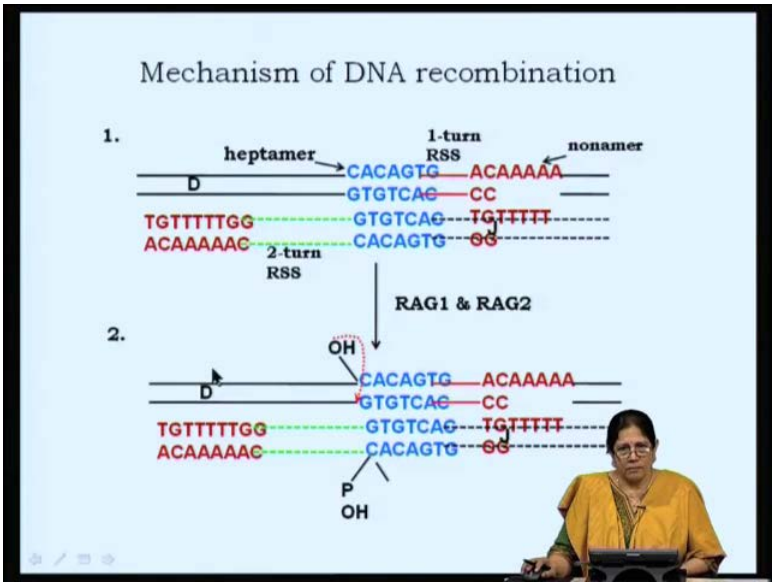

Now, how does this joining take place? This is quite a well-coordinated and complex mechanism. Now, first of all, both T and B cells only express a set of enzyme called

RAG1 and RAG2; recombination activated genes RAG and RAG2. We always tend to draw the DNA as straight lines, but we do know that these are quite looped. You know the DNA loops out. The RAG1 and 2 recognize the RSS. Now, RSS, the recognition signal sequences which are depicted in blue - the heptamer, and brown - the nonamer. Now, what shown in 1 is the DNA, now, which has the D segment which is going to join with the J segment.

So, the D and the J come together which is the random process; it could be any one of the Ds - 1 to 13 and any one of the Js - 1 to 7; they come together because RAG1 and 2 recognize; the sequences bring them together. RAG1 and 2 not only recognize the signal sequences, but they also nick the DNA on 1 strand and this is always at the joining of the coding strand and the RSS is a rather precise.

So, recognition of the DNA by the enzyme at the RSS nicking takes place on one strand. This generates a free hydroxyl radical which now attacks the opposite strand and cleaves the phosphorised trace bond. The RAG1 and 2 mediate recognition cleavage as well as now a trans esterification reaction, which now makes a hairpin loop. Now, what I am showing here is what is happening on the side of the D strand. So, you have now first cleavage happening on one strand and then the second strand, and the similar situation is on the strand which has the J gene segment. You have cleavage of the RSS or between the RSS and the coding strand.

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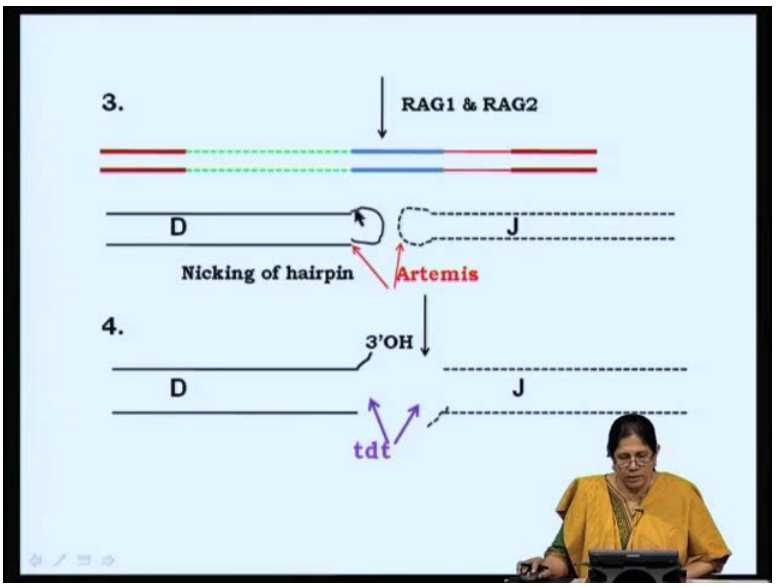

And what you have? RAG1 and 2 mediated hairpin loop structure formation. Now, you would like to… so you can imagine that all this region this entire region as well as this entire region now (Refer Slide Time: 30:32) gets exercised out and you have now the relevant D segment comes in close proximity to the J gene segment.

