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

Lecture - 17
Structural Variation in Immunoglobulin Constant Regions and Isotype Switching

In last lecture, we are discussing about the diversity of B lymphocyte and T lymphocyte. And what is the maximum number we can reach in different diversity of the 2 different lymphocytes today or in this lecture actually we are going to talk about structural variation in the immunoglobulin constant region. So, and later on we are going to talk about how this isotype switching because different constant region of immunoglobulin are known as isotype.

So, any particular antigen if it is recognized by the hyper variable region or the variable region of antibody its constant region may be a IgG type IgM type IgA type, but the specificity towards the antigen is specific. So, against any particular antigen, what can happen one antibody binding site can hold it? But it is constant region, which is responsible for the effector function can be IgM or IgG IgA IgE. So, different type of constant region is possible. So, today we are going to talk how this structural variation in the constant region is generated.

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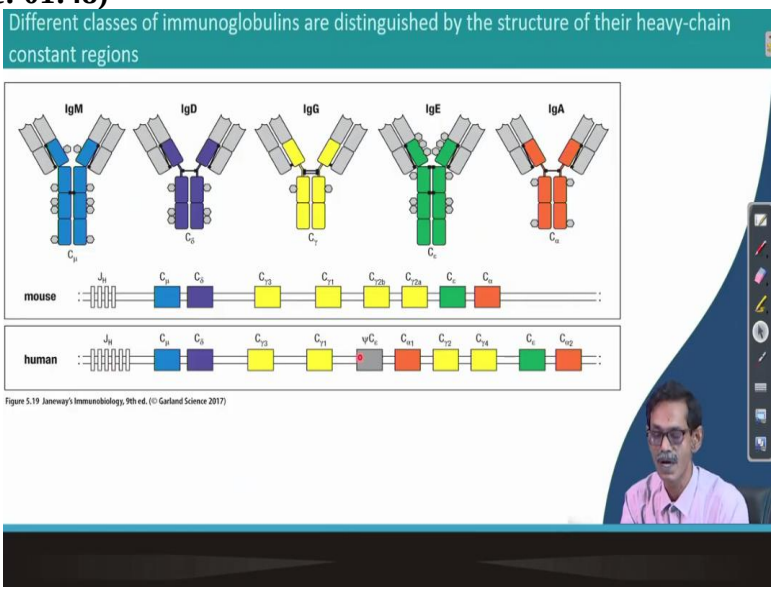

So, these particular slide you already have seen the upper part when you are discussing different type of antibody or antibody structure. We showed you that IgM IgG IgD IgE IgA different, so,

IgM IgG, IgD IgE IgA varieties of these 5 different isotype of antibody we have discussed is structural difference little bit also we will discuss, but today what we are going to tell about that how this constant region is coming to a particular type of variable region we have to remember that constant region is often antibodies only contributed by the heavy chain.

So, if we see how this heavy chain is rearranged, then we can tell what can be the isotype of the antibody. So, if you see below just below this antigen antibody structure, you see there is a series of same color code boxes, but these boxes this is basically the constant region gene segment. Now this is the upper panel is mouse, and lower panel is human I mean this is the human one. So, if you see the mouse in and human both cases JH is there.

So, what is there before that are the phi prime end of this gene, this is a DNA strand. So, phi prime of this JH we had D and before that we had B. So, after the J region, we have different constant region. If you see here, the constant region is designated as C and suffix by 1 Greek letter, what are those Greek letter if you see this mu delta gamma 3 gamma 1 gamma 2b gamma 2a epsilon and alpha you can easily correlate.

I mean if you see the name of the antibody gene like isotype IgM is coded by C mu IgD coded by C delta IgG is coded by gamma and there are different subtypes if you see gamma 1, gamma 2a, gamma 2b and gamma 3. So, there are 4 different subtypes of IgG and then we have IgE and then IgA is coded by C alpha. So, what is happening after this VDJ recombination VDJ recombination is over and after that it is giving the only variable region.

So, after variable region constant region is coming which constant region is going to come it depends I mean depending on that what isotype will be the final product is determined so, VDJ recombination in heavy chain is common. After that if mu comes then it will be IgM if delta then IgD if gamma then IgG if epsilon, then IgE if alpha then IgA. If you see the human genome and the arrangement of this constant domain, they are almost similar. But there is 2 alpha subtypes alpha 1 and alpha 2.

