Essentials In Immunology

Prof. Anjali A. Karande

Department of Biochemistry

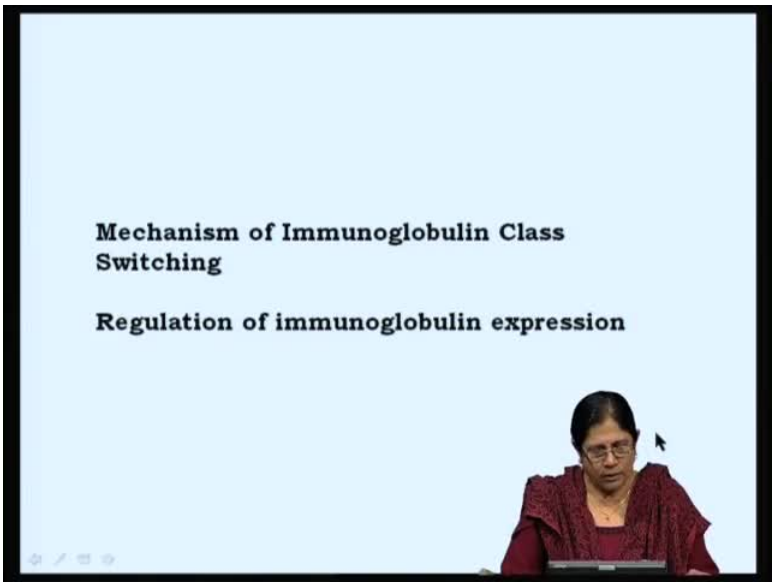
Indian Institute of Science, Bangalore

Lecture No. # 11

Immunoglobulin Class Switching Regulation of Immunoglobulin Genes

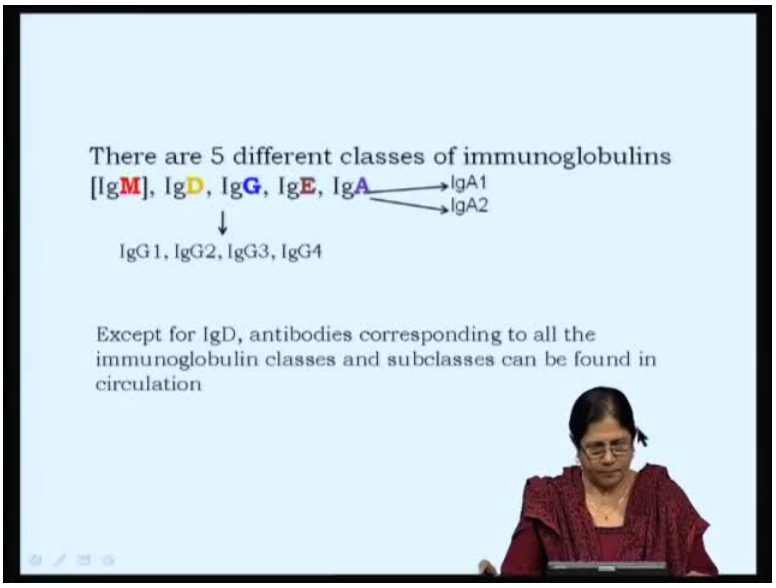
In the last lecture, I did introduce to you the mechanism of immunoglobulin class switching. Today's class, we are going to deal with in-depth analysis of what goes on when immunoglobulin class switching takes place.

(Refer Slide Time: 00:33)



Later on, I will also talk a little bit with regard to regulation of the immunoglobulin gene expression.

(Refer Slide Time: 00:39)



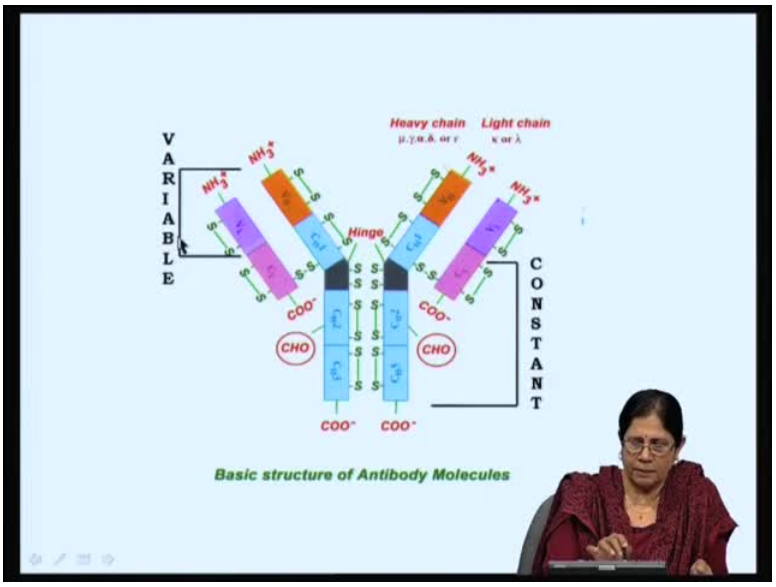
Before I come to class switching, I would like to introduce the different classes of immunoglobulin, because this is relevant with respect to the constant domain genes, which undergo class switching. There are five different classes of immunoglobulins: IgM, IgD, IgG, IgE and IgA. Now, when I say these five classes, we are referring to what is present in the mammals, human as well as mouse. Like I said earlier that these are the two systems that have been studied in depth by immunologists, mouse and human; and, what is true of mouse, is also of true of human.

Now, why do I have the IgM in bracket is because always, when the immune system responds to a particular pathogen or antigen, the first class of antibodies that are secreted in circulation are IgM. You might remember that naive B cells have the B cell receptors as IgM as well as IgD. The five classes of immunoglobulins are as mentioned here: M, D, G, E and A. These refer to the association of the heavy chain. So, these immunoglobulins, the classes are called by their heavy chain. Of course, in case of IgM, the heavy chain is mu; D – delta; G – gamma; E – eta; and, A – alpha.

The IgG has been divided into four different subclasses. In my next lecture, I will be telling you all the structure and function of immunoglobulins, and you will have the opportunity to see how these four IgGs differ from each other. IgA on the other hand is IgA 1 and IgA 2. These two subclasses are based on the fact that one exists as a monomer and the second one exists as a dimer. That again, will be dealt with in the next

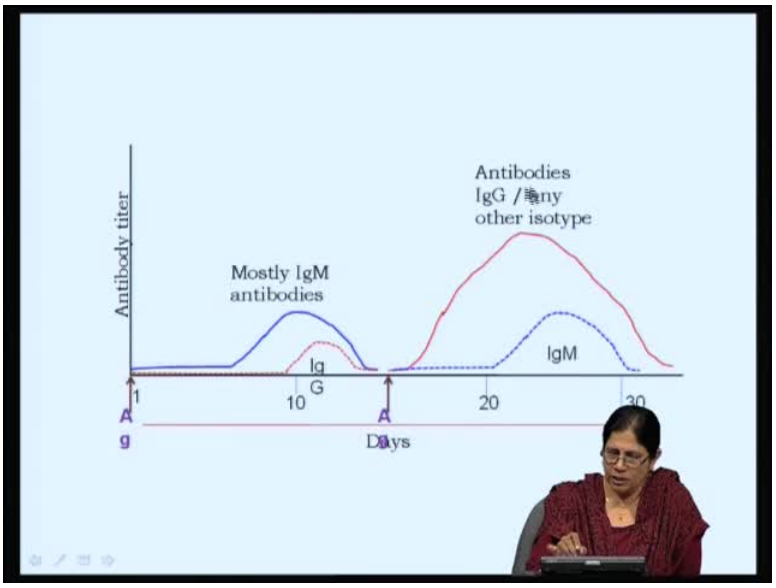
class. I would like to retreat here that IgD – the antigen receptor, which is expressed simultaneous with IgM; except for IgD, antibodies corresponding to all the immunoglobulin classes and subclasses can be found in circulation in response to a particular immune response.

