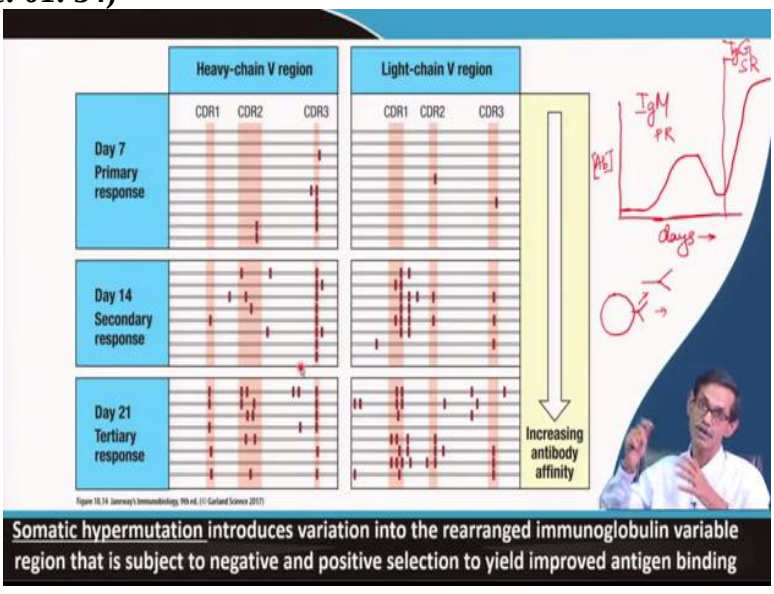
Immunology
Prof. Sudip Kumar Ghosh
Department of Biotechnology
Indian Institute of Technology - Kharagpur

Lecture-16
Generation of Diversity (GOD) of Lymphocyte Antigen Receptors (Contd.,)

So, we are still continuing the same generation of diversity in last lecture. In last lecture, we discussed actually there are 3 points we already discussed like how the diversity is going to happen first is variable number of VDJ region, then combination of different light chain and heavy chain and third is the one of the very interesting and very important reason for diversity is that in an ((()))(00:45) addition it is also called junctional diversity of antibody or T cell receptor.

Now, there is a fourth and one of the most important thing important 2 reason one is it is only specific for B lymphocytes, it is not happening in T lymphocyte. And these particular reason is not happening during the development this mechanism or this phenomena is happening only after the exposure to antigen and this is happening only in case of B cell not in case of T cell and this is called somatic hypermutation.

(Refer Slide Time: 01: 34)



So, let me go a little slowly at the beginning, because this is also very simple and straightforward thing, what is happening you see, that CDR region or HV region, the hyper variable region are the most important or only important you can say which interact with antigen that you remember

for different where I show you by showing my hand and some figures are already. So CDR1 CDR2 and CDR3 are more important. And this particular interaction between antigen and antibody is non-covalent mostly hydrophobic, hydro electrostatic Vander Waals.

And it all depends what kind of amino acids are there on the interacting region. So, if this is antibody binding region, if there are some positively charged amino acids here and if there are some negatively charged amino acid here it is very tightly it will boil but if certain happen so, that they are suppose there are 4 negative charge here and there are 3 positive charges here equally placed.

So, the interaction between this and 4 here 4 here will be much better just one amino acid change, same way can increase the affinity or the interaction power one amino acid change in that interacting region, interacting region means whatever the antibody structure ultimate interaction or the final interaction is happening between the CDR1 CDR2 and CDR3 and antigen epitope part. So, one amino acid change in either CDR1 CDR2 or CDR3 can change the affinity.

And specificity both like there is one glutamic acid converted to aspartic acid not much will change because both are acidic. So, similarly hydrophobic to hydrophobic similar substitution will not make much change, but it will complete so suppose one acidic amino acid it is replaced by a basic amine acid definitely it will be a big change. So, one amino acid change in the binding or interacting side of the antibody is very important.

So, that change can do 3 things one that changed may not have increased or decreased or may not change the affinity or avidity or their interaction power between specificity rather specificity and affinity. So, it remains same so, change does not make any difference, change can reduce the affinity or specificity or that change can increase the affinity or specificity. So, no change, increase decrease and 4 thing.

It can completely change the specificity or affinity that means, one antibody used to bind antigen A, but change happened it is not binding at all with antigen A it can find something else much better to bind. So, these anything can happen. So, what happened if you remember in our in

when we are discussing the primary and secondary immune response, I am coming back to that. So, in primary and secondary response what happened there was initially.

So, this is the days and this is antibody concentration. So, initially first 6, 7 days there is no change and then it is gradually increase stay there go down and in signal response It is like that, and like this. I said 4, I mean, what is the difference this is the primary response and this part is secondary immune response. So, the primary and secondary immune response, there are a few differences which I mentioned or you already know by now.

What are those one less time in the secondary response to react, more amount of antibodies produced in secondary response in comparison to the primary. And third one is more specific in secondary response. So, specificity increase, and the other one I think I mentioned, but if not then I am mentioning again and explain later again, in primary response, most or all the antibody is IgM.

Whether in secondary response, it is IgG. That happened that is called isotype switching, but this is the constant domain region because they affected function is changing, there is constant domain is responsible for a affected function, but how once if you remember the clonal selection hypothesis, clonal selection hypothesis is telling that 1 B cell having a receptor will not change in its lifetime or the same B cell will produce the same.