There one you can see here that psi C epsilon. I do not know how many of you are aware of this psi, designated gene, psi actually psi before the genome. I mean before any gene segment of gene indicates it is a pseudo gene. There are many pseudo genes present in human genome. Pseudo gene actually, the gene. I mean, this is not related to here, but just for your information pseudo gene, many of you may know already, pseudo genes are the gene which does not express.

But prediction I mean, if you see their sequence, it is a complete gene. To possibility media to possibilities you can find like why one particular gene is presenting chromosome but is not expressing because gene expression first thing you need it should be transcribed. So, for transcription, you need a promoter. And if promoter is not there, it is not going to transcribe that he is also called pseudo gene because gene is there.

But it is not transcribing that is why no protein another possibilities that even the promoter is there, but there is maybe a stock codon inside so that we would not get the complete protein product it will be truncated, so, final product will be incomplete protein or no protein. So, that is also maybe the cause of pseudo gene. So, here, this psi C epsilon is pseudo others all our functionality clear.

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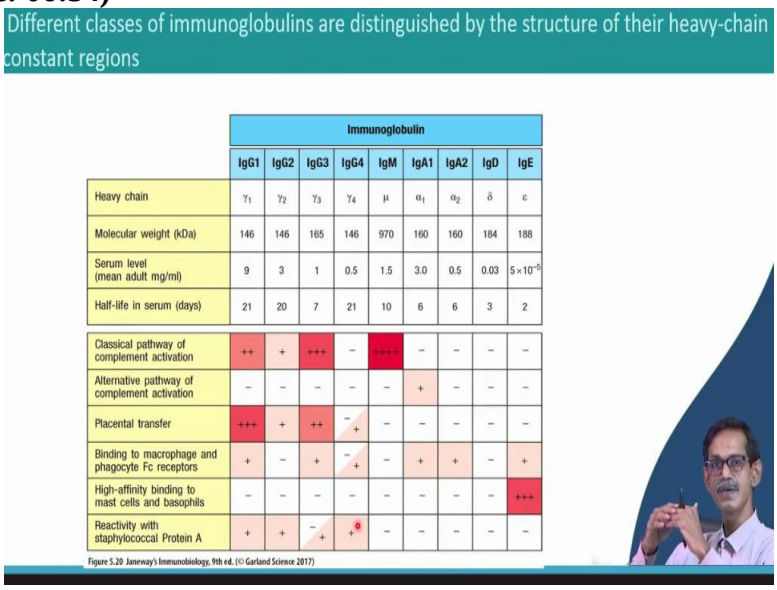

So, this table, actually is very, I mean, you do not have to remember do not be scared, you do not have to remember all these figures. But this table if you go slowly, I am not going to spend much time on it. This is actually saying that which is a heavy chain like IgG1 Ig2 Ig4 IgM IgA1 IgA2

IgD and IgE you can understand these as human 1 because IgA1 and IgA2 basically is a subtype of human not presented mouse and this is their molecule weight.

I am going to tell a molecular weight how you can estimate that or if you want to do verify that what I am telling or the written in the book is right or wrong, then you can do some very simple experiment to check that. So these are the molecules weight. So normally what we say is the average molecular weight of IgG is 150. Just to be it is not exactly so IgG has different, you see IgG1 is 146 2 is 146 IgG3 is 165. So averages are close to 150.

So IgG molecular rate is 150 kilo Dalton this is in kilo Dalton and if you see IgM which is huge, but, if you go back I mean if you see the structure IgM only had 1 extra constant domain. So, 1 extra concerned domain is contributing only 110 approximately 110 amino acids. So, these 110 amino acids will not contribute that much. So, why this is suddenly so high it is almost 5 times that we will see later and IgG is little more because it also has 1 extra constant domain.

So, their serum level if you see that maximum serum level or at any time and adult in cause check this isolate the blood and purify the serum and in serum if you measure how much IgG is there, then it is the maximum amount. And IgM is also good IgA is also not I mean in between, but there are others are not as much their half-life is also given. So, IgG has a maximum apply. So, from this what we can predict is we can predict that most of the adaptive immunity.