(Refer Slide Time: 03:24)



I would like to again, once more introduce to you constant versus variable regions on immunoglobulin. Now, the same thing also is seen as antigen receptor as well as antibodies. You have the variable region, which has been chopped out here. This variable region is formed by the recombination that we discussed in the last class; V-D-J recombination as happens in the heavy chain and V-J recombination that happens in the light chain. Now, needless to say this variable region is the region, which is required for antigen recognition. And, variables suggest that each immunoglobulin is different in the type of sequence that it bears on the heavy and light chain at this terminus. Two thirds of the molecule remains constant, and therefore, this is called the constant region. All immunoglobulin, let us say, IgG 1 would be quite identical in its sequence in the constant domain, but very different at the variable domain.

(Refer Slide Time: 04:45)



Now, before I come to again the class switching itself, let us see, when does class switching takes place. Remember, I told you that class switching is a phenomenon that happens in case of B cells, which are T cell dependent. I will remind you of certain molecules, which are required for this class switching, but just look at the antibody titer that one can see in response to an antigen. What is written here on the y-axis is the antibody titer and the x-axis shows the days of immunisation.

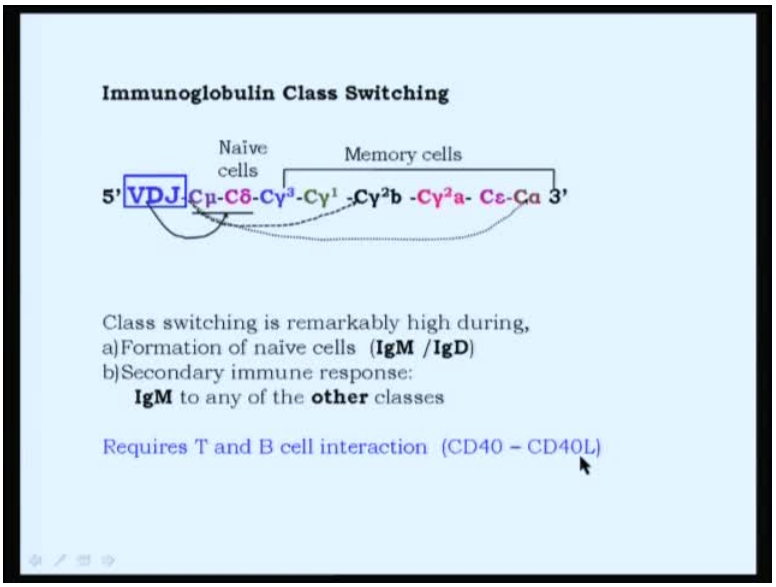
Now, let us say, this is a rabbit and has been injected on day 1; an antigen and the animalist test blood every other day, and the serum obtained from this test bleed is then tested for the presence of antibodies specific to the I g or the antigen. Remember that the total immunoglobulin repertoire will remain almost the same with respect to its amount of immunoglobulin. But, the antibody titer, the antibody to the antigen, which has been injected, will differ.

Now, what one can see very clearly that there is a lag phase of at least about 6 days, after which, the circulating antibodies to the antigens start to increase. And, the class of immunoglobulin that one sees in blue is mostly IgM. If one looks closely, one will be able to see that there is a very small population of antibodies that are of IgG class. This is much later than IgM; and, as has been via experiments that class switching from IgM to IgG begin at around day 7 in the primary immunization. When the same antigen is injected after the circulating antibody titer comes down, then there is almost no lag phase

and there is generation of a robust immune response, and the antibodies that are present here would be IgG or any other isotype. Once again you will see that there is a small amount of IgM, which would represent those naives B cells, which have been recruited in this particular immune response. Usually, one would not look at these IgM; will not be able to detect this IgM in an immunoassay, because the affinity of this population would be much lower than this, and therefore, these are totally obscured. Nevertheless, there are IgM antibodies in the response here, (Refer Slide Time: 07:55) which would be the primary response with respect to some cells, naive cells that have been recruited. Nevertheless, let us just look at this.

Now, again, I would like to tell you that the affinity of these two populations also varies. This is high affinity compared to this (Refer Slide Time: 08:16). But, what I would like to emphasize here, that the immune response that is generated in the primary would be mostly IgM; and, there is generation of memory cells, which is known to class switch to IgG type of antibodies, IgG class of antibodies or any other class.

(Refer Slide Time: 08:41)



Let us look at what are the other classes. When we come to immunoglobulin class switching between the IgM, heavy chain, which is called C mu and C delta, because these always are co-expressed on naive cells, you can see that these can class switch from mu D to any one of the isotypes. What are these isotypes? They are the constant domains remember, which we have seen in the immunoglobulin gene organization. This

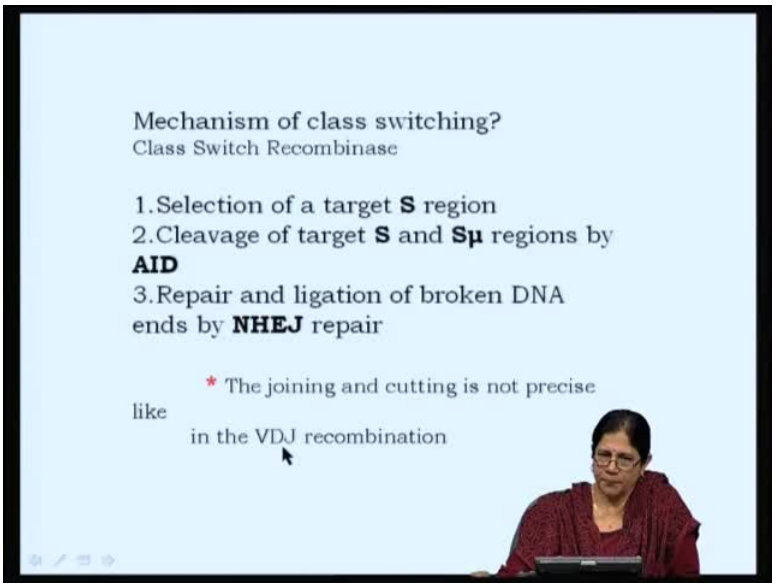
is exactly that the immunoglobulin constant domain genes, which are always in this particular sequence: mu, delta, gamma 3, gamma 1, gamma 2b, gamma 2a, constant eta, constant alpha. Now, just like to remind you again that gamma in human is gamma 1, 2, 3, 4; whereas, in mice, it is 1, 2a, 2b and 3.

When naive cells are being generated, you have the recombination event taking place in the heavy and the light chain. And, you already have the V-D-J or V-J combined. Now, it is only in the memory cells that you have the V-D-J, V-J, which now get associated with any one of the constant domain; like I said, in the primary, we will always have V-D-J, C mu, C delta. It is upon the generation of memory cells, subsequently, the same C mu delta can class switch to any other isotype.

When do you see class switching? If one thinks in terms of switching from mu to delta itself, which happens by alternate splicing at the mRNA level, then class switching is remarkably high during the formation of naive cells. Now, the cells are first… Remember, in the ontogeny of B cell development, you will see that IgM is the first immunoglobulin that is expressed on the cell surface. And soon after that, also IgD and there is then these double positive cells so to say, which have both IgM and IgD expressed. Remember always that IgM and IgD here would be identical with respect to the antigen binding.

In the secondary response, class switching is remarkably high, where IgM to any one of the classes of immunoglobulins, switching happens. Again, I would like to tell you, which I have specified in my earlier presentation, this class switching requires T and B cell interaction just like also, affinity maturation. All these happen in the memory cells, and therefore, those B cells, which are T cell independent, there would be no class switching; there will be only IgM. T B cell interaction – remember, this happens by way of recognition of the CD40 receptor present on the B cell with the CD40 ligand, which is expressed on the T cells upon activation.