I mean the whatever the receptor it is expressing at the surface when it is activated, it will produce the same sequence for antibody. So, these 2 are same. So, now, if some B cell get activated in the primary response, how it is making a different or more specific antibody because B cell is not changing or new B cell is not coming in the same B cell how come they are producing. So, I mean, when it was analyzed, like how the antibody become more specific what was found there just special event happening in germinal center.

Basically in the lymph node or in the secondary lymphoid organ, when it is interacting and more making more not in the first time mostly in the second time. So, primary response is very low, but gradually in the secondary response it is the rate is very high, the event is somatic

hypermutation normally what happened, normally during cell division, we do not have that many mutations are there every cell will die, I mean, every protein will change their sequence.

And India will lose their activity, we cannot survive. So, what is happening? In this particular case, there is a special event called somatic hypermutation happen many theories are there, I mean, some are experimentally valid like there is a protein called activation induced cytidine deaminase AID that makes this kind of change happening, what is happening and another thing also there because during this period I mean during the cell division, what happened?

The mutation rate normally when cells divide, our cell divide DNA replication happened, DNA replication is not error free 100 percent error free. It is not possibly this an enzyme it is doing a reaction anytime it can do a mistake, and the rate of mistake is 1 in 10 to 10. So rate of mutation that is normal cell division found is 1 in 10 to 10 but in case a B cell replication or proliferation in during their infection or in lymph node or in the secondary lymphoid organ.

The rate of mutation or the rate of error in DNA replication in 1 in 10 to 3 is much high. And what is happening as a result? There are a lot of mutation happen in all over the sequence but particularly here and you if you know it is fine otherwise you just go and check there is a term in genetics called mutational hotspot, there are some part of the DNA is always there, which is very prone to mutation.

So, what is happening and another theory, I mean, I do not know how you like it or not, another theory is there is that during this multiplication or replication, this B lymphocyte or T lymphocyte and B lymphocyte particularly T lymphocyte also there T lymphocyte qualified very fast and if you want to do anything in hurry, you will do some mistake, and that is also reason the DNA polymerase if you will push them to synthesize the DNA faster.

There is a chance or mistake is very high or more mostly ID induced. So, what is happening? If you see this figure what you see here, this is heavy chain variable region and this is light chain variable region primary day 7 primary response say like, the B cells are proliferating. They are

converting to plasma cell producing antibody during that time also mutation occur. What is the rate of mutation you see, these are the mutation this red spot or the mutation.

You see, very few mutation are here and there in light chain there is almost no mutation and heavy chain there are few mutation but mostly they are present in CDR3. In day 14, secondary response, you see the number of mutation is increased and most of the mutations are in this shadow region, or CDR region CDR1 CDR2 and CDR3. See, most of the mutations are within the CDR region.

And if you go there to 21 that are responsible for further infection, the mutation is much higher. So these mutation can be either good or bad, because some mutation always possible that some mutation can introduce the stop code in between the gene. So that B cells will not produce a receptor anymore, it is a random. So, what will happen, that B cell will die. If this B cell is produced, get a mutation, which reduced the efficiency or if the specificity that will be no use.

There we need better antibody or our immune system need better antibody better quality antibody which can deep bind better quality. So, as a result, I mean if you see with time, more and more infection happened, you see the affinity of the antibody towards the antigen is increasing and that is because the CDR1 is getting mutated. Normally this hypermutation is not very common or not at all common it is happening only in B lymphocyte.

Fortunately, it is not happening in T lymphocyte. Why I will tell? I cannot tell you exactly why but how it is advantages that somatic hypermutation is not there, that I can tell you like, it is absent in T cell receptor diversity. So, this is advantages in this way. Here, what is happening B cell is continuously producing? And these mutation accumulation can give either nonproductive like the B cell will die or less affinity or equal affinity or higher affinity.

More and more affinity increase the lower affinity or low grade interaction containing B cell receptor with will just not grow anymore or not multiply anymore. So, it is a kind of selection going to happen as positive selection kind of thing that better will only attach to the antibody and will B cell receptor and B cell receptor proliferation is going to happen. So, that is how affinity

maturation is going to happen. So, in this affinity maturation is happening in case of secondary or tertiary immune response.

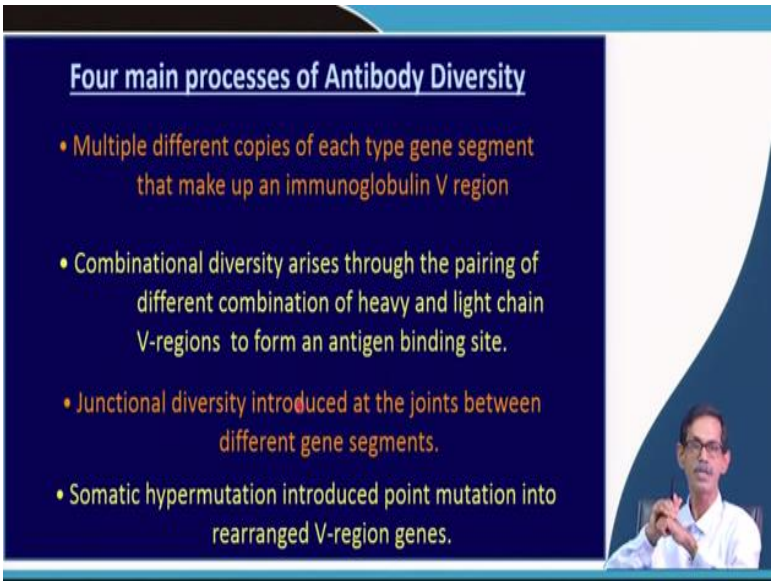
And that is the reason that in many cases, vaccine if you see when we are immunization, that booster dose is given. So, first you give say for tetanus this is very common, you know, most of us got caught while playing or while cycling and if you go to hospital immediately. They will ask when did you take the vaccine or when did you take the anti (())(15:04) injection, and they depending on that, they will give say.