What we are seeing or what effect we are enjoying actually the immune system and how they are protecting major player in antibody mediated immunity is played by IgG that is why they are more in amount they are more in their half-life is also more that means, they stay in blood for longer time. So, IgG is one of the very important antibody isotype which plays a major role in adaptive immunity mediated by antibody and bottom part.

If you see bottom part has effector function some of the effector function we already discussed like opsonization neutralization, complement activation. So, you see these plus and minus, which indicates plus means, it is helping in the way for example, classical pathway complement activation. Best performer of this activation is IgM, you see 4 plus and the number of plus in we

are indicating how good it is or negative means it does not take part in the complement activation.

So, you can see only IgM and 3 of the IgG are mostly taken care of this complement activation IgM is the best player and a IgG2 is the least efficient player alternative pathway complement activation that I mean this is you do not have to remember right now, but you will learn when the complement will teach complement system of immunity, then you will understand what is this alternative pathway, only IgA1 is involved.

Same way if you go through this then you can understand which antibody is doing what and here is one thing which we did not discuss called placental transfer, which is very important because when babies are growing inside mother's womb, then mother's blood is actually protecting the baby inside the womb, because that time immune system is not ready, they are antibody production, their exposure. So, any kind of protection of a baby inside the mother womb is protected or taken care by the antibody present in mother's blood.

So, if antibody cannot cross the placental barrier, then it cannot help. So, not all antibodies are crossing the placenta. So, there are a few like IgG1 and you can see from here, so, and gradually you can see what is happening here at the bottom. One thing is written that reactivity with staphylococcal protein A reactivity with staphylococcal protein A. I will come to that. Not exactly in relation to the infection, but we will discuss or I will discuss what this protein A can do on how we use this protein A.

Protein A actually the surface protein of staphylococcus aureus. It has a very good property, which is not good for health, but the technologists are the scientists developed some technique to purify the antibody that we will discuss some time if time permits. So, this reactivity with the staphylococcal protein A is you see mostly the IgG are interacting with staphylococcal protein A. So, what it is actually doing these protein A is the surface protein of staphylococcus.

They have a specific property it can bind to the constant region of IgG. So, by that they can eliminate the IgG to do its proper function, this is a defense mechanism from bacterial side. So,

now, we can see from this table most of the antibody present in our serum is IgG and staphylococcal antigen can clear them by binding them. So IgG actually cannot do much to protect from staphylococcus. So that property that means protein A binds to IgG is also exploited to purify the antibody that we will see if time permits.

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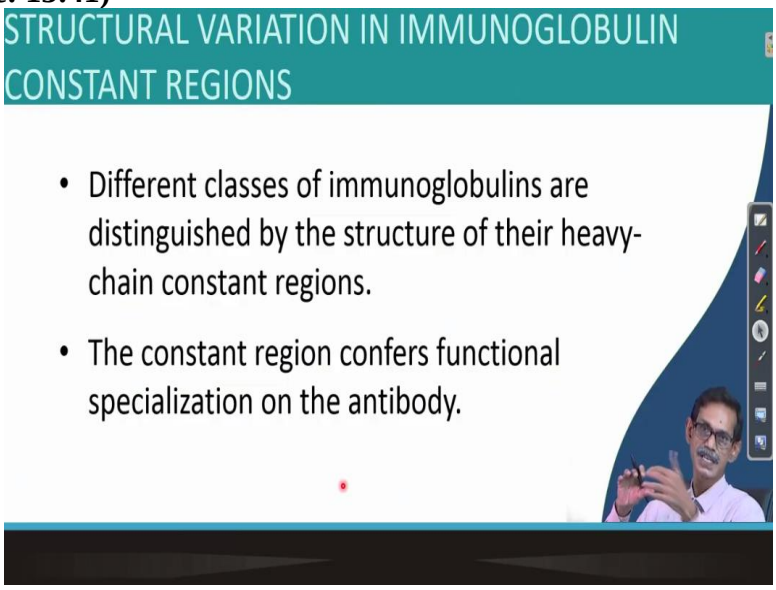

So now, different classes of immunoglobulins are distinguished by the structure of their heavy and constant region. And these constant region confers the functional specialization of the antibody that I am just this is a summary of the just the last slide. So different classes of immunoglobulins are distinguished by the structure of the heavy chain that we already have seen in the last few slides and the constant region conferred the functional specialization like which one is going to do the complement activation which one will do the opsonization. The last table the bottom part is actually saying by this statement.