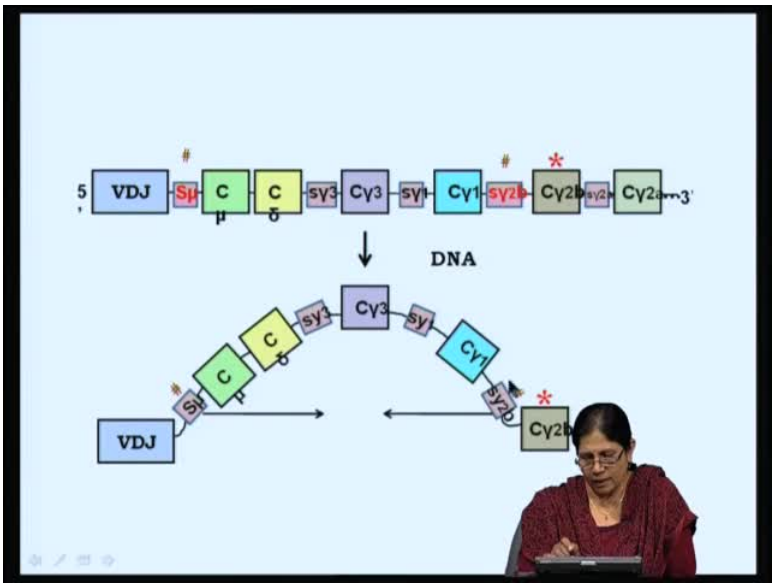
(Refer Slide Time: 12:12)



Mechanism of class switching – class switching happens by a very well-organized class switch recombinase machinery. Now, recombination might ring a bell; we have been talking about RAG 1 and RAG 2. That is also a part of recombination machinery. Though the V-D-J recombination and class switching recombination are similar, there are distinct proteins and enzymes, which are required for both these events to take place. While the V-D-J recombination is very specific, where recognition of sequence is on the DNA takes place specific sequences, recombination for class switching is a little bit more imprecise.

Let us look at what are the events that take place when class switching takes place. First, selection of a target switch region; now, I have not introduced that and I will do it in a minute. Selection of a target S region by the recombinase machinery; cleavage of target switch, which would be IgM always; target S and switch of the mued heavy chain; now, this happens by one enzyme called AID, which is activation-induced deaminase. The third sequence in this event would be after cleavage, repair and ligation of broken DNA ends by NHEJ, that is, non-homologous end joining repair system. Again, I would like to say that the joining and cutting in case of the class switch recombinase is not precise like happens in the V-D-J.

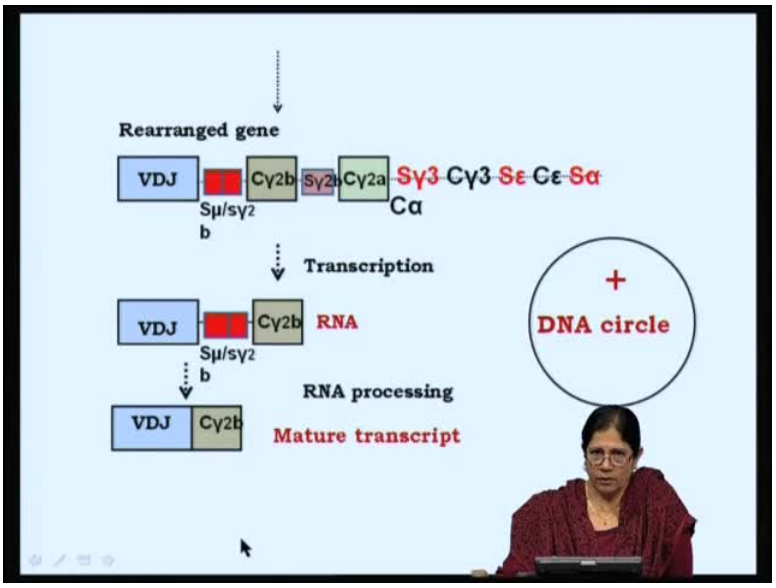
(Refer Slide Time: 14:16)



Let us look at where these switch region genes or genes segments are present. I told you a little while ago that recombination can take place between the C mu and any one of the constant domain barring C delta. Therefore, there has to be a switch region gene on the 5 prime side of every constant domain. Some of the constant domains I have not listed here just because of lack of space. Let us look at now the switch region genes, which are on the 5 prime side or every constant domain except C delta.

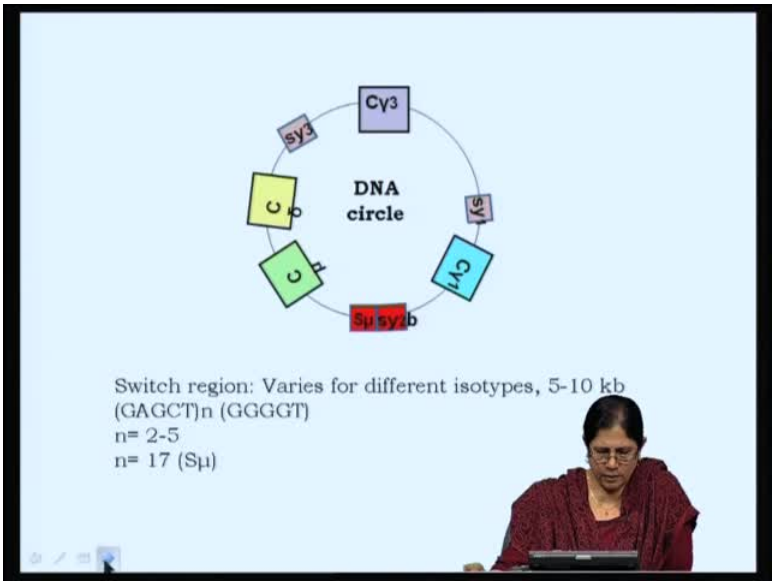
Now, let us say, in this particular instance, C mu is going to class switch with C gamma 2b. Therefore, recombination should take place between the switch mu and switch gamma 2b. Looping out takes place. Now, what brings about this looping out we will deal with, but let us just look at the events that are taking place. Now, VDJ – this should be 5 prime (Refer Slide Time: 15:38). Now, the recombined VDJ is associated primarily with the C mu C delta and now, needs to undergo class switching to C gamma 2b.

(Refer Slide Time: 15:59)



Now, the S mu and the S gamma 2b – these two come together; they join; you can see. And, this becomes now the rearranged gene again at the DNA level. The rest of the constant domain genes, which come after gamma 2b, still continue to be present. Now, it is at the transcription level that you will have the VDJ; you can still see the joint switch regions, C mu and C gamma 2b; and, the RNA transcript has been formed together with DNA circle. Now, what is this DNA circle? We will come to that in the next slide. After RNA processing, you have the mature transcript and there is a message for the protein.

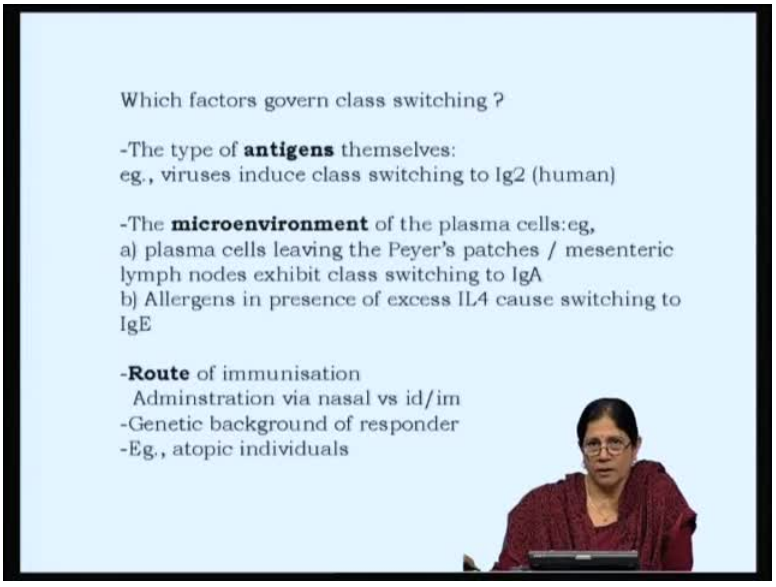
(Refer Slide Time: 16:58)



Before I come to what are these switch region genes, let us just look at the DNA circle that I talked about denoting the previous slide. Now, switch region is seen between the S mu and S gamma 2b on the rearranged DNA, and a part of it, which is exercised out is seen even as a circle in the cells, which are undergoing switch recombination. Now, the joining would take place even in the excised one just like the switch region (Refer Slide Time: 17:55) S mu as well as S gamma 2b, are a part here. And, a part would be here, which is seen in the next slide (Refer Slide Time: 18:03). Now, these are of course, when they are cut, these would not form circles, but the joining or the enzymes that would ligate would not differ or differentiate between the excised one as well as what is in the DNA, and therefore, you will have these circles.