If you say that it is more than a year ago, they will give number 1 and ask you to come after 6 months or 3 months or 9 months for the second for the third, what that second third immunization or the booster is doing, polio. You see, so, many times polio vaccination is going on the same kid. Because every time if you give a exposure to the immune system of that antigen, this affinity maturation will happen, better quality B cell receptor will be in the memory.

So, next time it can handle much better way from very beginning. So, this affinity maturation is very helpful in immune system as well as which is coming but first infection, it is not happening that much. You can see the rate of mutation or the total quality mutation. And again another important thing it is happening mostly in the CDR region. It is not that only CDR region mostly in the CDR region, rest that framework region, the amount of mutation is not much.

Even if it is, there is not making any difference as long as it is maintaining the structure, we do not have any problem because we need an antibody, which will interact strongly with antigen and save us or protect us.

(Refer Slide Time: 16:31)

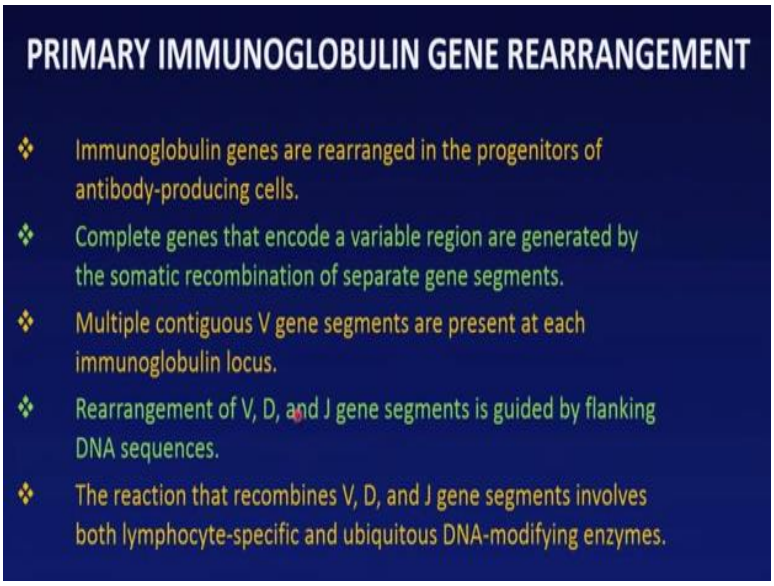


So, in summary, there are 4 main process of antibody diversity, whatever we have discussed, so far, there are 4 main process of antibody diversity, first, multiple different copies of each type of gene segment gene segment means now you know VDJ this kind of segment that makes up the immunoglobulin V region. So when we say the antibody variable region that is combination of PJ in case of light chain and VDJ in case of light chain.

And so multiple copies multiple different copies, that is very good, multiple different copies of different segment present for immunoglobulin variable region, true for both light and heavy chain combinational diversity arises to the pairing of different combination of heavy and light chain. We are all discuss this I am just summarizing again, light chain V region to form an antigen binding site junctional diversity introduced at the joint between different gene segment that also we discussed.

Somatic hypermutation induced point mutation into rearranged variable region. So, these are the 4 main reasons so now what are the points of antibody diversity careful, it is very clearly 4 main process of antibody diversity. So far we are talking about lymphocyte receptor diversity. Here we are specifically mentioning antibody diversity, why? I hope you can understand because first 3 true for both B cell and T cell receptor diversity, but the fourth one the somatic hypermutation is only happening in case of B cell not in case of T cell.

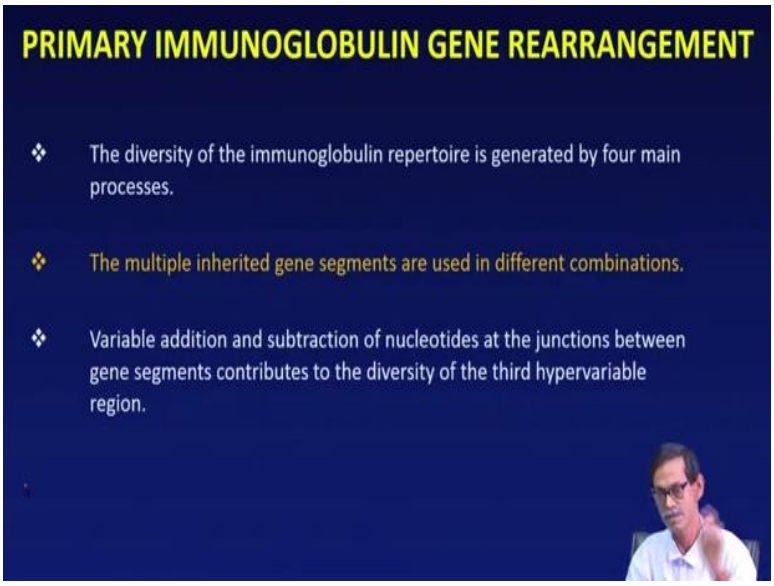
(Refer Slide Time: 18:31)



So, summarizing the whole thing, whatever I discussed in diversity and just reading once, or you can also read, like immunoglobulin genes are rearranged in the progenitors of antibody producing cells. That means not while they are matching before maturation, complete genes that encode a variable region are generated by the somatic recombination of separate gene segments. That you know, multiple contiguous.