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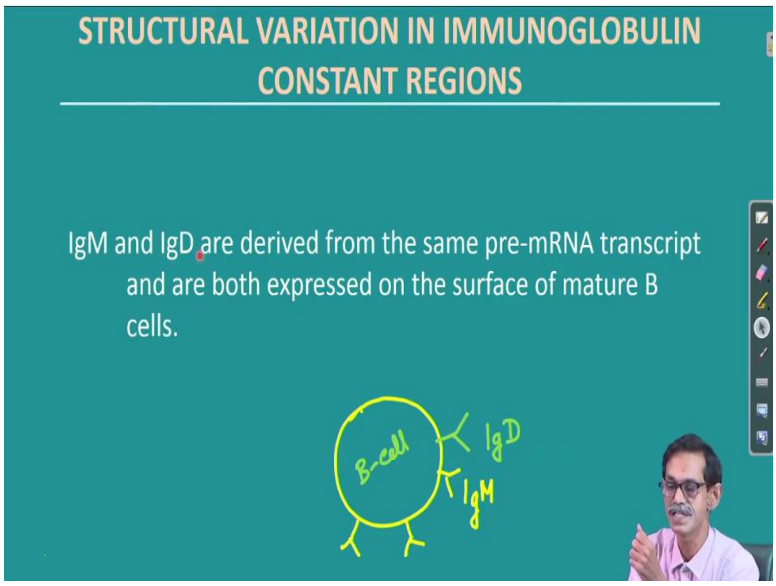

Now, IgM, IgD and IgM, IgD are derived from the same pre-mRNA transcript, and are both expressed on the surface of match your B cells. I am repeating again do it is written IgM and IgD are derived from the same pre-mRNA how it is possible so before going to go there, what I will do is what I am going to do is in B cell if this is the B cell before the antibody secreted what which form it is present in the surface it present as a receptor.

It is present as a receptor and upon activation the same receptor are synthesized in different form and secreted. So, what is there in this which form of the isotype is present as receptor actually there mostly IgM and they also have IgD normally IgD concentration in blood is very low and they do not secret. So, IgD actually take part in the initial signaling during the activation of B cells, this is B cell or B lymphocyte. So, during the activation of B cell that means, when B cell is there, receptor is there.

So, this is the receptor in B cell and Eve antigen come it binds and which gives a signal to the B cell that something you have to take care and along with that signal, this signal from t helper cell is also given this signal to the same B cell. So, when 2 signals comes together that we discussed in the during basic concept of immunology in the first few lecture we discussed. So, in B cell, actually 2 types of receptors are there 2 Isotypes of receptor same specificity, specificity or antigen specificity of these 2 IgM and IgD both are same.

But only their constant domain are different. And both of them attached to the B cell as a receptor, IgM and IgD both take interact with the antigen and gives the signal to B cell to proliferate and to convert transplant to plasma cell for production of antibodies along with the signal from t helper cells. So, how this IgM and IgD always come together? It is saying that IgM and IgD are IgM and IgD are derived from the same (())(17:37). Now, we will see what is happening how it is located.

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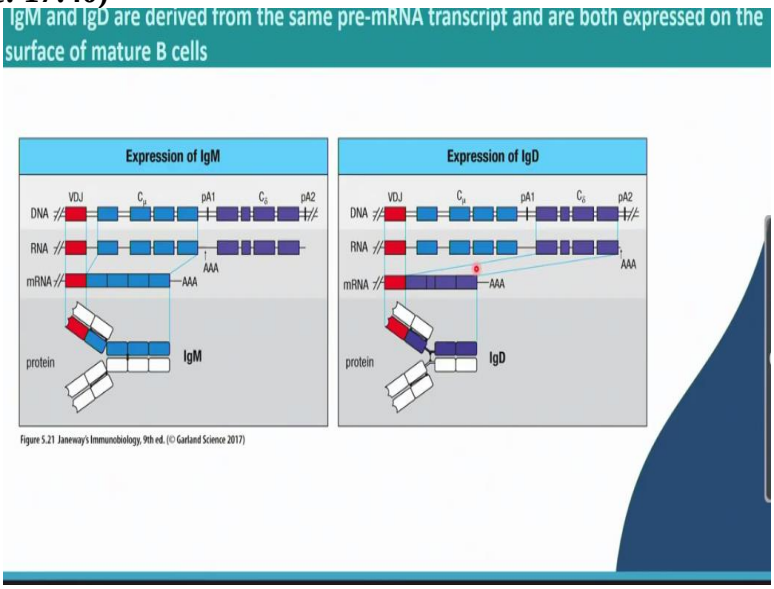