Now, this hairpin structure that is formed is cleaved very specifically by an endonuclease which has been characterized rather recently known as Artemis. This enzyme that I said is an endonuclease does not recognize any sequence; interestingly it recognizes this hairpin loop structure. The nicking takes place randomly and on both, and you have now free or cut DNA. I will be dealing with the way these nucleotides are then added by terminal deoxy ribosyltransferase; remember, this is one of the marker enzymes present in V cells. Now, you have you can imagine, see that, variability would take place here because there is addition of nucleotides at the cut ends.

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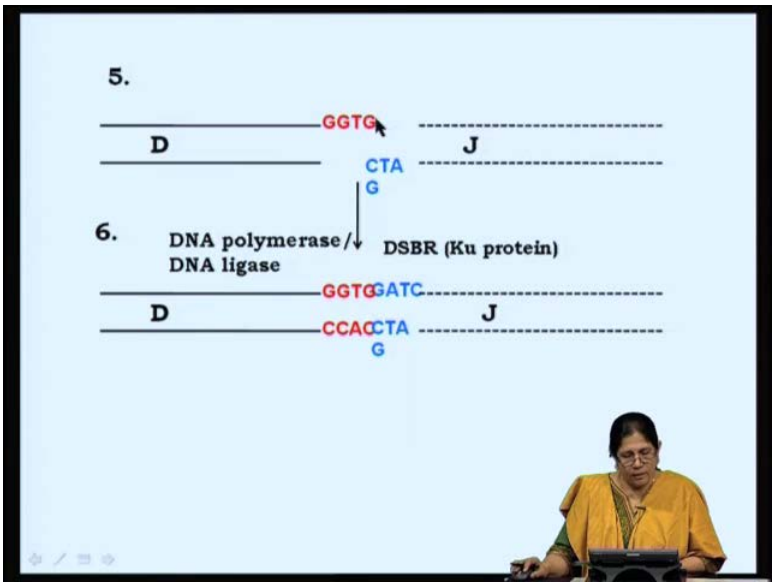

Then you have ligation. So, this is where the cut ends; there are additions of nucleotides and now you have double strand break repair enzymes which stitch the D and J together.

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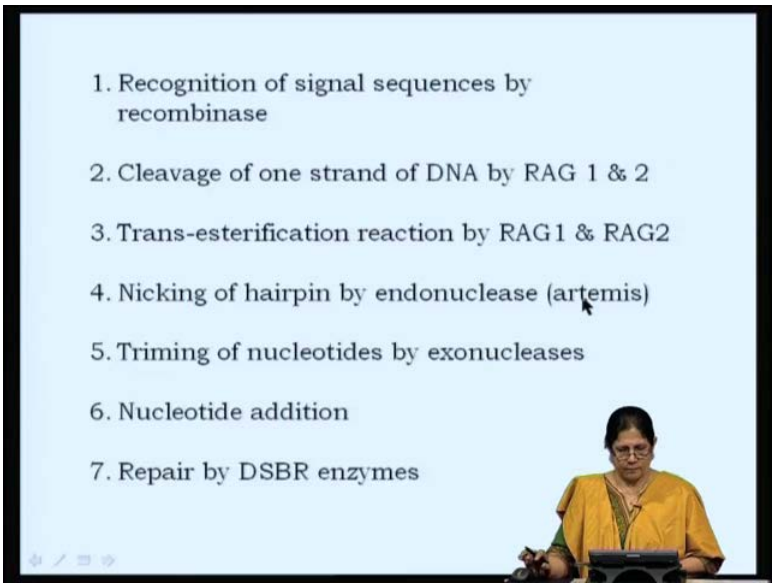

Now, you can see it very easily; what are the different mechanisms that govern or that takes place for this RSS recognition cleavage, and finally, the two in this case 1 D and 1 J coming together. I would like to go back go over this once more.