V gene segments are presented each immunoglobulin locus like V1 V2 V3, that is what exactly we mean rearrangement of V,D and J segment is guided by the flanking DNA sequence that is a heptamer and nonamer that also you know, the reaction that recommends V,D and J segment involves both lymphocytes specific that means rack 1 rack 2 protein and ubiquitous DNA modifying and like cool protein, then ligase then your DNA repair or polymerase and which will tell the complete the sequence.

(Refer Slide Time: 19:48)



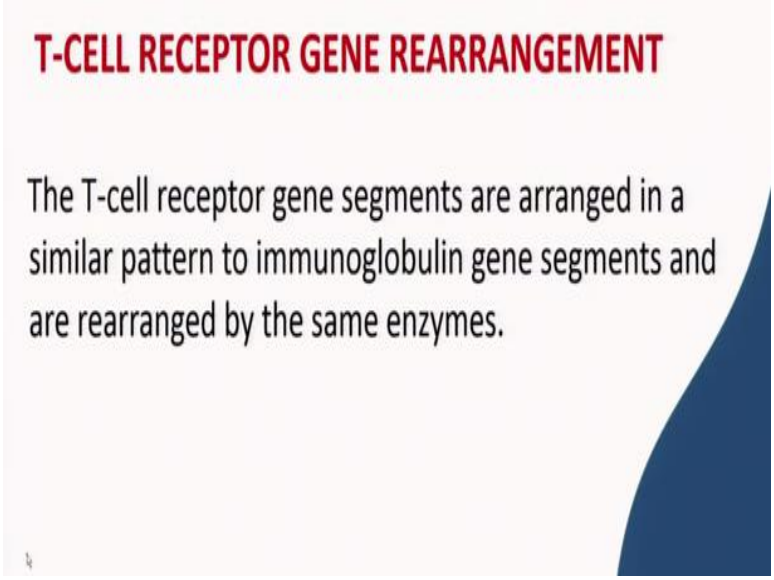
Then, the diversity of the immunoglobulin repertoire is generated by 4 main process that we just have discussed. So, what are the 4 different process the multiple inherited gene segments are used in different combination that is whatever we discuss VDJ same thing, but that is inherited here the spatial point that we inherited those segments from our pairs variable addition and subtraction of nucleotide at the junction between these gene segment that is a junctional diversity.

That is contributed a lot and that is a one of the most interesting discovery in antibody diversity development and to the third hyper variable region and third hyper variable region why because PJ recombination in light chain, if you go back my previous lecture, the light chain there was one nice light also. The light chain is PJ recombination that junction region come into CDR3. So CDR3 anyway, it is very important because CDR3 actually contribute to the center of the antigen binding site. So, if you see that this is the antigen binding site.

So, this is CDR1 this is CDR2 in such CDR1 and CDR2 and the fold in such a way then the protein CDR3 comes in the middle. So, slight change in the CDR3 in the center actually makes the interaction very strong or can make the interaction very weak. So, one chain, so, CDR3 is very important region, both for T cell receptor as well as B cell receptor, because CDR 1 and 2 basically 2 surroundings and the center part of the antigen interacting site is CDR3. And in case

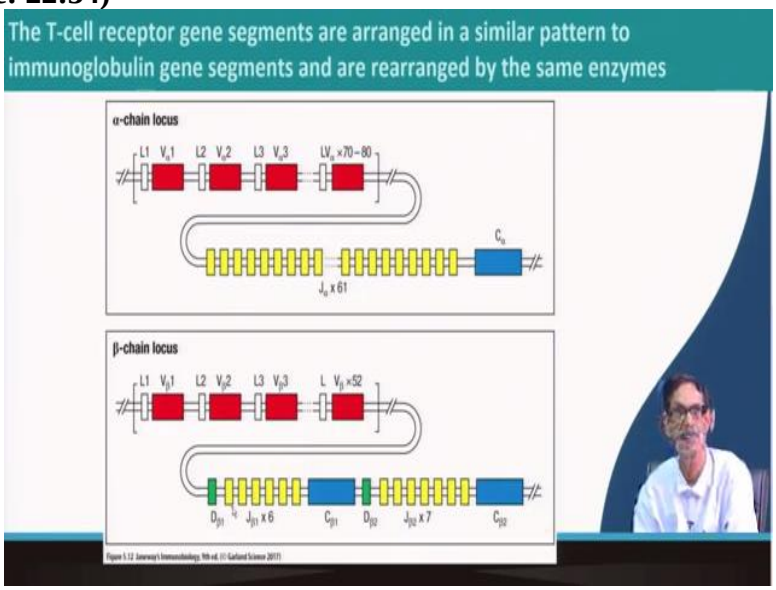
of light chain, which is very much contributed by this junctional diversity, because this is the BGA recombination site by which is already figuring.

(Refer Slide Time: 21:58)



Now, after discussing all this part from B cell receptor, let us discuss about the T cell receptor gene alignment. They are very similar with the B cell receptor. According to the mechanism I am talking about the T cell receptor gene segments are arranged in a similar pattern to immunoglobulin gene segments and rearranged by the same Indians. So, life is very simple and straightforward. Once you understand that part, you do not have to study much you just see few figures and understand what is happening that I will do I will also do the same thing to you too.