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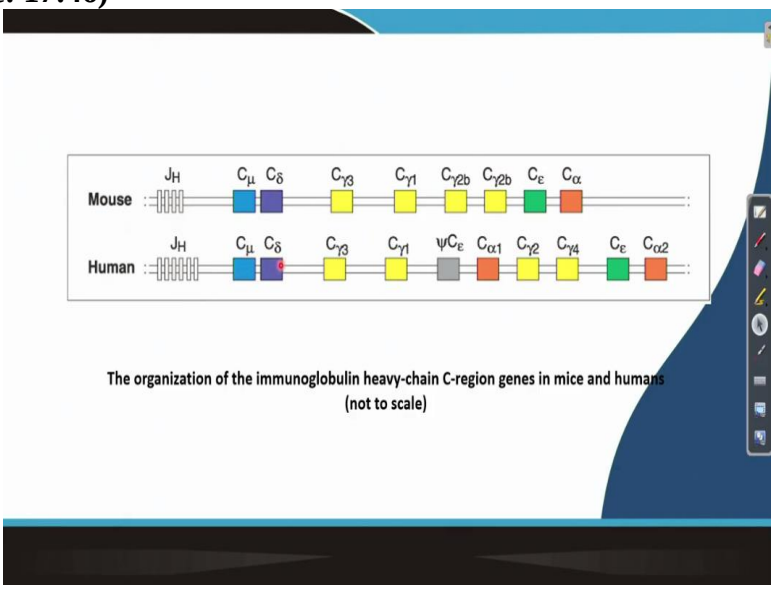

So, if you see, I mean this is the structure actually what is happening, do not be scared, it is very simple. So, if you see the structure or the organization in the genome, you see C mu and C delta

is side by side C mu and C delta both in case of mouse as well as in case of human. We I mean, I do not remember whether I told previously in class or not, but most of the immune system what we are studying what we understood.

So, far, much of them or most of them are studied in mouse, because mouse immune system and human immune system are very similar not exactly identical there are very there are many places where there are different, but most of the cases they are very similar even in the gene organization also, but we cannot do all this thing in human. So, mouse is a very good model to study human immune system.

You see here I am in this case C mu and C delta organization in mouse as well as C mu and C delta that means IgM and IgD constant domain organization in this human are exactly identical. So what is happening? How they C mu and C delta always comes together that we will see.

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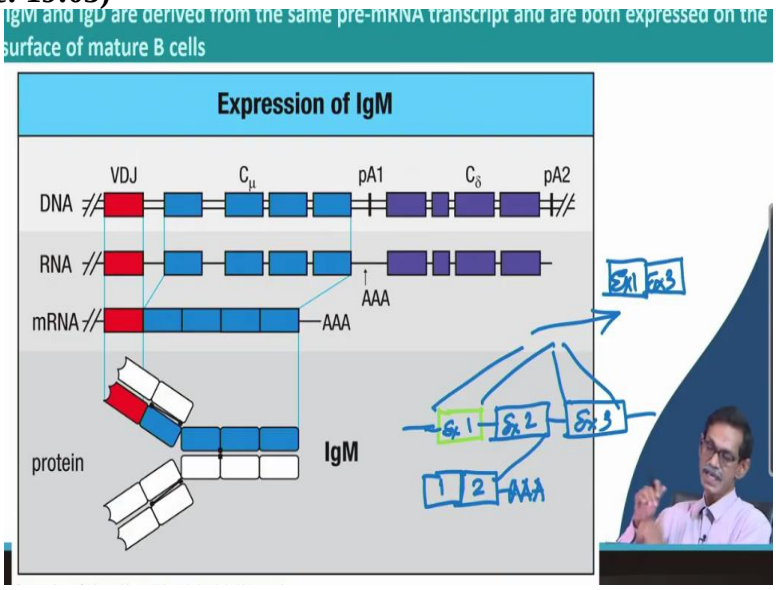

So, here, if you see actually how this I mean, if you just enlarge that C mu and C delta part, how it will look C mu and C delta part VDJ is already recombined, then immediately after that this is the DNA. I told you once and I am reminding again that as when there are 2 lines between these boxes, that means DNA and when there is one line that is RNA, because of just a mark in cartoon and also signifies the double strand single strand.