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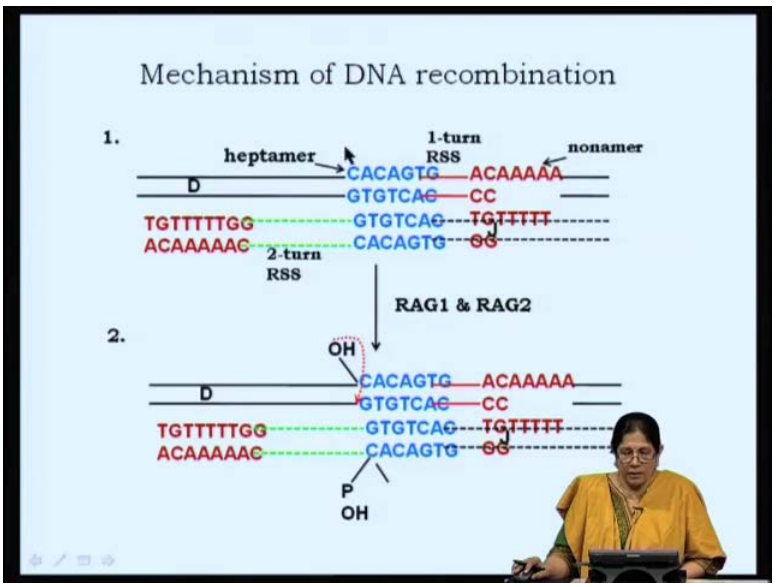

Let us see once again mechanism of DNA recombination. You have recognition of the heptamer, the 1 turn RSS separated by from the nonamer. Now, this is recognized by the RAG1 and 2 along with a another RSS which is a 2 turn RSS, where the nonamer and the heptamer are separated by 22 base pairs.

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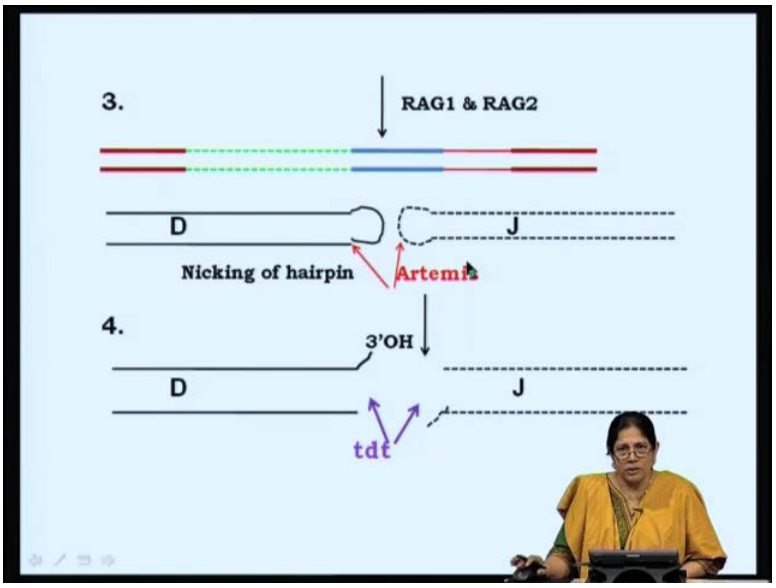

Once these come together, then the enzyme nick 1 of the strands of the DNA establishing a hydroxyl radical which then attacks the opposite strand bringing about a trans esterification reaction and making these hairpin structures. These hairpin structures, in turn, are recognized by specific endonucleases called Artemis, which can cut any part of the hairpin structure because neither the recognition nor the cutting is specific. These now generate open ends which are filled by DNA polymerases plus by addition of new nucleotides by the terminal deoxy ribosyltransferase and then double strand break repair enzymes which stitch the DNA together.