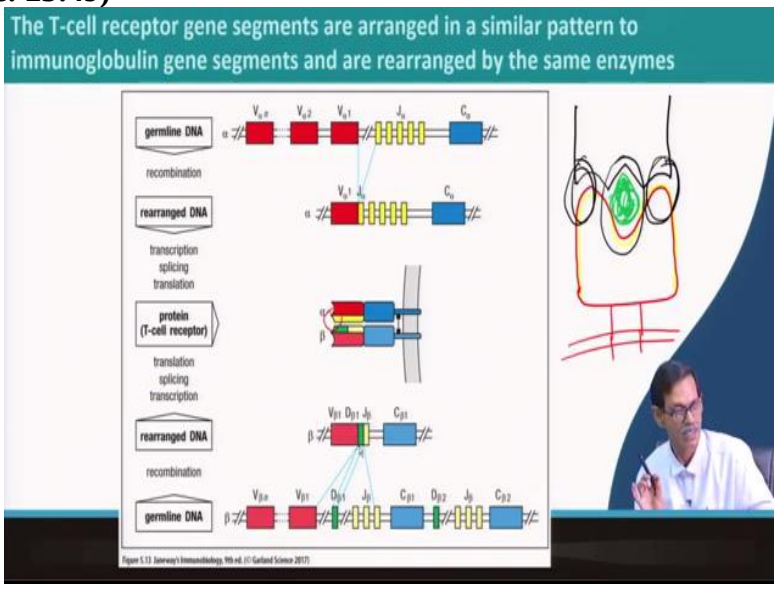
(Refer Slide Time: 22:34)



So, here is the alpha gene locus of the T cell receptor you see all V u1, V u2 V u3 and the numbers are different that we already discussed. And in alpha chain they have only V and J they are more number of J same heptamer and nonamer same rack 1 rack 2 same TDT everything is same. So, there is no point of discussing the same thing again for T cell receptor, you just whatever knowledge you have, you just put it here in beta chain of the T cell receptor.

I hope you remember the T cell receptor is composed of 1 alpha and 1 beta chain. It just looks like very much similar to fab. I will just remind you, if you just forget. So, you are in beta chain, which is very similar to heavy chain here again, which has a variable region. Then we have diversity region D, and we have gene segment, here also in beta chain DJT is first arranged and then the D J recombine DJ are recombined with the V segment, same way.

(Refer Slide Time: 23:49)



Here is a schematic diagram. If you see that V 1, V 2 V 3, like that, then J, then C, and then the D combine to BJ this is for the alpha chain, which contribute here, you see this Raiden yolo code. So, this is a very cross but still you can understand that even the JD region is contributing to this antigen interacting site. And very important I will show you already when you do not know much about MHC.

But I will tell something I hope you will understand and remember when I will discuss MHC in beta chain, this B D and J same with first DJ recombine and DJ recombine with V. And here you see these J segment does not have much role in the diversity war antigen binding or interaction

here only D and V is very important. So red and green news see this, but the overall structure is very important.

So in a T cell receptor central part very important why? If you remember correctly, T cell receptor cannot recognize antigen alone it must be presented by MHC. So, if you remember if I draw they MHC like we see we have seen like this yellow, it is not very clear let me change the color the MHC is red MHC is red. So, this is MHC is supposed to this MHC 2 and here you have a antigen like this, which is similar this antigen is like this, this is a chapter so now if T cell receptor interact, if T cell how it will interact, it will interact like this.

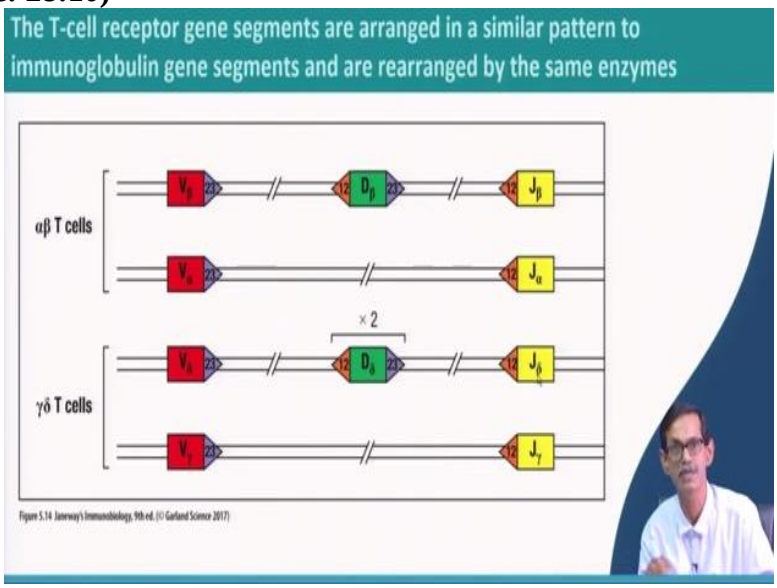
So, if you see this structure, so, these in part are the receptor outer part of the receptor, T cell receptor is interacting with the MHC only and central part mostly important for interacting with the antigen which is contributed by CDR3. So, CDR3 is very important, because this central region is because antigen binding or antigen recognition along with the MHC or even in case a B cell the interaction is mainly happening.

And this center part is combined by I mean the combination of both light chain and heavy chain in case of antibody and in case of T cell receptor both alpha and beta chain, so, the central part is very important. So, CDR3 contributing the central domain or central region of the antigen interaction site here hope you understand that why the center is more important with respect to antigen, because we have a certain specific number of MHC.

So, discuss what is that, but for the timing you just remember, believe me that we do not have huge number of variety of MHC, so, you have a limited number of MHC. So, recognition on my cell own MHC cannot be that many, so, there is not much variation in this region. So, only the antigen which is variety is unlimited, almost anything can be antigen anything can create problem in immune system.