Now, let us look at what are the switch region consists of. They vary for different isotypes, but they are approximately 5 to 10 kilobases; each one of the switch region gene segments. But, anything which is common between all of them is that they are GC rich. These sequences, which are 5 to 10 kilobases of course, also have some motifs, but these switch region genes are the sequence, reads as GAGCT 10 times followed by GGGGT. Now, this n can be 2 to 5 or n can be 17 as one can see in the switch mu region.

(Refer Slide Time: 19:22)



Now, obviously, there should be some factors, which govern class switching. Are there any factors that govern this? First thing what one can look at is the type of antigen themselves, because during evolution, from let us say, the jod fishes to mammals, which

are the most complex and humans, absolutely the most complex. In evolution, during this, there had to be some kind of development of the heavy chain, so that we become better and better; or, in evolution, animals became better and better by evading the pathogens or mounting a very well and robust immune response. So, how do these classes of immunoglobulins differ? IgM, IgD, IgE, alpha and so on; all these differ in their constant domain genes, and therefore, the effective functions. It is the VDJ or the variable region genes, which recognize the antigen. Now, there of course, the difference would be with respect to the affinity by which these receptors or antibodies bind to the antigen. But, it is the effective functions of the rest of the molecule, which actually brings about the actual function of these immunoglobulins.

One example I can give you is with respect to IgG 2a, which is an isotype that is made in response to viruses; and in humans, this would be IgG 2. And, the FC regions of these immunoglobulins can bind to some of the cells of the innate immune systems. Now, thereby, this would be an antibody driven mechanism, where the antibody binds with the pathogen; through the FC receptor, they are brought in close proximity to the cells of the innate immune system, and thereby, now, bringing the pathogen close to let us say, a macrophage, so that engulfment is faster, better, more efficient. So, amongst the factors that govern class switching, the type of antigen themselves would be the first. So, you have now an example that I gave you of viruses.

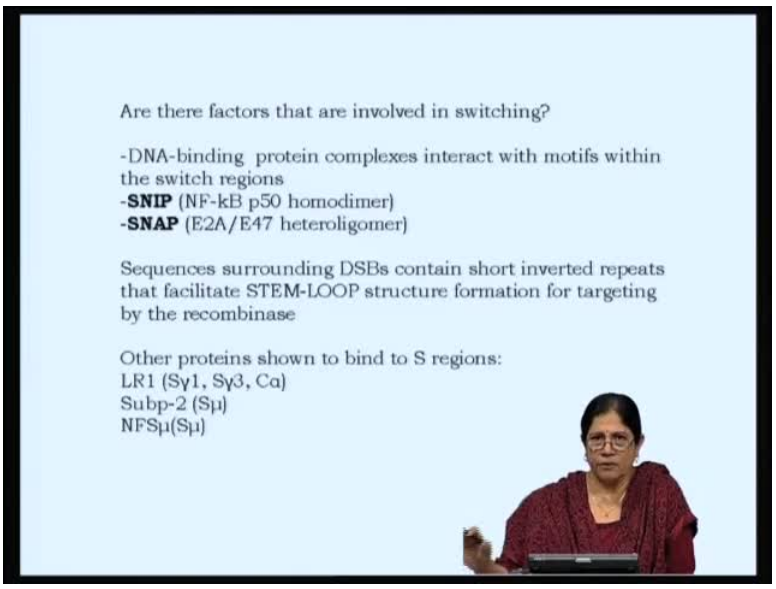
The second would be microenvironment of the plasma cells themselves. Example, plasma cells leaving the Peyer's patches or mesenteric lymph nodes exhibit class switching to IgA. IgA is seen mostly in body fluids. If you look at the different immunoglobulins, maximum secretion of any immunoglobulin class would be IgA; we will come to that in the next lecture. But, Peyer's patches, which are present the intestine; so, plasma cells which leave that region would class switch to IgA, because of the microenvironment. Allergens in presence of excess interleukin IL 4 causes switching to IgA. Now, again why, because IgE brings about mast cell degranulation; again, this will deal with in the next class.

Another factor that would be governing would be route of immunization. Administration of an allergen, let us say, through the nose or the nasal passage, may give rise to IgE. However, the same allergen when injected intradermally or intramuscularly, would give class switching to IgG. Therefore, route of immunisation is one of the factors that govern

class switching. Finally, the genetic background of the responder – this I can explain by way of people who are atopic individuals, which would mean they respond by way of hypersensitivity or allergic reactions. Now, these individuals already have a background; the genetic makeup is such that they respond to allergens by way of class switching to IgE. Now, the same allergens would not evoke such a response in other individuals, who are not atopic.

We have gone now to see factors at the molecular level. Are there any factors that are involved in class switching? Now, people have looked for factors, such as transcription factors or factors, which now in this case, would allow the class switch region of the IgE to come in close proximity with the class switch region of mu or IgM. And, this interaction would mean that the cell would now allow V-D-J recombined region to combined with the constant domain E. All this would happen only if the chromatin reorganization takes place in the switch region genes.

(Refer Slide Time: 25:16)

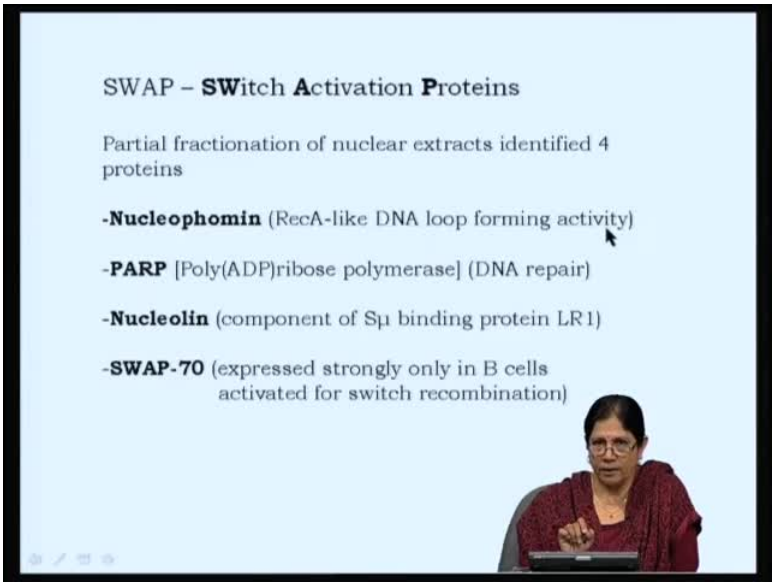


So, people have looked at what would be the protein complexes that interact with the switch region or motifs within this switch region. And, there are two such candidates that have been discovered called snip and snap. Snip is NF kappa-B p50 homodimer and snap – E2A/E47 heteroligomer. Now, what is also been seen is sequences surrounding the double strand breaks when the switch region. When the cutting takes place by the activation induced deaminase, these double strand breaks contain short inverted repeats

that facilitate stem-loop structure formation for targeting by the recombinase. This looping out is absolutely essential as I have shown in the previous slides. And, this would mean that there should be some proteins, which allow this stem-loop structure to be formed.