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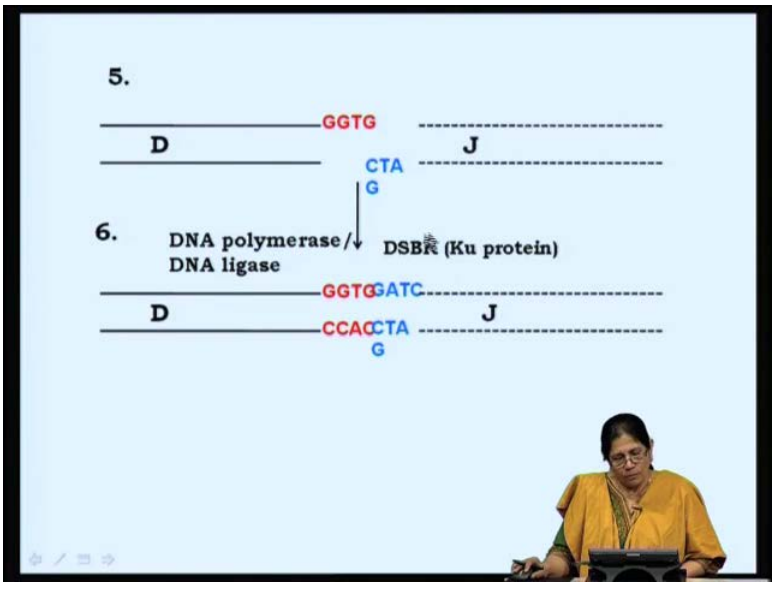

Now, the Ku protein is not only specific for in B cells; these could be present in several cells.

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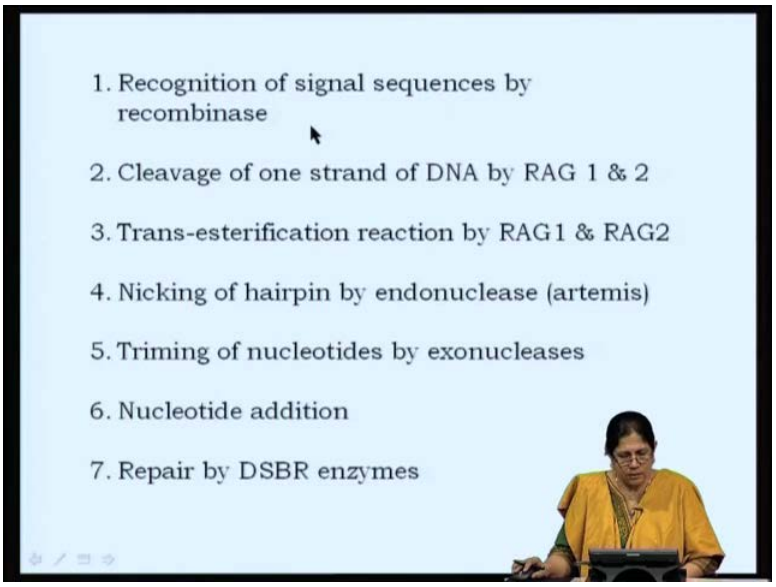

So, recognition of signal sequences by recombinase machinery that is the RAG1 and 2; cleavage of 1 strand of DNA by RAG1 and 2; trans esterification reaction by RAG1 and 2; nicking of hairpin by endonuclease Artemis; trimming of nucleotides by exonucleases; then nucleotide addition and repair by DSBR enzymes.

