Essentials in Immunology

Prof. Dipankar Nandi

Department of Biochemistry

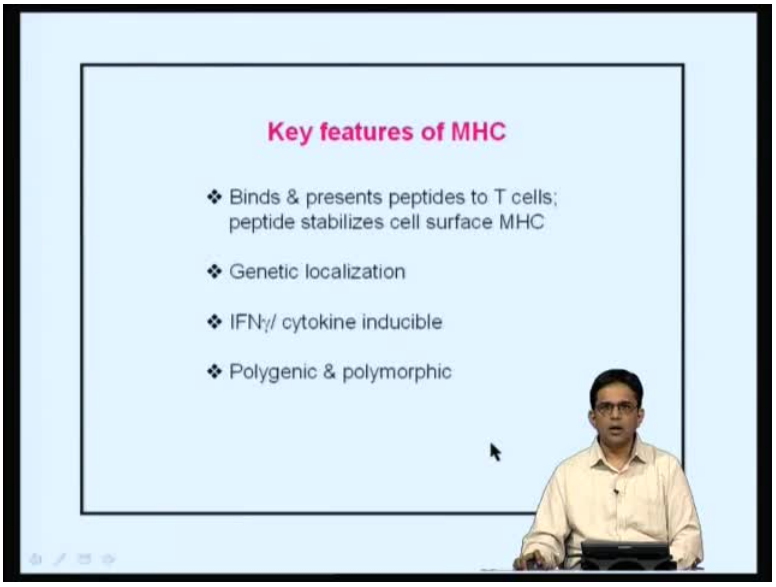
Indian Institute of Science, Bangalore

Module No. # 11

Lecture No. # 23

The Major Histocompatibility Complex: MHC Class I Pathway

(Refer Slide Time: 00:36)



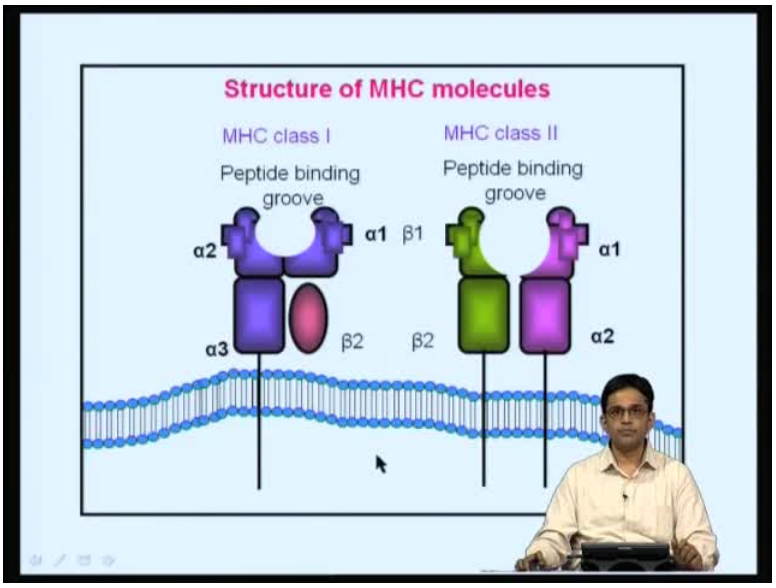
So, today, we will start on the MHC class 1 pathway. In the previous class, we had studied the MHC, the discovery of what MHC, and MHC products, which are important in so many different immune reactions, and so if we look at what are the some of the key features of the MHC, the first is– MHC molecules bind and present peptides to T cells, and what the peptide binding does is, actually, it stabilizes cell surface MHC. So, if we have MHC molecules, in the absence of peptides, these are unstable. So, that is the very important point that students have to remember.

The second thing is genetic localization. The MHC class 1, class 2 molecules are encoded in the major histocompatibility locus, and this is known as H2 in mouse and HLA in humans, and this is a particular defined localization. Now, what is important to remember is that apart from MHC class 1 and class 2 molecules, there are other components of MHC, as well as the non-immune components that are part of this…, of this complex, and we will be looking into that a little bit more in this class, and also in the subsequent class.