So VDJ is next to VDJ is the C mu. In previous slide, its C mu was on only one box, but here actually, this C H 1, C H 2, C H 3 and C H 4. There are 4 domains in C mu and this region in between is hinged region. But in other case but here there is no hinge because in IgM there is no hinge. So, this is C mu and there is a 1 pA1 p1 is what in eukaryotic system you know in eukaryotic system there is a in every mRNA there is a polyadenylation site that means, poly A has been added in mRNA and eukaryotic mRNA contains poly a tail and the 3 prime end.

So, it needs a signal there is a site specific site in the genome also. So, after the C mu there is one poly a signal, then immediately after that, this is a C delta constant domain in C delta, this is C H 1, this is C H 2, this is CH 3. This is C H 3, C H 1, C H 2 and C H 3 and these boxes and actually talking about that hinge region. So, in previous slide what we have seen that C mu and C delta next to each other, but this one box is not really a box, this box is actually a several domain of C mu.

So, now, what happened during expression you first you need you need to have a transcription. So, when it is transcribe it transcribe the whole thing, so, VDJ C mu and C delta transcribe together. So, this is that whole transcript VDJ is common in C mu and C delta. In this after this transcription event, this is a precursor RNA, you know precursor RNA undergo splicing and after splicing, the mature mRNA come and this mRNA is responsible for what will be the protein sequence.

So, this is 1RNA sequence, this RNA sequence is having 1VDJ plus C mu segment and C delta segment in 1 splicing, what is happening VDJ is there and then only C mu is spliced as there is already a poly a signal, so, what is the mRNA have only C mu part which is all this internal intron type intron are spliced out and they come together. So, product of this mRNA is actually IgM. And this is actually a very good example of alternate splicing.

If you do not know what is alternate splicing please go and check that what is alternate splicing, but in there many genes are going to undergoing alternate splicing and giving 2 different product of 2 different protein. So, in very brief alternate splicing is suppose there are 2 exon of let me

change the color there are say exon say exon 1 and exon 2 exon 3. So if this is the precursor RNA 123. So in one splicing exon 1 and exon 2 come together, 1 and 2.

So these 2 supplies together and they are one poly A. In other one, it is possible, I am going this way, what happened? Exon 2 and exon 1 joint so, as a result, you will find exon 1 and exon 3. So, what is going to happen? So, there is only 1 mRNA so, 1 protein is having 1 and 2 another protein is having 1 entry. So, though there are 1 mRNA but you can have 2 different protein of 2 different sequence from this is alternate splicing.

So, here we are going to see the same we are going to see the same example I mean same alternate splicing. So, 1 mRNA 1 precursor which are both C mu and C delta as well as VDJ. So, if I consider the whole thing I mean there are so many introns and exons but I am so, to make my life easy said VDJ is 1 C mu is 2 and C delta is 3 here one is what is happening 1 and 2 is combining and giving IgM.

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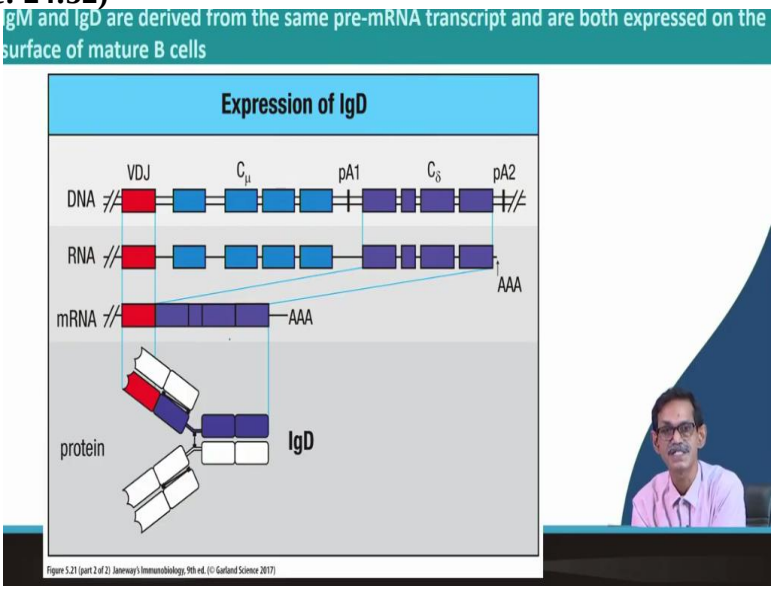