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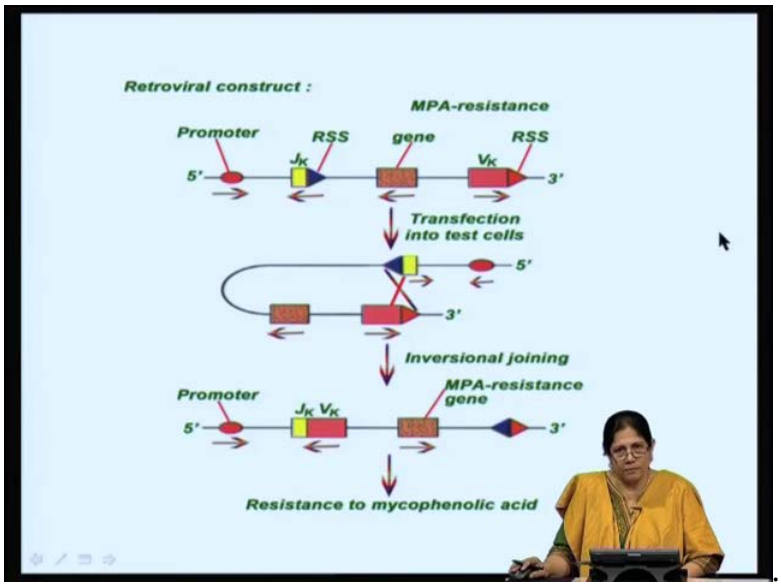

Now, we know all this its text book knowledge nowadays that how could people arrive at the presence of RSS and the recognition signal sequences are recognized by RAG1 and 2 and then joining takes place.

Now, I would like to tell you about this very allegiant experiment that was carried out about 20 years ago. A retroviral construct was made were the construct had a promoter which was not in frame with the J gene segment with its RSS on the 3 prime side. Now, then followed the gene that conferred a confers resistance to a drug Mycophenolic acid. Now, the gene is placed between J K RSS and V K RSS; I just described earlier that the joining of D and J are the same thing; what happened in the case of V and J happens in the kappa light chain.

Now, the gene is placed between these two, such that only if recombination between these two takes place, can the gene be in frame with the promoter and therefore, thereby get expressed. Now, this construct was transfected into test cells I think they have taken fibro blast and in the same fibro blast they also transfected the RAG1 and 2 genes; RAG1 and 2, only when RAG1 and 2 were also transfected and expressed in the cells that the cells became resistant to Mycophenolic acid. This is a very simple experiment; it would appear, but generate the generation of this construct was what was difficult. Let us look at this a little bit closely.

So, if RAG1 and 2 in the cells were expressed, they would bring about the same recognition and cleaving as I described earlier. This would then allow V and J to come together, which would then allow the promoter to be in frame with the MPA resistance gene, or let me put it the other way, by inversion joining; therefore, the MPA resistance gene would come in frame with a promoter and therefore, would get expressed. Only those cells that received… the all cells received the construct, but only those cells that received also RAG1 and 2 constructs and the protein was expressed, did they become resistant to MPA or Mycophenolic. Now, this was shown. The same experiment was also utilized later to show the presence of TDT and associated with generation of diversity which I will come to later.

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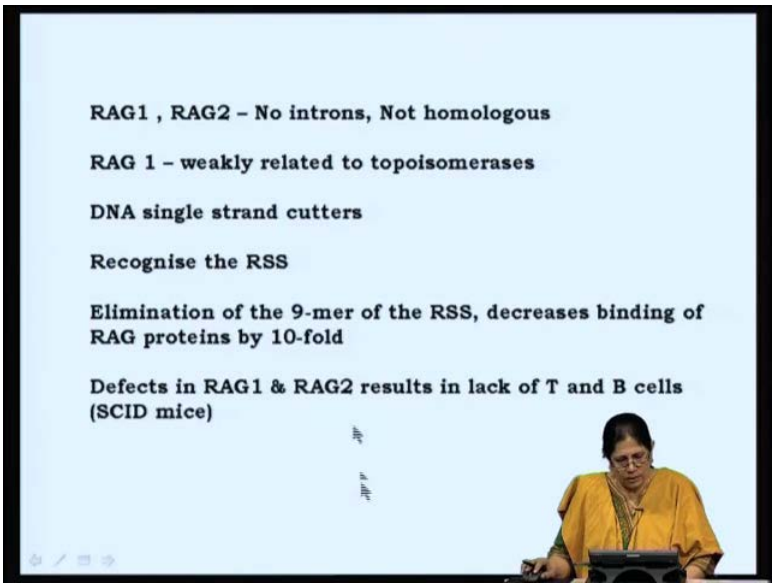

Now, RAG1 and 2; these two I had told you, are enzymes which are so specific and are absolutely required for generation of, in fact generation of B and T cells. Defects in RAG1 and 2 result in lack of T and B cells. There are set of mice, a strain of mice called SCID mice; SCID for Severe Combined Immune Deficiency.