The third important point is that they are interferon gamma inducible or cytokine inducible. So, why should this be? So, the moment you have an immune reaction to…, you want to amplify these, and part of a way to amplify is the use of inflammatory cytokines. So, interferon gamma is a good example of this, and what interferon gamma does, it will result in more expression of MHC class 1, class 2. So, how does this help? Because you have more MHC class 1 and class 2, then the chances of a particular antigen specific or pathogen encoded peptide that is recognized by T cells is also higher. So, consequently, your chances for immune reaction or generation of immune response is also more. So, that is something that, again, you should be sort of familiar with.

The other aspect is that MHC molecules are polygenic. That means, there are several genes encoding. So, for example, in humans you have MHC class 1 that is encoded by a, b, and c, and in MHC class 2, human ones are d p, d q, d r. So, there are these different genes that are encoding MHC class 1 or MHC class 2 molecules.

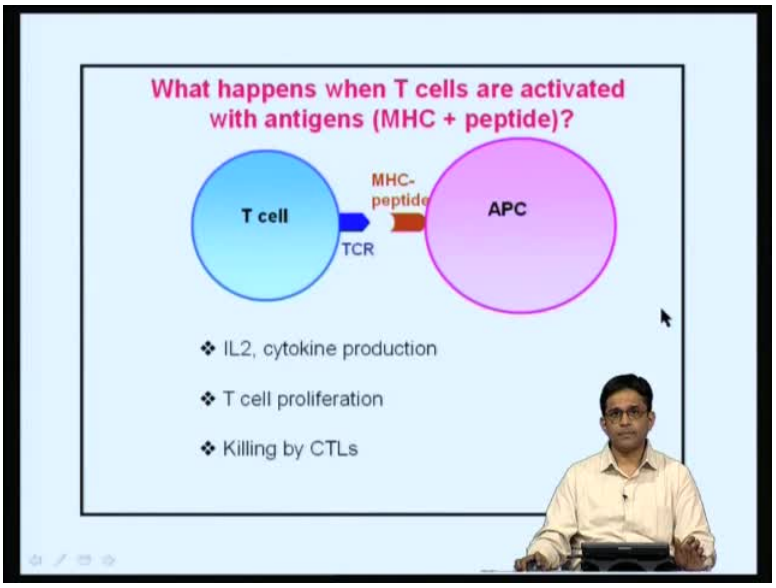
(Refer Slide Time: 03:26)



The other point is that they are polymorphic. So, within k, within, let us say, a, b, c, you have different forms of HLA a so or HLA b. You have HLA bo 5, HLA b 27, so on, and so it results in polymorphisms, and so, what is the importance of polymorphisms? What polymorphisms allow, is for different…, because there are the polymorphic residues are usually present in the peptide binding region. You will have the array of peptides that can bind to MHC molecules is increased, so that is an important point that students should be familiar with, and this is a part of some of the key features of MHC molecules, and this is… we will just briefly go over the structure of MHC molecules.

So, MHC class 1 molecules, you have a heavy chain, and the heavy chain has three domains– the alpha 1, alpha 2, and the alpha 3. Now, if you can see, the peptide binding groove, it consists of the alpha 1 and alpha 2 domains, and so, therefore, the polymorphisms that are responsible for binding different peptides will be localized primarily in this peptide binding groove region.

(Refer Slide Time: 04:15)