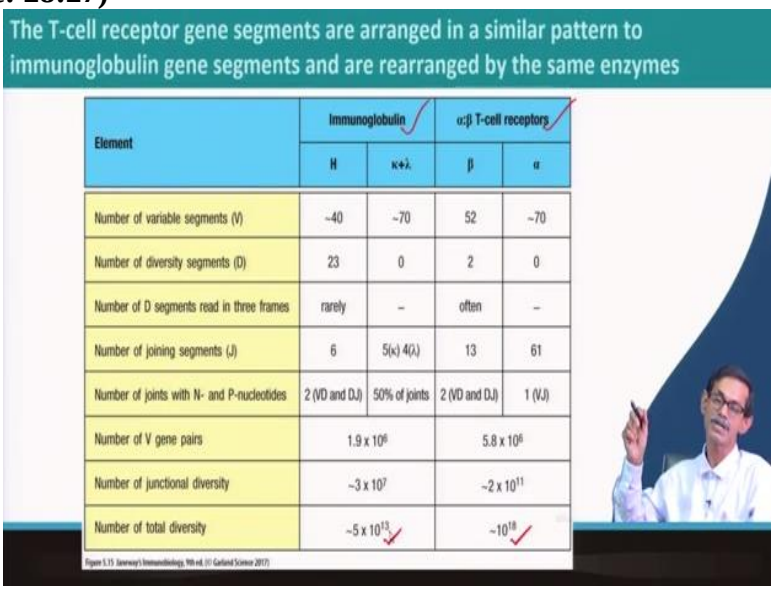
So, that should be recognized by T cell which is definitely should be foreign. So, if this foreign thing, so, the central part that is very important which is contributed by the CDR3 both alpha and beta chain and this region is that way is very important, but every other mechanism is very same.

(Refer Slide Time: 28:10)



Alpha beta and gamma delta they are very much similar, they all have 12 23 rule they follow both gamma delta T cell receptor and alpha beta T cell receptor, there is nothing, but their arrangement may be different, but the mechanism or the principle is same.

(Refer Slide Time: 28:27)



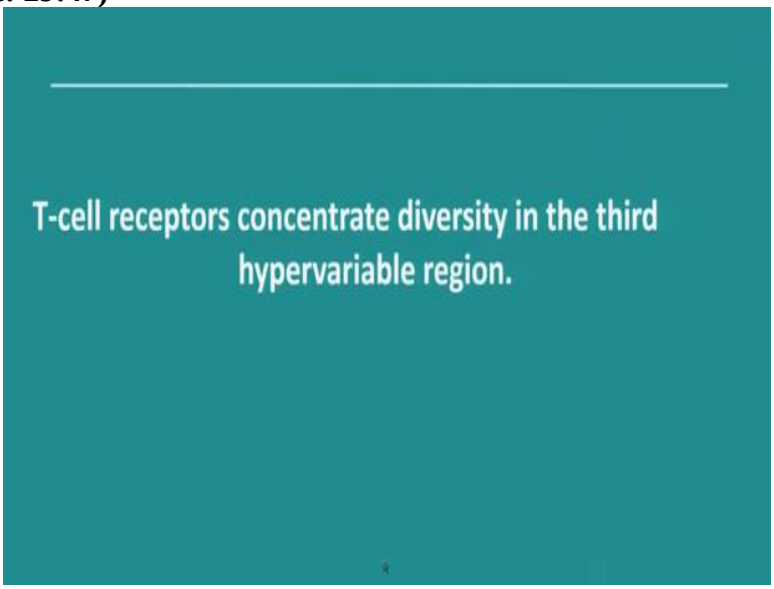
Element	Immunoglobulin		αβ T-cell receptors	
	H	κ+λ	β	α
Number of variable segments (V)	~40	~70	52	~70
Number of diversity segments (D)	23	0	2	0
Number of D segments read in three frames	rarely	–	often	–
Number of joining segments (J)	6	5(κ) 4(λ)	13	61
Number of joints with N- and P-nucleotides	2 (VD and DJ)	50% of joints	2 (VD and DJ)	1 (VJ)
Number of V gene pairs	1.9×10^6		5.8×10^6	
Number of junctional diversity	$\sim 3 \times 10^7$		$\sim 2 \times 10^{11}$	
Number of total diversity	$\sim 5 \times 10^{13}$		$\sim 10^{18}$	

So, if you consider this, this is a summary, I am not going to go through read and waste time in this case. So, this is the number of V segment average V segment of this case of immunoglobulin and this some are in case of T cell receptor all are so, this is immunoglobulin, this is T cell receptor only for beta and alpha, alpha beta T cell receptor, this is the number of gene, this is the variety, like number of region pairs.

How many possible this is all possible calculation that are staying it is not that easy that we did so. Finally, total number of diversity after junctional diversity, it is 5 times 10 to a 13 in case of immunoglobulin and in case of alpha beta T cell because this number is much more and this is number of junctional diversity is also very high. So, they are 10 to 18 here in case of 10 to 13 here the somatic hypermutation is not calculated.