Now, mutations in RAG 1 and 2 in these mice do not allow rearrangement of the immunoglobulin genes and if the immunoglobulin genes cannot be rearranged, then there is there are no B cells made. Therefore, not only B cells also T cells. So, such mice lack the acquired arm of the immune system completely.

Now, what are these RAG1 and 2? Just for interest, these are strange enough though they seem to be present in highly organized animals; RAG1 and 2 do not have any introns. So, they are primitive genes are these 2 related to each other; no they are not homologous, but RAG1 is weakly related to topoisomerases. You might remember topoisomerases from your DNA and molecular biology subjects; these are the cut DNA.

RAG 1 and 2 are single strand cutters. They cut only single strand, but then the OH, the hydroxyl group that is now generated is what attacks the opposite strand, but the enzyme themselves cut only single strands; of course, they can cut only if they recognize and place themselves on that particular segment and this is through the ability to recognize the signal sequences or recognition signal sequences.

I talked about the heptamer and the nonamer. Are both of these essential for the generation of this recombination? Interestingly, though everybody looked for the palindrome or the heptamer, it is the nonamer that appears to be very important because elimination of this 9-mer of the RSS, the eighty rich nonamer decrease the binding of the RAG proteins by tenfold. So, absolutely essential; both heptamer and nonamer are required for the recognition, but the affinity with which the enzyme is bind decreases a lot when the 9-mer is taken away.

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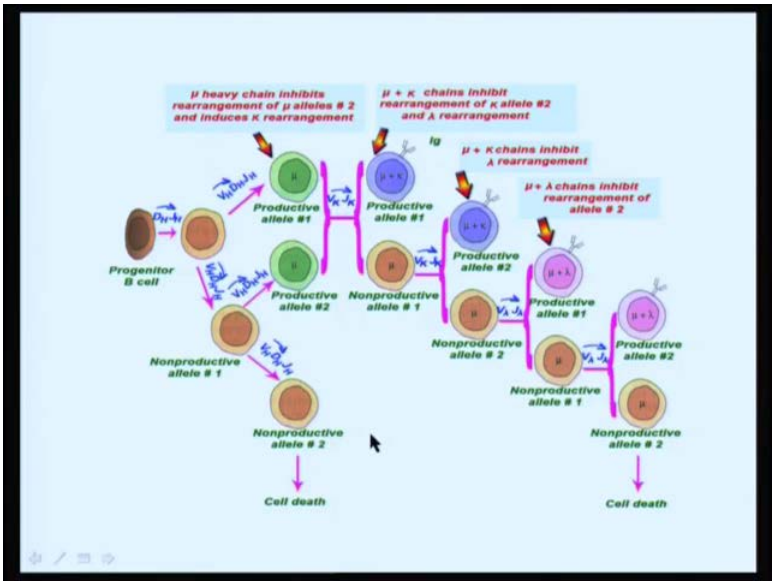

Now, I have come almost to the end of my talk, but I would just like to show this, the final slide that shows what is the what are the events or chain of events that take place for the formation of the entire immunoglobulin. Now, I have been talking about light chain gene separately and the heavy chain gene separately. Let us look at what starts first but I have not told you all that always the heavy chair assembly happens first.

If you remember the ontogeny of the B cells, you know my first lecture that you have the progenitor B cell which is already committed to become B cell already starts expressing RAG1 and 2, and therefore, at the level where they these progenitor cells come in contact with the stromal cells, they start now proliferation because of the interaction between interleukin 7 and interleukin 7 receptor. They already started now the recombination of the immunoglobulin genes at the heavy chain. Now, the heavy chain, I told you, starts with the combining of D H and J H.