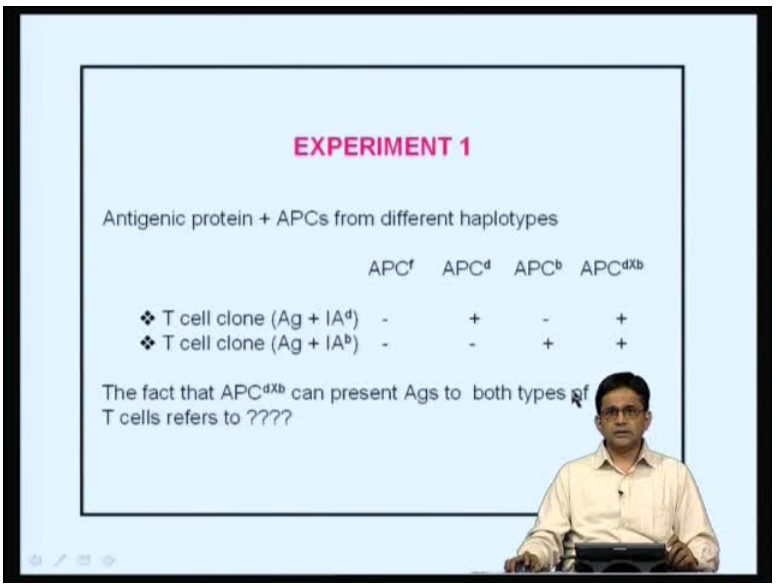


The alpha 3 is important for binding to beta 2 microglobulin. Now, in MHC class 2 molecules, there are two chain– that is, the alpha and the beta chains, and you can see, the peptide binding groove is consists of the alpha as well as the beta chains. So, what happens? So, MHC molecules present peptide to T cells. So, what is the consequence of this? So, this is what is shown over here. This is antigen presenting cell is presenting a MHC peptide complex to a T cell, and you have a cognate TCR MHC reaction. By cognate, what I mean is, you have a specific reaction, means, this TCR is specific for this MHC peptide, and so, once you have this and there are other features also involved in this, but mainly, this specific interaction comes from the binding of the TCR to the MHC peptide complex.

Once you have this, so the T cell gets activated. It produces IL2; it produces other cytokines; then, IL2 is important because it allows for growth of these cells. So, you will have this clonal T cell that will now… you will have more numbers of these of the same cell. So, in order for that, you need growth factors, and IL2 is a T cell autocrine growth factor. So, the T cells proliferate and in case of CD8, it is what will happen is this cognate interaction will result in differentiation from a CD8 to a CTL, and so, CD8 positive T lymphocytes becomes a cytotoxic T lymphocyte by the process of differentiation, and it is the CTL which can now kill the target cells containing MHC peptide complex.

So, this is a very important point that students should be very familiar with– what is the job of the MHC molecule? It is to present peptide. And where are these peptides generated? These peptides are generated within this particular antigen presenting cell. Once, so whatever is happening inside the cells, the T cells can peruse it, or you know, get a feel for what is happening, and the T cell, over here, will recognize this using the specific T cell receptor. Once you have this, you have T cell activation.

(Refer Slide Time: 06:05)



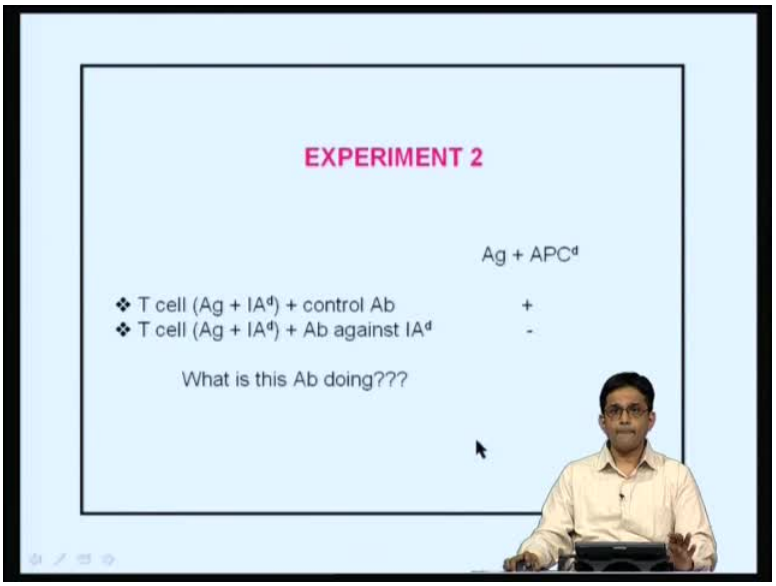
Now that you have some knowledge about MHC molecules, we will be trying and doing some experiments to better understand this process. So, you have said, you have antigen presenting cell and you have a T cell. So, we will have, what I have shown over here is antigen presenting cells from different mice with the haplotypes. By haplotypes, what I mean is, their MHC molecules are different so that is it shown as f here, d here, b here, and this is a f 1, which is a d by b.