There are other proteins, which have also been shown to bind to the switch region gene segments. And, they are LR1 in case of the switch region for gamma 1, gamma 3; and it should be Sa; it should not be Ca; this denotes constant. But, we are talking about the switch; (Refer Slide Time: 26:35) this should be S alpha. There are also those step binds specifically to the switch regions of the mu heavy chain, the subp-2 and NFSmu. Now, I would like to emphasize here that though these factors have been seen to bind to these motifs and switch region genes, but none of these have been actually demonstrated to bring about class switching in vitro condition. By DNA binding studies, these molecules have been identified.

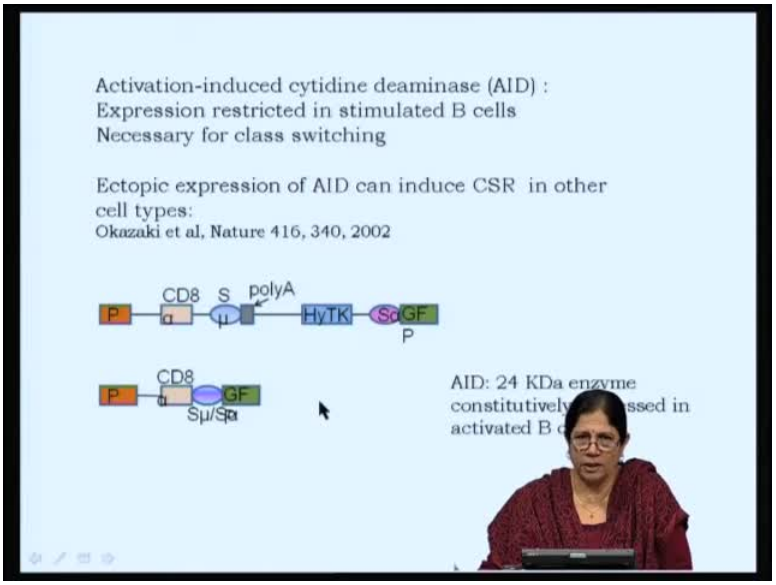
(Refer Slide Time: 27:11)



Now, the other way to look and identify molecules that would bring about class switching would be to identify the DNA binding proteins in the nuclear extracts of cells, which are undergoing class switching. And, four such proteins have been identified and actually the functions of which in fact, would be required for class switching to take place. The four proteins are Nucleophomin – this protein is a RecA-like DNA loop forming activity; PARP, which is a polymerase required for DNA repair; so, PARP is

poly ADP ribose polymerase; nucleolin – this is a component of the S mu binding protein LR1, which we referred to in the previous slide; and, SWAP-70 – this is expressed strongly only in B cells and it is involved in switch recombination.

(Refer Slide Time: 28:19)



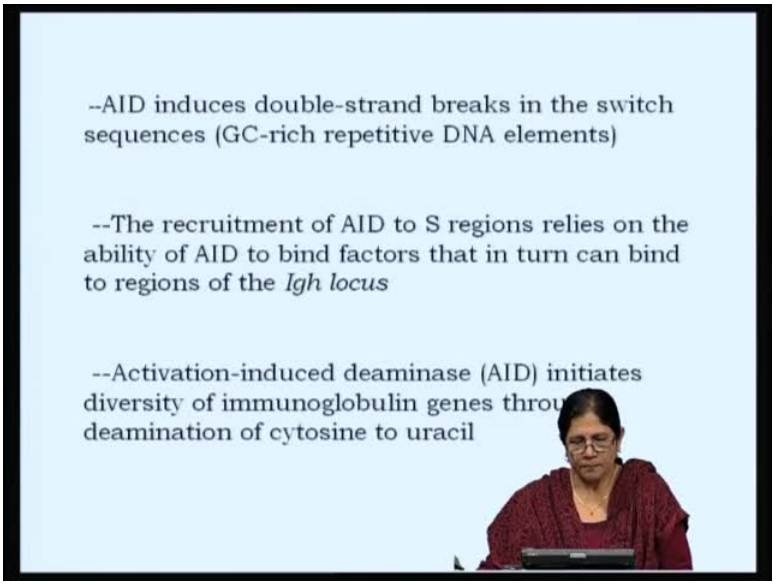
The last, but not the least, the most important protein in fact, which has been shown to induce class switching is AID. In fact, this is the only enzyme that has been shown in vitro to induce class switching and this is what I am going to explain to you. Now, this was a paper that was published in 2002, which showed the importance of activation-induced cytidine deaminase. And now, one can imagine that this enzyme was shown to bring about class switch in fibroblast cells.

Once again, as in some of the previous experiments that I discussed with you, people have this Okazaki et al – they have made an interesting construct, which was transfected in fibroblast cells. So, they have the construct where there is a promoter, which next to the promoter is CD8 alpha. Now, CD8 is a receptor; you might remember from some of the lectures that you heard so far, CD8 alpha, beta. These are the two, which form a co-receptor for the T cell antigen receptor. Now, this is membrane bound (Refer Slide Time: 29:49). Therefore, they have included this in the construct.

Now, there are two switch region genes; those that are for S mu or the heavy chain of IgM and switch region for alpha, there is a polyadenylation signal, which is on the three prime side of the C mu. Now, after S alpha, the switch region alpha, there is GF P, that

is, gene coding for green fluorescent protein. Now, when the cells were first selected, they were transfected with this construct and selected by the thymidine kinase gene, which was the selection marker. And then, all the cells containing this construct were then transfected with AID, the construct that now encoded the gene for activation-induced deaminase. This enzyme is a 24 kilo Dalton, which is constitutively expressed in activated B cells. So, when this was transfected in the fibroblast along with this stably transfected construct fibroblast, then the cell started to flurries green; how did this happen? That the switch region combined because of the activation induced deaminase. And now, the CD8 alpha chain fused with the GF P. And since, this is a cell surface express protein, the cells that allowed class switching to take place also flurries green. And, this was a rather elegant experiment, where AID was shown to induce class switching and in a system where it does not normally take place.

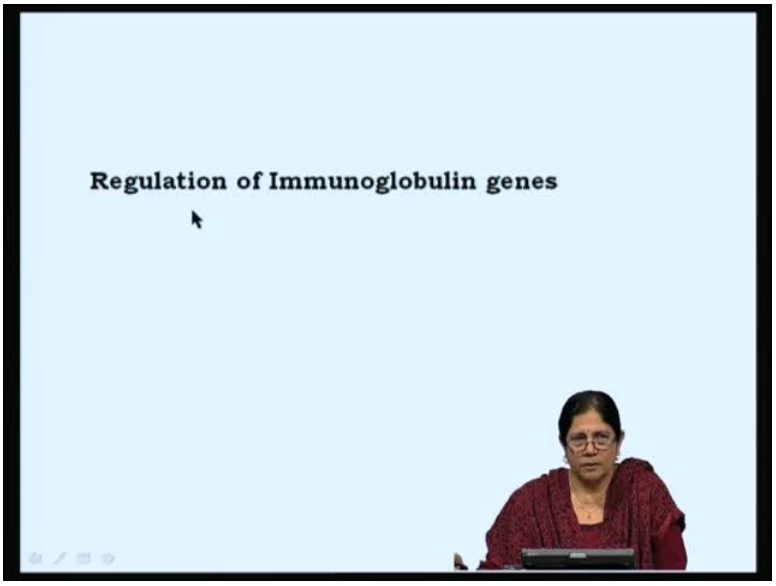
(Refer Slide Time: 32:00)



The paper also starts to say a few aspects that I will deal with now, that aid induces double strand breaks in the switch sequences. So, though there is no specificity with respect to this double strand break happening while keep sense specificity is ,because remember, RAG 1 and 2 recognize specific sequences in the hepatoma and the nonama of the RSS (recognition signal sequences). There are no RSS in the switch regions, but AID recognizes certain motifs in these GC-rich repetitive DNA elements that form the switch region and induces double strand breaks.

Now, the recruitment of AID; how does this happen to the switch region is not by the recognition of the switch region by the AID, but there are factors that bind to these regions, and inturn, bind to or recruit AID to this particular locus. Now, another thing again, activation-induced deaminase as one can imagine, deaminase initiates also diversity of immunoglobulin genes through deamination of cytosine to uracil. This is another factor that governs the variability, but what we are dealing with is the constant domain and the class switching.