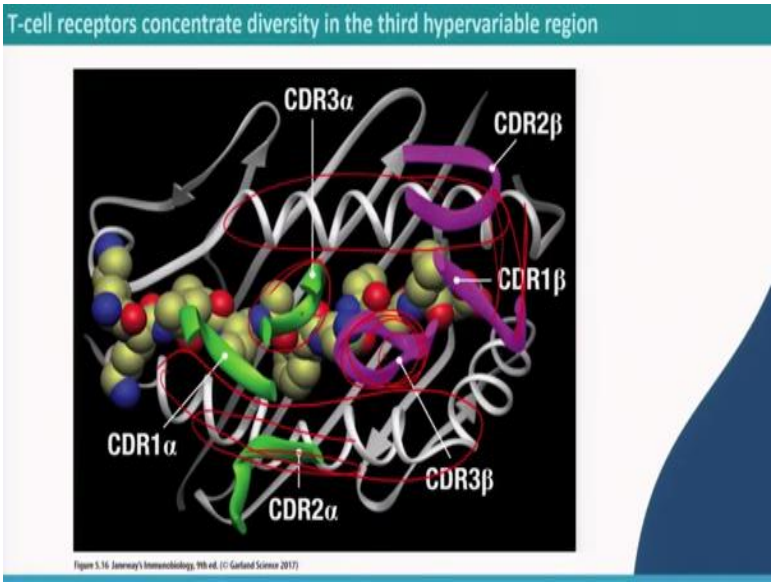
So, this is a number of possible receptor one person can have among which many are very similar to or against our own protein or a cell protein, so, that we will be deleted. So, those who are surviving finally and circulating in our peripheral blood, that is enough.

(Refer Slide Time: 29:47)



So, T cell receptor diversity in the third variable region that just I discussed. In the in my hand sketch is the same thing.

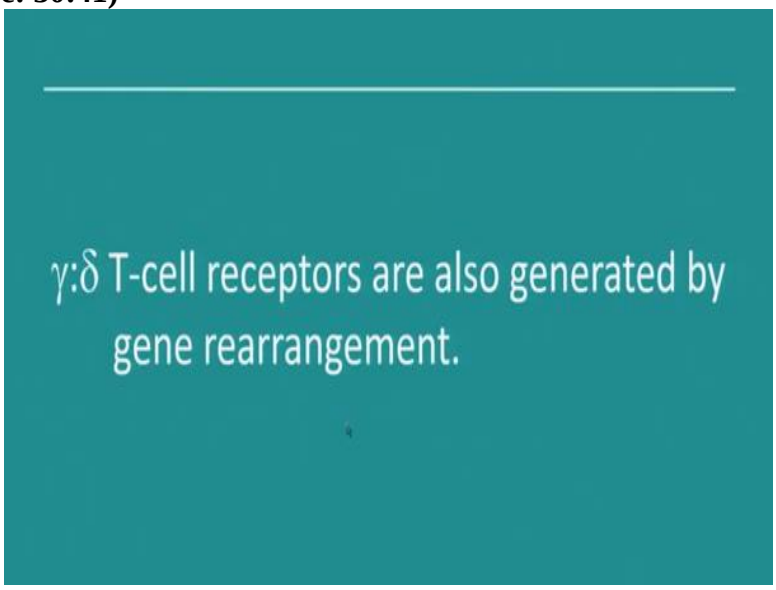
(Refer Slide Time: 29:56)



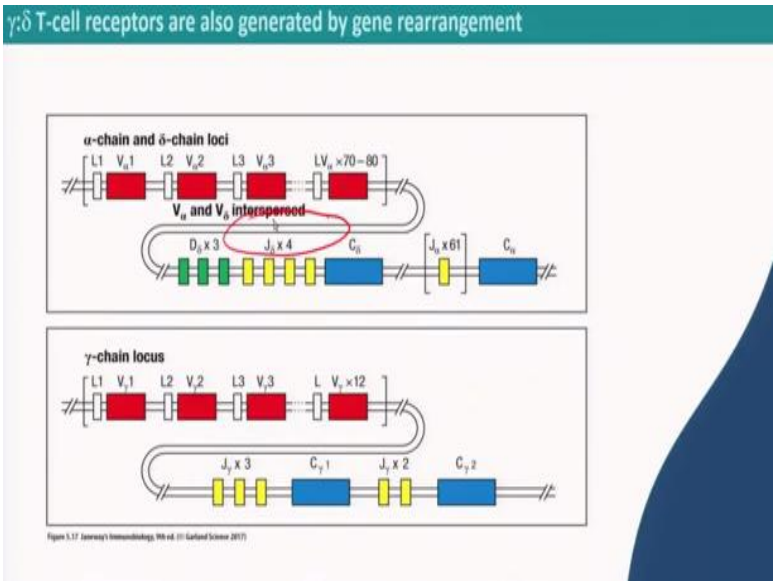
Here you see the CRD2 and CDR3 this is the MHC region. So CDR2 and CDR1 alpha 2 alpha 2 beta 1 beta they are mostly interacting with the MHC region, but the central region, this is the antigen, this ball and stick model this special model, this is the antigen who at CDR3 is interacting this pilot is the CDR3 and this is the CDR3.

So, CDR3 alpha CDR3 beta both are interacting with the antigen mostly and rest 1 and 2 is mostly interacting with the MHC part. So, that is how this is in crystal structure in much more detail, which I discussed in the very android.