Now, the APCs will present antigen in complex with the MHC, but it needs to be recognized. For that, we need specific T cell clones. So, you have a T cell clone here, so this particular T cell clone recognizes the antigen or peptide in complex with IA of d. Now, if you remember IA, IA is a MHC class 1, is a MHC class 2 molecule, and d, again, refers to the haplotype. This is another T cell clone. This recognizes the antigen in complex with IA of b, so these differences are due to polymorphisms, and they are very distinct.

So now, when you put the APC and the T cell together, you should get IL2 production if it is a cognate reaction. So, we will address whether you will get IL2 production, or some indicator of a response for a cognate interaction between the TCR and the MHC. So, do you think the T cell clone that is specific for this haplotype will recognize APC? And you have to remember, in these experiments, the antigenic protein is given. So, it will not recognize because it is MHC is different, but if it is a different APC and the antigen is given, and if you have this particular IA of d and the d, then it recognizes.

Now, again, with b, it will not recognize this one. This will not recognize, but this clone will, because it is IA of b, over here. Now, the APC, which is the f 1, which has the d and b will generate a response in both the clones, because it expresses one allele of d and one allele of b. So, what does this refer to? This refers to co-dominance. This is the phenomena of co-dominance, and that is because we have one set of chromosomes from our father, one set of chromosomes from our mother. So, we have 2n. So, that is why both types of MHC molecule will be expressed on the APC. So, as a result of this, you will get this APC molecule will be able to present both the antigen in both in the d as well as the b. So, this is very important concept, and I hope students understand this very well.

(Refer Slide Time: 08:53)

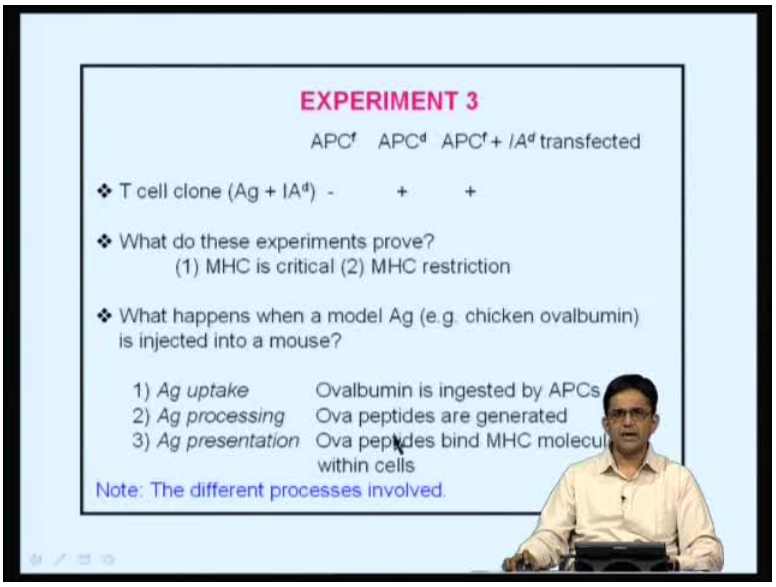


Now, we will try and go towards the second experiment. The reason why I show these experiments is because by experiments, you can really test your learning, and it is a good

way to learn. And so, that is why it is also a little bit of fun, also because instead of just me telling you all the facts, it is important for you to understand the experiments that go on. So, over here, we have these APCs with antigen, and then you have this particular T cell.

Now, what has been done here is, you have an antibody molecule against IA of d, and you can see the control antibody, which does not recognize the particular MHC molecule, you will see IL2 production, if that is the read out. Whereas, if you have an antibody that blocks IA of d, then you are not able to see. So, what is this antibody doing? What this antibody is doing is, the antibody is blocking the interaction of the MHC with the T cell receptor– again an important concept. These are the reagents by which a lot of studies with of involving MHC were done; that is why it is important for students to understand it.