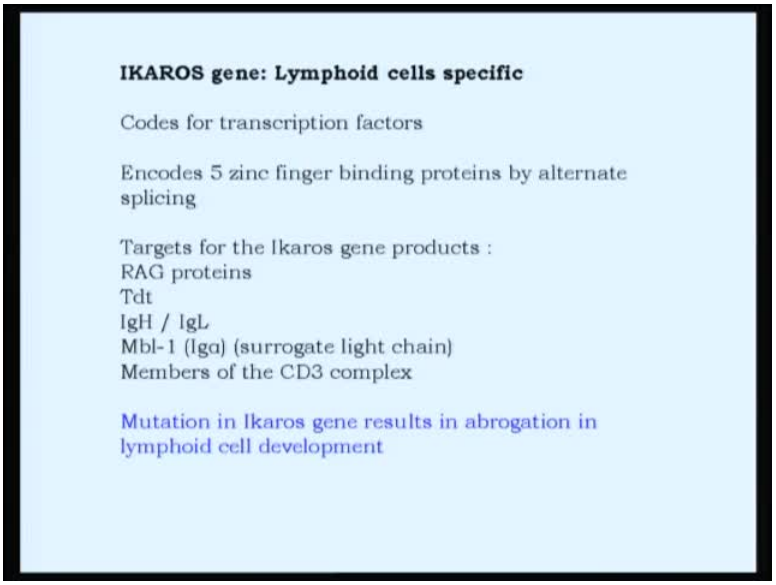
(Refer Slide Time: 33:44)



Now, just to summarize this part, in the primary immune response, always, the V-D-J recombined region associates with IgM, IgD and expressed on the cell surface. Therefore, in the first immune response that is generated, this is a naive cell. When such a naive cell encounters an antigen to which it has this specific receptor, undergoes an activation process; starts to proliferate and differentiate. Now, the immunoglobulins that are synthesized as antibodies by cells originating from this first cell in the primary immune response would always secrete IgM. There are few cells that become memory cells. And, these when they encounter the same antigen again and when they get help from T cells by way of CD40 ligand presentation to the CD40 receptor, these undergo class switching whereby the very same VDJ now associates with any one of the constant domain gene segments, which is dictated inturn by the type of antigen that had been introduced.

I would like to say here, though I keep saying VDJ, which is already recombined and it is identical, remember, from the previous lecture, that memory cells also express error-prone polymerase DNA polymerase, which can bring about mutations and increase in the affinity of these immunoglobulin antigen binding genes. So, the class switching happens, so that the immune system gets better and better at eradicating almost all the type of pathogens or soluble factors, which are foreign to us. Therefore, in the next class, it will be easy for you to recognize why do we make IgM; why do we make IgG, the different classes of IgG, subclasses of IgG or IgE or IgA.

(Refer Slide Time: 36:18)

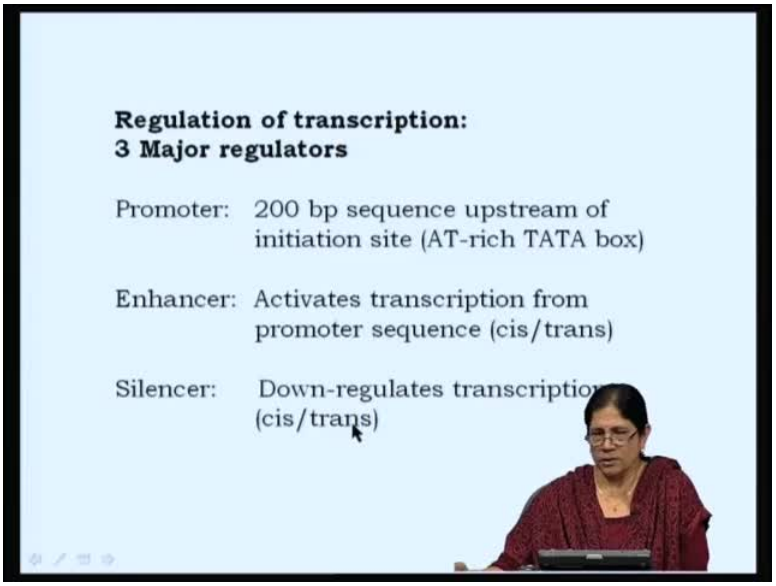


Now, let us go back to looking at the regulation of the immunoglobulin genes. Now, let us go back to see why RAG 1 and 2 are expressed only in lymphoid cells; there has to be something that regulates the RAG 1 and 2 expression. So, when people started to look for factors in the T and B cells, which now allow expression of RAG 1 and 2, they found a gene called IKAROS. IKAROS – like I said a minute ago is highly lymphoid cells specific. IKAROS codes for transcription factors. This transcription factors encode five zinc finger binding proteins and this is by alternate splicing.

Now, the targets for the IKAROS gene products: primarily, RAG proteins 1 and 2; terminal deoxyribosyl transferase; remember, this enzyme adds N-nucleotides to the cut ends of the hairpin structure that is formed once again by the RAG proteins. The targets for IKAROS is also immunoglobulin heavy chain and immunoglobulin light chain.

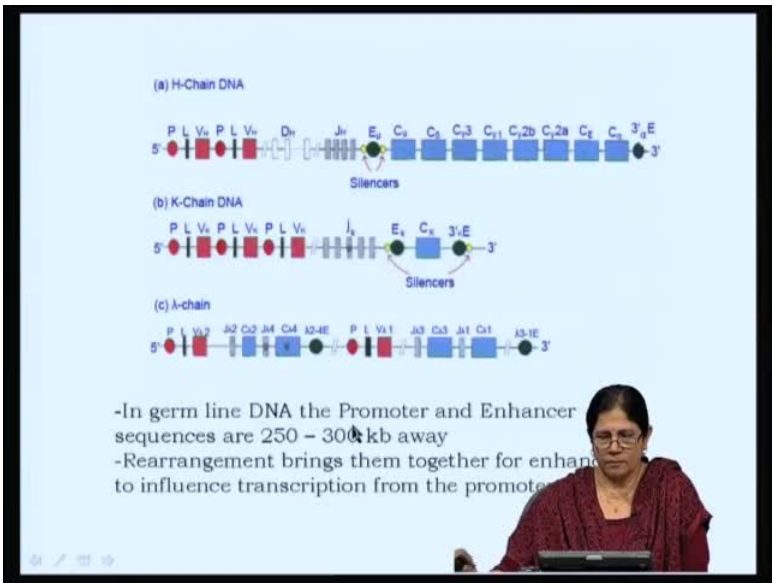
Another target is Mbl (immunoglobulin alpha); remember, this is the immunoglobulin alpha, which is present as a co-receptor; this should not be there – not surrogate light chain; but, the co-receptor for the antigen receptor. Also, members of the CD3 complex; CD3 complex – you have already studied the antigen receptor in case of the T cells and you know that CD3 is the co-receptor associated with the TCR. Mutations in the IKAROS gene results in abrogation of the lymphoid cell development; therefore, one can imagine that not only RAG 1 and 2, but RAG 1 and 2 themselves come under the control of IKAROS gene, and therefore, mutations of this gene itself will abrogate the lymphoid cell development totally.