(Refer Slide Time: 30:41)

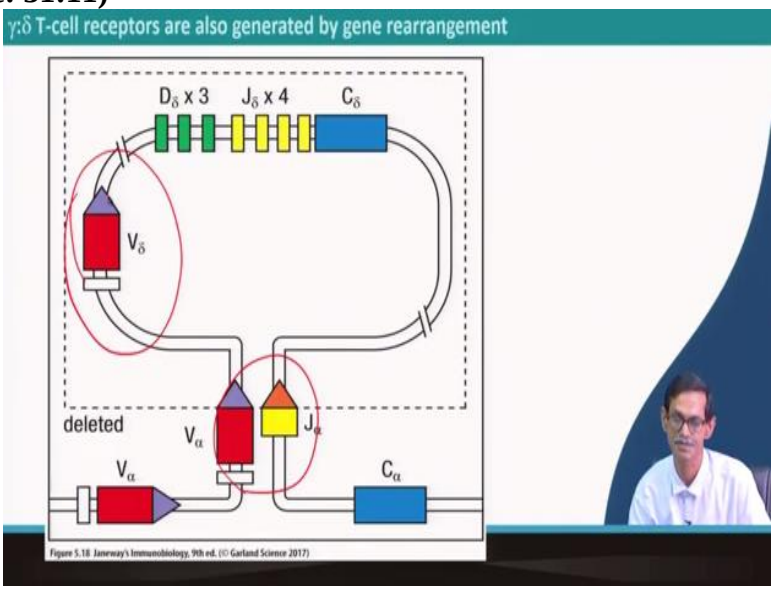


(Refer Slide Time: 30:47)



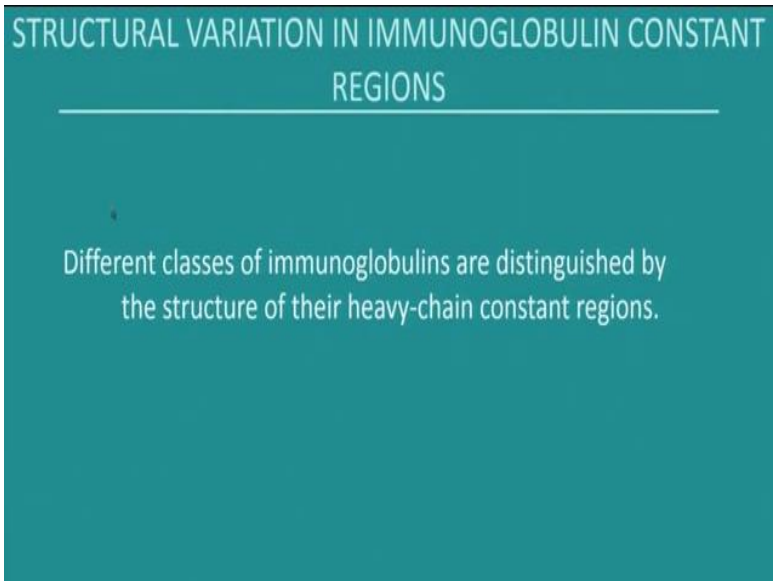
Gamma delta T cell receptor also generated by same element. So, they are actually what happened this is very nice. So, what happened is V delta segment is present here actually. So, if V alpha and DJ recombine these are automatically eliminated. So, if there is 1 alpha there will be no chance of V delta. Am I clear?

(Refer Slide Time: 31:11)

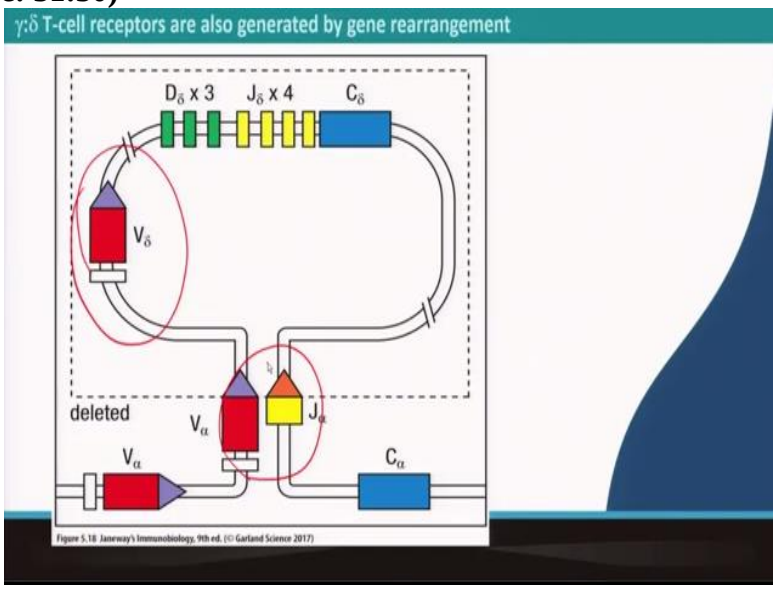


If not then see, this is the V alpha region and this is the DJ region. So, if 1 J and V alpha segments, so, V alpha and J alpha combine here what happened the whole V delta segment is going out from the chromosomes as forming the loop. So, there is no question. So, this is just to know tell you that V delta is intercourse between them V alpha and G alpha region.

(Refer Slide Time: 31:38)



(Refer Slide Time: 31:50)



Way to picture it other (FL). So, this is this look permission you already know because we are seen in case of immunoglobulin like how this B 1 and G 1 recombine, how the loop element, so, this loop will be eliminated forever. So, if there are a B alpha join, there will be no question of V delta joining. So, this is one of the nice explanation. So, why T cell receptor is either alpha beta or gamma delta type.

So, if alpha beta T cell receptor there are many other reason, theory or hypothesis for that how it is decided, but this is once V alpha is join V delta is not possible. So, this is the T cell receptor

diversity so far. Hope you understand. And after that you read the book. I hope you will understand if there is any question differently. You can clear it later. Bye for today. Thank you.