(Refer Slide Time: 09:54)



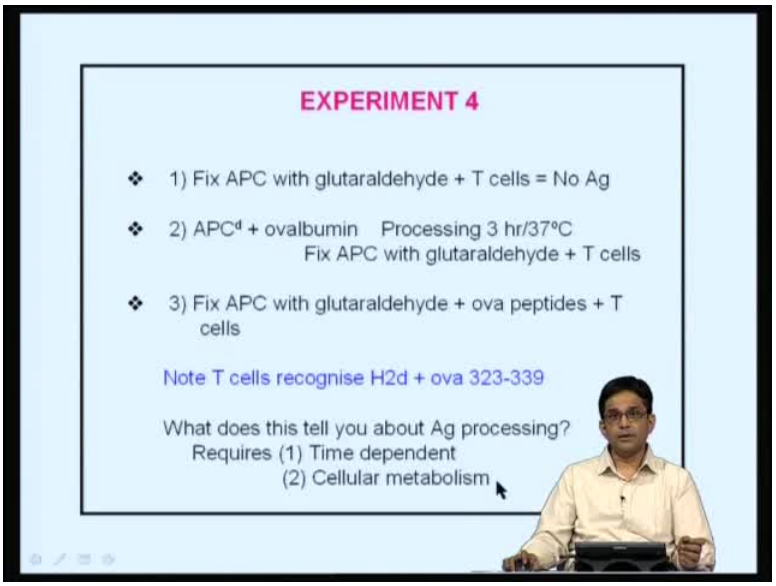
Let us go to the third experiment. So, this is a T cell clone with IA of d, and you have given it APC of f. Now, we had seen that previously, and you would obviously not get an interaction. Now, with the APC of d, you do get an interaction, and that is, actually, the positive control in this experiment. Now, if you take these APCs and you transfect IA of d, which means you transfect IA alpha chain and IA beta chain, and now, you see that it is able to respond, and it is able to respond because now, the APC f is expressing the right MHC.

So, what do these experiments prove? First is that MHC is critical, and there is… the T cell will recognize MHC molecules because they are restricted by it, which means, the T cell receptor recognizes only this particular MHC molecule, and it will not recognize any other MHC molecule, because that is how it has it has been taught and has been educated.

Now, if… what happens when a model antigen, for example, I have put, over here, a chicken ovalbumin is injected into mouse, you first have the antigen uptake. So, it is ingested by antigen presenting cells. The ova peptides or the antigenic peptides are generated. These antigenic peptides need to bind MHC molecules within cells. Now, there are different processes that are involved over here. Very important you have antigen uptake. You have antigen processing, where, from the antigen too, antigenic peptides are generated, and the third important point is antigen presentation, where these antigenic peptides are presented on MHC molecule.

So, very important parts for you all to remember that you have this different process, and you should be able to distinguish the difference between antigen processing and antigen presentation. They are not the same– people often use it in the same way, but there are very distinct process involved here.

(Refer Slide Time: 12:09)



Processing is the way by… is the mechanism by which peptides are generated. Presentation is the way by which peptides are presented on MHC molecules. So, this is

important point that you all should remember. So, we have one more experiment. Now, if you take antigen presenting cells and you fix some with glutaraldehyde, now what does glutaraldehyde do? Glutaraldehyde will cross link all the cell surface protein, because that is what is being done here, and then you put in T cells, and then there is no antigen over here, and you will not get a response.