(Refer Slide Time: 38:36)



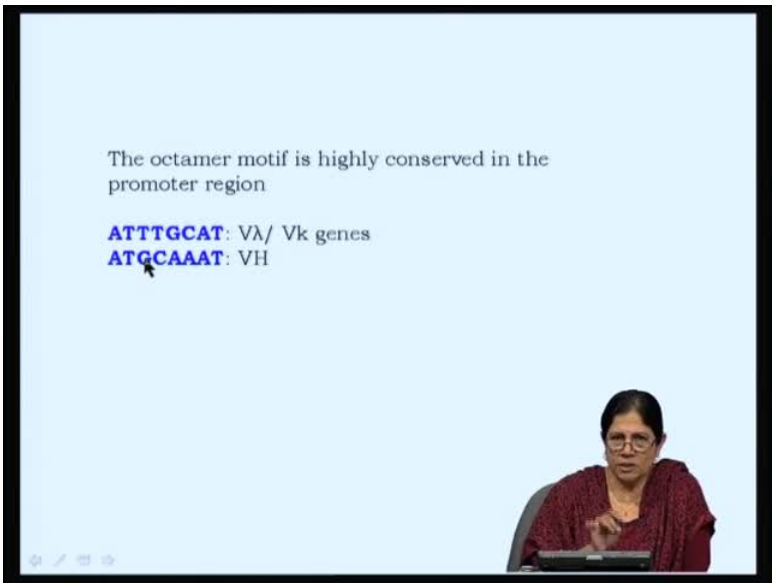
Let us look at the regulation of transcription of the recombined immunoglobulin genes. There are three major regulators like the same in almost every gene. There are promoters, enhancers and silencers. Promoters – all of you would know already; promoters are approximately 200 base pair sequences, which are upstream of initiation site and AT-rich TATA box, which is the initiation site. Enhancer – these are sequences that activate transcription from the promoter sequence and they could work in cis and trans. Silencers, which down-regulate transcription. So, you have enhancers and silencers, and the name themselves denote that. Silencer also down-regulates transcription – both cis and trans.

(Refer Slide Time: 39:36)



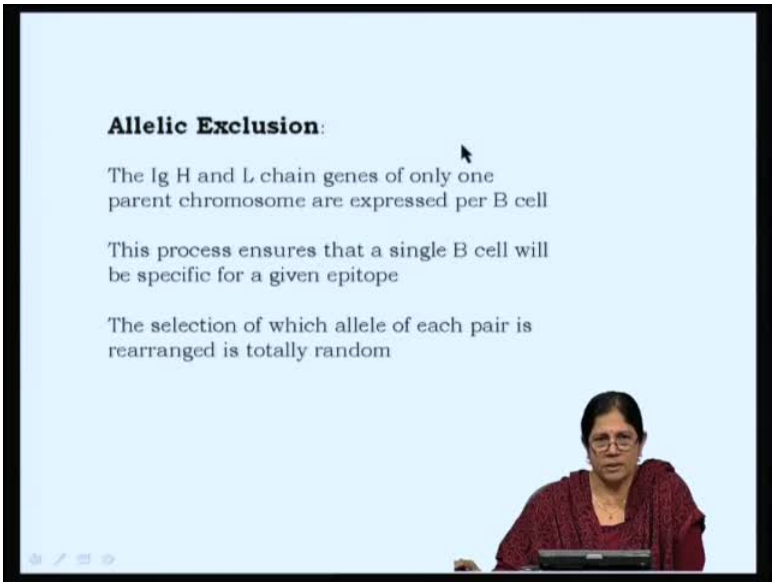
If you look at the heavy chain, this is what I wanted to show you that the promoter then has to be a promoter on the 5 prime side of every leader sequence, which is of the 5 prime side of every V gene segment. So, you can see the presence of promoter. And, every variable gene segment, which is a part of the variable domain of the immunoglobulin gene has a promoter not only in the heavy chain, but you can see also in the light chain. Now, there are enhancers with charge on the 5 prime side of the constant domain, also in the kappa and the lambda light chain. In the germ line DNA, the promoter and the enhancer are 250 to 300 kilobase away. And, rearrangement of any one of the V with any one of the J and D would bring these close together; because of which, transcription is influenced.

(Refer Slide Time: 40:55)



Are there any specific motifs present in the promoter region of the immunoglobulin genes? Yes, there are octamer motifs, which are highly conserved. And, one can see the motif ATTTGCAT in the lambda and kappa genes, the light chain genes; whereas, in the heavy chain promoters, it is ATGCAAAT. It is interesting; these sequences are very conserved in all immunoglobulin genes on the V segment.

(Refer Slide Time: 41:32)

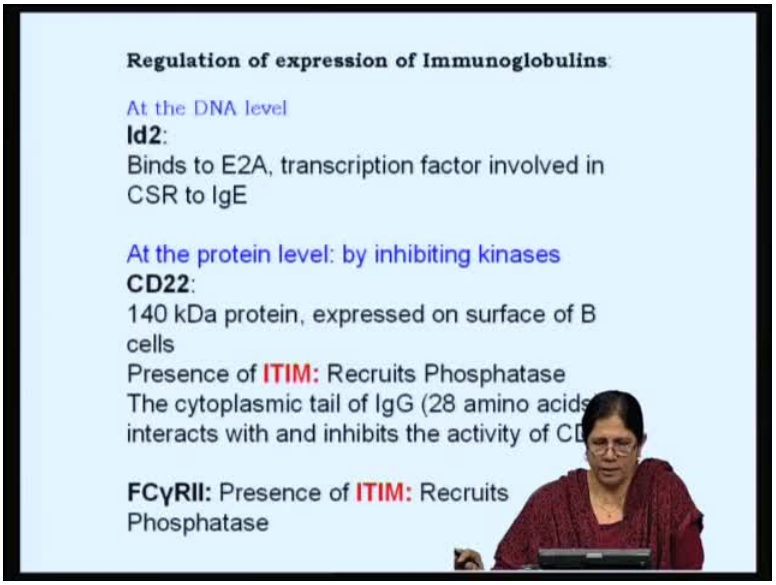


Something I would like to again introduce to you, that this phenomenon of every B cell, which is already committed to look at one type of or recognize one type of antigen.

Recombination happens only in one of the alleles; never on both alleles at the same time. And, I am going to deal with this once more; but, there is a coordinated way by which recombination takes place. It is known that the immunoglobulin heavy and light chain genes of only one parent chromosome are expressed per B cell; the other one is excluded.

Now, allelic exclusion – this allows or ensures that a single B chain will be specific for a given epitope. The selection of which allele of each pair is rearranged is totally random. All of us do get both paternal and maternal alleles and they are equal. However, in the B cells, which heavy chain allele is now taken or rearranged first or is successful, is totally random. But, this is important, so that one B cell recognizes only one epitope of any antigen, and therefore, makes antibodies of only one type.

(Refer Slide Time: 43:20)



Now, regulation of expression of immunoglobulin genes happens at the DNA level by several factors. But, it is not very easy to talk about all of them. I would like to talk about those, which are very important. For example, Id2 – these are more recent literature and immunology in fact is young science. And, there are more and more additions to the information that we already have at regular intervals. So, regulation of expression of immunoglobulins can happen at the DNA level as well as at the protein level. I will give you examples of this at the DNA level.

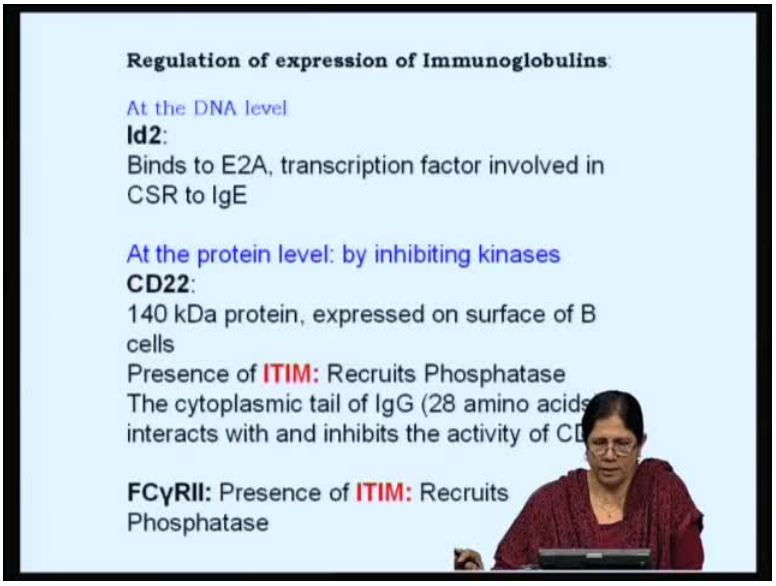
One should have minimal IgE type of an immune response. Where would one want an IgE type of an immune response? For example, in case of pathogens, which are large; let us say, helminths worms that enter our system, where the macrophages cannot engulf; neither will the antibodies be able to kill. However, there needs to be some factors, which should now eradicate these worms; and, the immune system is prime to do that. Is the IgE antibodies that actually participate in this? And, I would like to say that, let say, one is infested with worms; then, there are factors, which allow the class switching to take place between IgM to IgE. Now, this happens through transcription factors called E2A, which bind to the switch region of IgE, making that available not to undergo class switching.