Another this case what is happening 1 and 3 is coming 3 means C delta constant domain so, 1 and 3 is making IgD. So, 1 mRNA so, they are so close in the genome, 1 promoter and 1 product. So, as a result, if we go back if we go back to this slide, so, as a result in B cell as a receptor, we are seeing IgM, Ig2 and IgD is together? As a result, IgD and IgM is coming.

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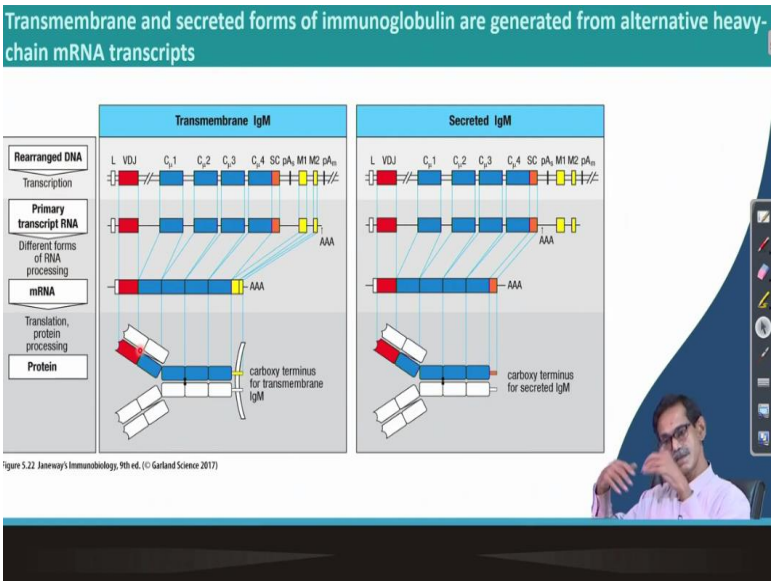

This is the alternate splicing product of single mRNA which is giving expression of IgM as well as IgD. Now, question, I mean, I hope it is clear. Now, I will explain in the very similar thing. Now, I told several times that same receptor molecule, the receptor molecule this receptor molecule is synthesized as a secretory protein upon activation. So, same receptor molecule secreted as antibody molecule upon activation of the B cell, how this thing happened?

Because receptor has a transmembrane domain and secreted protein antibody does not have that transmembrane domain otherwise that cannot be secreted here is again similar alternate splicing is happening. Now, we have to see more detail of that gene in the previous slide, it was less detail. Now if you see a closer look of the IgM, what we will see, VDJ is there we will see C mu 1 C mu 2 that is C H 1 C H 2 C mu 1 C mu 2 C mu 3 and C mu 4 all our as usual before, and then what was not there.

In the previous slide, there is 1 SC 1 M1 and 1 M2 SC M1 and M2. SC is for secretion, which is a tail but not transmitted and M1 and M2 is basically contributing the transmembrane domain of the receptor antibody molecule or B cell receptor molecule. So, here again alternative splicing is happening 1 product so, this is DNA and 1 single MRN is happening here you see there is 1 pA s and pA m this pA s and pA m both are polyadenylation signal.

So, transcribe after transcription, everything is coming, everything is here. But so, in one product what is happening VDJ is coming all this C 1 C mu 1 C mu 2 C mu 3 C mu 4 all are coming, but this SC portion is not coming. So, it is not spliced instead I mean in after that this M1 and M2 which is contributing the transmembrane domain of the B cell receptor and joining. So, all these are different exons.