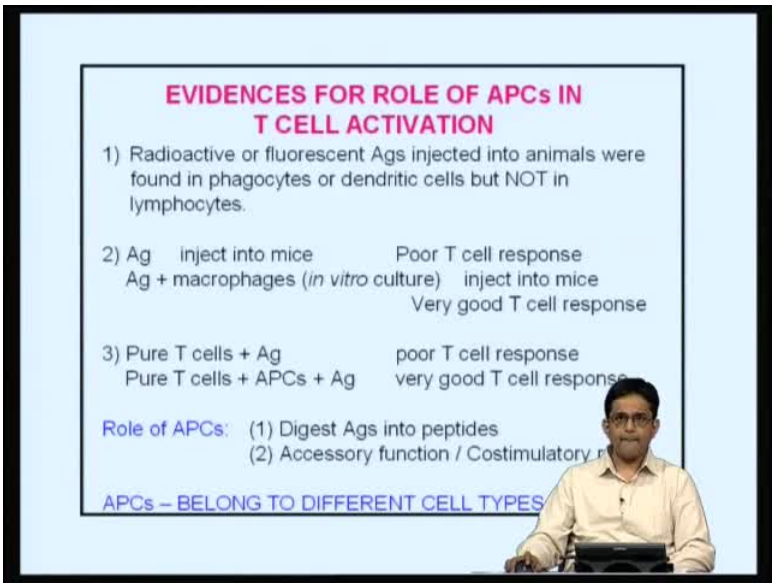
Now, however, if you take the APCs, this is with d, and you have given… put them with ovalbumin, which is the antigen, and we allow for processing for about 3 hours at about 37, now you fix the APCs plus glutaraldehyde with your T cells. You will get a response because these APCs, there is sufficient time given for this, for processing, and at the right temperature also, because the cells need to be able to be viable and to be able to be process it.

The third important point, and so there is no big surprise over here, in the second part, that the T cells are able to recognize it. The third important part is where you fix the… fix APCs with glutaraldehyde. Now, you add the ovalbumin peptides from outside, and now, you add T cells. Again, you will get a response. So, what is happening over here is the ova peptides will bind to MHC from the outside; they may displace off some endogenous bound peptides. So, they will now bind to it, and now, this MHC peptide complex is recognized by your cognate T cells.

So, over here, what is shown over here T cells recognize H2 of d, which is the haplotype plus this particular six-mer peptide that is derived from ovalbumin. So, what this tells you is there are two aspects of antigen processing which are important: first is antigen processing is dependent upon time, because if you do it quickly, maybe sufficient peptides will not be generated.

Third is dependent upon cellular metabolism. So, the cells have to be able take it up and it takes and process this. You have to break it down and then generate this. So, the question is– how are these generated? How are antigens taken up by cells, and how are they broken down into peptide? What is the machinery that is involved in this, and that is really the crux of this class.

(Refer Slide Time: 14:22)



What are the evidences for the role of antigen presenting cells in T cell activation? So, there are several– we will just discuss a few. One is, if you take radioactive or fluorescent-labeled antigens and you inject them into animals, and was found that these are present mainly in phagocytes or dendritic cells, but not in lymphocytes. So, while lymphocytes are the effectors, the actual processing is done by other types of cells, and these are the antigen presenting cells. So, what are some of the antigen presenting cells? So, for example, you have Langerhans cells; you have dendritic cells. Dendritic cells are the most physiological antigen presenting cells and your macrophages, so on so forth.

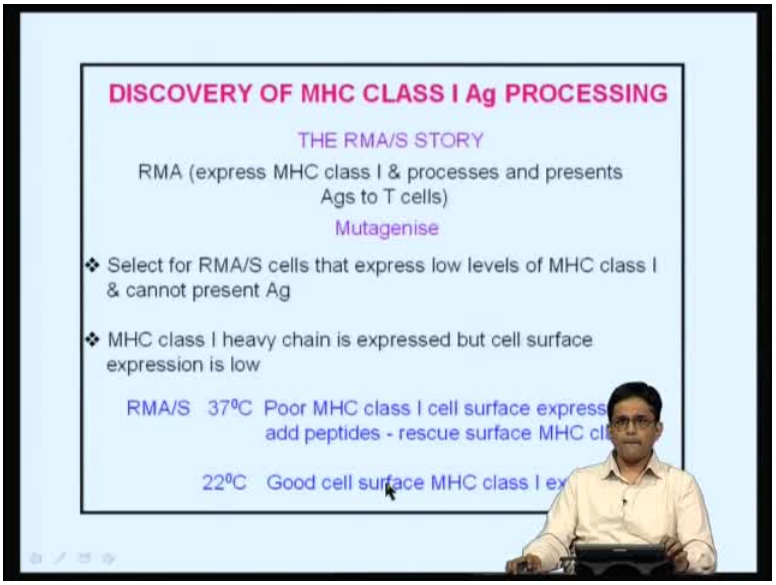
The second is– now, if you take an antigen and you inject it into mouse, you get a poor T cell response now, but if you take antigen plus macrophages in vitro in culture, and you inject it, you get a much better response. So, that is because antigens just by themselves are… you do not get a good response because you are not able to generate it. What you would have to do, over here, is actually take the antigen and mix it with Freund's adjuvant, or some other adjuvant, and you are now able to generate a good, a better immune response, and there are some aspects of this that were covered in the part 1 on innate immunity.