Now that cell has choices. If the D H and J H joining takes place, then the cell goes to would be called productive allele 1. Now, this after D H and J H join, then they recruit any one of the V and you have V D J recombination; that means the heavy chain which is already recombined and can be transcribed.

In case this recombination cannot take place, then the cell has another chance on allele 2 for V D J recombination. If this also does not take place, you do not have V D J recombination; then the non-productive allele, even in the allele 2, then such cells undergo epitosis. If in the first instance, like I said, if this productive allele, then these cells go onto the light chain assembly and there also the sequences that of the two chains now every every gene would have two alleles of the two chains; the kappa light chain gene assembly takes place first; first allele 1, then allele 2, and then if the kappa light chain assembly also does not take place, then the cell choses to allow the recombination happening in the lambda light chain allele 1, and then if not then allele 2. So, what you can see very clearly over here that you have productive alleles and non-productive alleles.

Now, because the cell can choose between, well has choice that if the first allele is not productive, recombination starts with the second allele. Now, in case of the heavy chain, you have non-productive allele 1 and 2, such a cell would die. So, there is no question of the light chain assembly taking place; however, should this either allele 1 or allele 2 become productive and you have V D J combination; then it is possible that the cell can now try to recombine the kappa light chain gene allele 1, if not allele 2 and then has 1 more chance with the lambda light chain gene.

Now, because of this highly regulated reorganization, that is heavy chain first then the kappa then the lambda light chain, one can see that the number of immunoglobulins that are associated with the lambda light chain are very few.

In human, it is much better than mouse. They are 60 percent of the immunoglobulin gene which are associated with the kappa light chain and 40 with the lambda light chain; however, in mouse, for some reason, in fact the lambda light chain gene is very constructed in the mouse even with respect to the variability. The light chain gene, the lambda light chain gene constitutes only about 5 percent of the total immunoglobulins. So, with this, I will end here and my next class will be on the generation of diversity.

So far what I have talked about the mechanisms that go to combine the immunoglobulin genes such that the constant genes come in close proximity to the already combined hyper variable domain which constitute V D J in case of the heavy chain and V and J in case of the light chain.

Now, once these are combined, the genes are ready to transcribe and gets expressed on the cell surface later on in the ontogeny of development of the B cell. When such B cells encounter an antigen, they are able to secrete the very same immunoglobulin genes as antibodies.

So far, I have I like said that we only talked about the organization of the immunoglobulin genes, but now what is most important is how do this organization alone help in generation of this tremendous diversity? That we can see now. It was quite interesting to find that we have the set of genes corresponding to the V D and J gene segments; the permutation combination that can take place in the heavy chain and the light chain and coming together of the heavy and light chain genes still could account for around a 10 million different antigens, but we do know that the diversity that they immune response can recognize is to the order of a 100 million. How does this happen?

Now, apart from just the sequence that are present in this V D J gene segments, quite interesting that there is presence of error prone polymerase in the memory cells which gives raise to this hyper mutation. Now, why did I even think about talking about hyper mutation is that of the two theories that was proposed in the beginning, you know, in the before 1960s one of the theory was germ line and the other one was hypermutation - the germ line theory.

Like I said, both the theories were actually rejected, but the germ the second theory that is hypermutation said that immunoglobulins are identical from one B cell to another, but it is after the encounter with an antigen. There is hypermutation of the immunoglobulin genes, such that now there is a conformational change in the immunoglobulin hypervariable region, inducing now a close fit with the antigen.

Now, of course, the hypermutation that I am going to talk about in my next class is not this hypermutation that people thought about at the beginning, but there is an error prone DNA polymerase which is capable of inducing small mutations in the variable region of

the heavy chain, and to some extent, also the light chain which changes the affinity of the antigen binding to the receptor.

So, the mutations are small. There are times with the mutations can bring about abrogation of binding with the antigen, but the immune system only regards those that bring about an increase in the affinity, and therefore, we see a difference in the affinity in the second human response to the same antigen as compared to the primary immune response.

Thank you.