So, in first case, this SC is not coming instead of that 2 yellow which is a M1 and M2 that the transmembrane domain is coming and you can see this yellow is indicated as a transmembrane domain part which is a carboxy terminus. So, as a result what is happening this is the final mRNA. So, these productive converted to protein which will have a transmembrane and which will remain attached to the membrane clear but the same transcript is happening upon activation in plasma cells, but splicing is happening differently what splicing is happening here.

Here is what is happening instead of M1 and M2 this SC portion which is secreted is a IgM portion which is not as big as this whole transmembrane domain, but they have a little tail and these pA s which is the polyadenylation adenylation signal is coming. So, here what is happening same VDJ same IgM constant domain but instead of M1 and M2 this SC portion is coming. So, what is the take home message from here 1 mRNA multiple actions.

Out of that VDJ and all constant domain region 1234 are always there in one case secretory domain is there in another case transmembrane domain is there. So, normally in B cell when it is expressing not activated not antigen is there or not converted to plasma cell, they are expressing the gene along with the transcript in domain so; they cannot leave the cell and remain attached to the membrane or plasma membrane. That is why it is that this receptor and do is doing its job and this carboxy terminus or the cytoplasmic tail part is responsible for signaling.

So, upon activation, same gene, everything is same, but just the slight change in the splicing, make the same molecules to secretory protein that then it secretes to blood and doing its own job like neutralization, opsonization complement activation and many other things clear. So, but if you see the variable region is not changing that what we say that every B cell will produce a single type of antigen specificity.

Because once one VDJ recombine give a specific specificity against a particular antigen that remain constant it is not changing and the same molecule or same VDJ I also act as receptor also secreting as antibody, so, there is no change. So, throughout the lifecycle of that 1 B cell, it may change by hyper mutation, but once it is changed, these VDJ will remain same. So, same molecule is going to be receptor as well as antibody molecule. So, this is the reason why this particular receptor molecule converted to antibody without changing it specificity. So, this is the mechanism or this is how it is happening.

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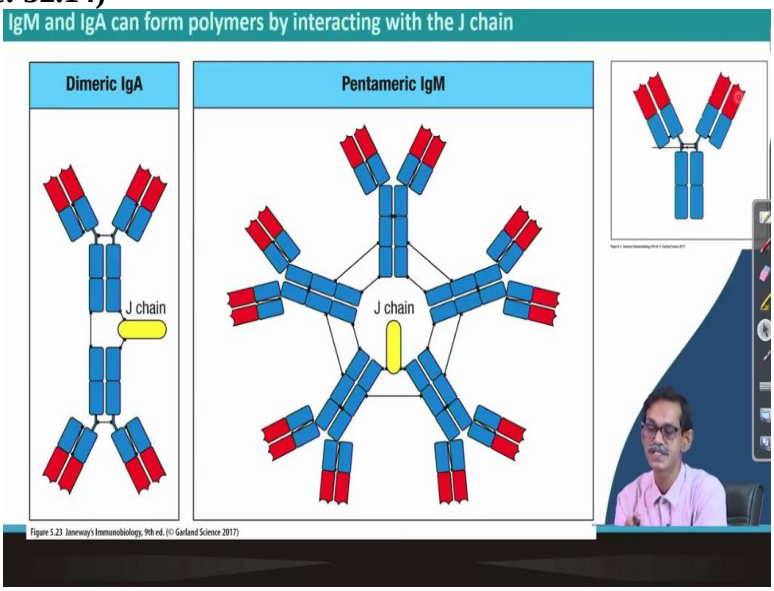

So I will go quick 2 things. One, why IgM was so, high molecular weight because IgM always stay as pentamer so 5 molecule if you see antibodies like one, this is a IgG, but IgM always remain as high together the pentamer that is why it is molecularity so high and this is a very common question how in pentamer IgM how these monomers are attached peptide bond or what no you see all a disulfide bond, even the J chain which is connecting these J chain is also connected to monomers by disulfide bond.

Similarly, IgA remain as dimer same J chain as and a disulfide bond, IgA remain as dimer and IgM remain as pentamer. So I am repeating again IgM as pentamer IgA as dimer. So if you remember what is the valency of antibody, normal IgG balances is 2 what is the valency of IgM. So IgM valency balance is 10 because each 1 can bind 2 so balance of IgM is10 valency of IgA 4

other like IgG Ig all have that balance is 2. So we will see you in the next class. Thank you very much.