What is also seen is the pure T cells plus antigen. You get a poor T cell response because T cells, by themselves, are not good APCs. However, pure T cells plus APCs plus

antigen, you get a much better response. That is because you have now the APCs that can process this antigen and present it to T cells.

So, the role of APCs, primarily, is to digest antigens into peptides, and b, it has a accessory or co-stimulatory function, which means, you need to send a signal for T cell activation through the specific signal is through the MHC peptide, but you also need– it has to be done in that proper context, and for that, you need a co-stimulatory ligands to be up-regulated, which are recognized by co-stimulatory receptors on T cells, and you get a much better T cell response.

(Refer Slide Time: 16:30)



Now, for the MHC class 1, we will start off with there are basically two stories that I will like to share with you: the first is the RMA S story. Now, what was this was… this happened several years back. RMA is a cell line that expresses MHC class 1, and the scientist who are doing this were trying to look at the N k mediated killing of this particular cell line, and so, this cell line was mutagenized and it was selected for a cell, which is known as RMA S.

Now, this RMA S has low levels of MHC class 1, and it cannot…. So, as a consequence of that, it is a poor antigen presenting cell compared to RMA. So, the wild type was RMA and the mutant that was derived from RMA was RMA S, now, the difference between the two is that RMA S is a poor antigen presenting cell; RMA, the parent, is a

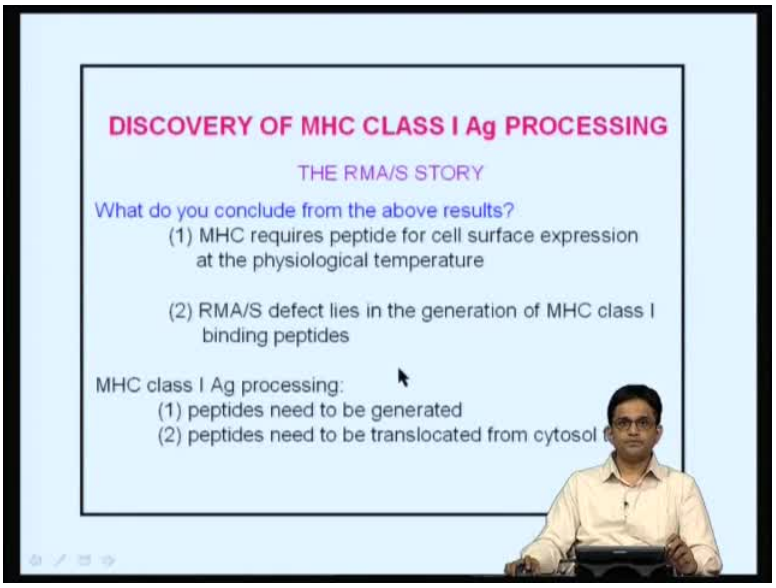
good antigen presenting cell, and why this is needs to be figured out, but what was found is that the MHC class 1 expression on RMA S was low.

So, one obvious response for being a poor antigen presenting cell is that the MHC class 1 response, MHC class 1 expression is low, but here is the interesting part about it: now, RMA S at 37 showed a poor class 1 response, which is class 1 expression, which is what I had said.

Now, however, now, if peptides were added, peptides that bound to MHC class 1, then you could increase the expression of cell surface MHC. So, this is telling you, this is what I had said, that MHC molecules, if they are empty, which is without peptide, they are unstable. They get internalized very quickly; they cannot stay upon cells because the conformation is affected. They are not stable; however, if you add peptide, then peptide increases the stability of MHC class 1 molecules increases their…, and increases their half-life on the cells surface, but the real interesting fact was that if these MHC class 1 molecules were, or if RMA S was cultured at 22 degrees for some time, you got very good cells of this expression.