In case of the pathogen like helminth, it is required that the immune system makes IgE type of antibodies, because these antibodies as soon as they are secreted, they are bound to the mast cells. When the molecules from the antigen are recognized by these IgE, which are sitting on the mast cells, cross linking of these receptors take place; thereby, there is secretion of the large granules, which are already present in the mast cells; preform granules. The preform granules now attack the helminth. No cells can engulf these helminth, because they are too large; not like bacteria, which can be phagocytes. So, there is an arsenal of molecules that are now thrown on the parasite on the extracellular parasite. Now, all these happen only because of presence of IgE, which is made in response to the pathogen. And now, the IgE is sitting on the FC receptors, which are specific for IgE on mast cells. Now, to prevent such switching to take place between IgM and IgE for all antigens, there are transcription factors like E2A, which are downregulated by Id2. Now, these Id2 are present all the time, so that now, they suppress E2A, and therefore, suppress the class switching from IgM to IgE. This is happening at the DNA level.

Now, you can have regulation of expression of immunoglobulins by way of antibody production. So, you inhibit the cells from proliferating, differentiating to antibody producing cells at the protein level. And, this can happen by, let say, receptors like CD22, which I have not talked about before. CD22 is present on surface of B cells. CD22 is a 140 kilo Dalton protein. It is a glycoprotein in fact. Now, this (Refer Slide Time: 47:46) has in the intracellular domain, ITIM; does this ring a bell? Can you remember ITAMs and ITIMs? When I was talking about signaling and B cells, I talked

about ITAMs. ITAMs are immune receptor tyrosine-based activation motif and ITIM – immune receptor tyrosine-based inhibitory motif. Now, both ITAM and ITIM to get regulated or get activated by way of phosphorylation… But, while ITAM now recruits kinases, ITIM recruits phosphatase. So, that is what CD22 does. But, how does it do that? Interesting that the cytoplasmic tail of IgG – again, I hope you remember this that the cytoplasmic region of the IgG has 28 amino acids as opposed to only three amino acids of IgM and IgD.

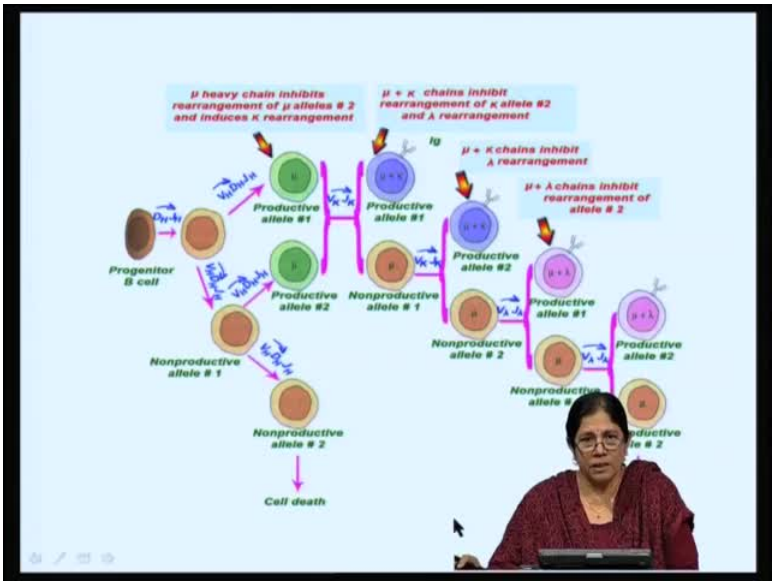
(Refer Slide Time: 43:20)



Now, what are the cytoplasmic tail do? It does not have ITAMS; you remember, in immunoglobulin, the antigen receptor itself does not have any motifs. But, what does this 28 amino acids do is, it inhibits or it interacts with the CD22 phosphatase molecule intra cellularly and inhibits the activation of the CD22; thereby inhibiting the ITIM, and thereby, inhibiting the phosphatase; which would mean that a B cell that is making IgM and another B cell, which is making IgG, it has been seen that always, the IgM produced by a cell is much lesser in number of the molecules made as compared to IgG. So, there is regulation by inhibiting kinases. Does this remind you of another molecule which I talked about in reference to signaling? Yes, you are right; this is FC gamma RIIb, which is a receptor, which is present on all the B cells. Remember this that when the immune response to a particular antigen is very high and you have now circulating antibodies present, the circulating antibodies when bound to the pathogen let us say, the circulating antibody can now bind to FC gamma RIIb and colligation takes place, because another

epitope is recognized by the antigen receptor of the same B cell. This brings about a conformational alteration in the FC gamma RIIb. And, this undergoes phosphorylation for recruiting phosphatase, which would now start lowering the activation index of that particular B cell; inturn, inhibiting the immune response. So, there are a score of different molecules both at the DNA level and at the protein level, which ensures that the antigen receptor mediated activation of B cells; and bringing about now, activation of the synthesis of antibody producing plasma cells can be modulated such that immune response does not go haywire.

(Refer Slide Time: 51:21)



Now, the regulation really in total would be also at the level of the recombination event itself. Exactly, how this happens is not very well known. But, as I go along, I will tell you where this recombination can be seen to be regulated; primarily, at the progenitor B cell, which undergoes recombination at the heavy chain gene first. Heavy chain gene at the variable region – recombination takes place first between any one of the D with any one of the J. Remember, this is important for any one of D J then recruiting any one of the V. Now, if the recombination is successful, then the cell goes to recruiting any one of the V, a productive allele one. Then, if this happens, the products of this allele would inhibit recombination, which can happen in the second allele. So, there is regulation over here. Now, it is believed that the signal joints that are formed when V-D-J recombination is taking place, these inhibit the allele 2 in this particular cell from undergoing recombination.

Incase this recombination fails, only then the second allele starts the recombination process. Interestingly, only when V-D-J recombination has taken place and the heavy chain transcription and translation happens, this can associate with remember, the surrogate light chain. The surrogate light chain itself cannot recognize an antigen, but can associate with the heavy chain such that surface expression takes place. Now, this surface expression is the clue for the recombination of the light chain genes to start. This surface expression also inhibits the second allele of the heavy chain from starting. So, that means regulation at yet a second level.

Now, always, recombination in the light chain genes starts with the kappa light chain gene assembly, allele 1. And, if that does not happen, if there is non-productive allele 1, then the allele 2; if both the alleles of the kappa light chain genes fail to reorganize themselves, then allele 1 of the lambda light chain is recruited; and, if that does not happen, then allele 2. But, remember all along, it is a very coordinated process. At no point of time, does recombination of the heavy and the light chain occurs simultaneously? No, the kappa and lambda happen simultaneously. This ensures that one of the alleles only of the heavy and one of anyone of the light chains kappa or lambda recombine. And now, you have an immunoglobulin molecule on the B cell surface, which has the capacity to recognize only one type of antigen or epitope.

So, with this, I will end the lecture here. My next lecture deals with the structure of all the immunoglobin classes and the effective functions. I will like to keep saying this that during the course of evolution, we have now, the immunoglobin genes, which in the first vertebrate that it appeared, is only IgM, and subsequently, the other classes of immunoglobin started appearing. And, we have now, in the finest form of the immunoglobin classes by way of five different classes of immunoglobin, each of them with specific function.

Thank you.