So, what this says is that there is no… inherently, there is no problem in MHC class 1 expression. So, the MHC class 1 expressed. But somehow, at 37, there is a problem, because at low temperature, where you do not need peptide for very good cell surface expression, the MHC class 1 molecules come up on the surface, and they are stable at low temperature. It is a 37 or the physiological temperature, which is a problem. So, this is a very important point that students should be aware of.

(Refer Slide Time: 19:16)

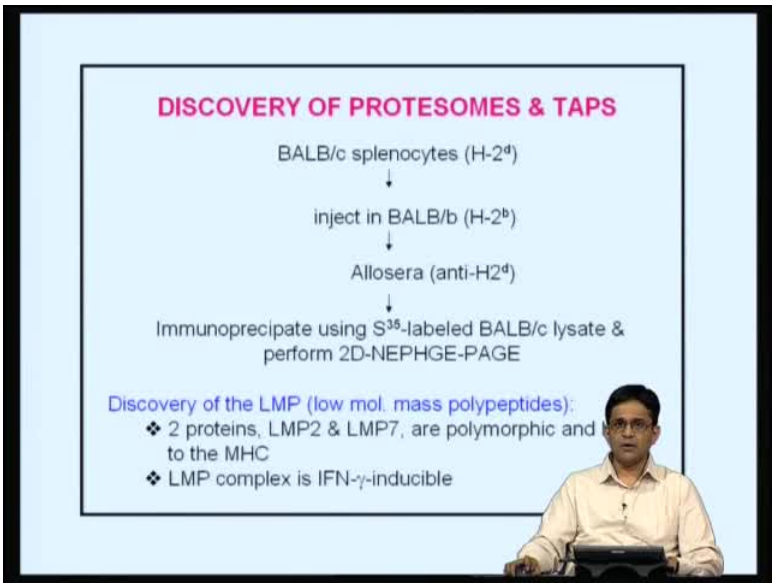


So, this is the main points that I had talked about, so that MHC requires peptides for cell surface expression at the physiological temperature, and the RMA S lies in the generation of MHC class 1 binding peptides, because there is no inherent defect in MHC class 1, because if there were an inherent defect, you would have the problem again at 22 degrees; you do not see that.

At 22 degrees, MHC class 1 expression is fine. It is only at 37, and the fact that you can add peptide and stabilize MHC class 1 expression, suggests that the problem is really with the peptide binding to these MHC class 1 molecules.

Now, here is the thing about MHC class 1 antigen processing– first is, peptides need to be generated, so they need to be generated. So, the question is– peptides need to be generated from antigen. They need to become peptides, so there has to be some machinery that is able to do this.

(Refer Slide Time: 20:47)



The second important fact is that MHC molecules– they start off ER, and then they travel to the cell surface by a process known as vesicular trafficking. So, the peptides, if they were generated in the cytosol, they need to be translocated into the ER, and that is the very important point. So, the peptide generation is probably in the cytosol, and then it needs to be translocated in the ER, and that is where it might bind MHC class 1, and then be trafficked up to the cell surface. So, this is an important aspect that you need to keep in mind when you are undergoing… when you are reviewing this part of the data.

So, now, we will go from here to another set of experiments. So, this is with discovery of proteasomes and the transporter associated with the antigen presentation. So, over here, as I said, there are ways of making allo MHC sera. So, if there are differences, if you have two and this is the advantage of congenic mice, and this is the contribution primarily of the people in the Jackson lab who found out of… George Snell and all, who found out the congenic mice.

So, what happens over here is that you have two strains of mice. They are genetically identical except for one locus, and that locus the MHC. So, if you take BALB c splenocytes, which are H 2 d. They are distinct from BALB d only in the MHC complex. So, if you…So, you would be able to generate an allosera against this, and that allosera will recognize only MHC. So, that is what was done. You have BALB c splenocytes; they were injected into BALB b, and you generated an allosera, and you got H 2. This

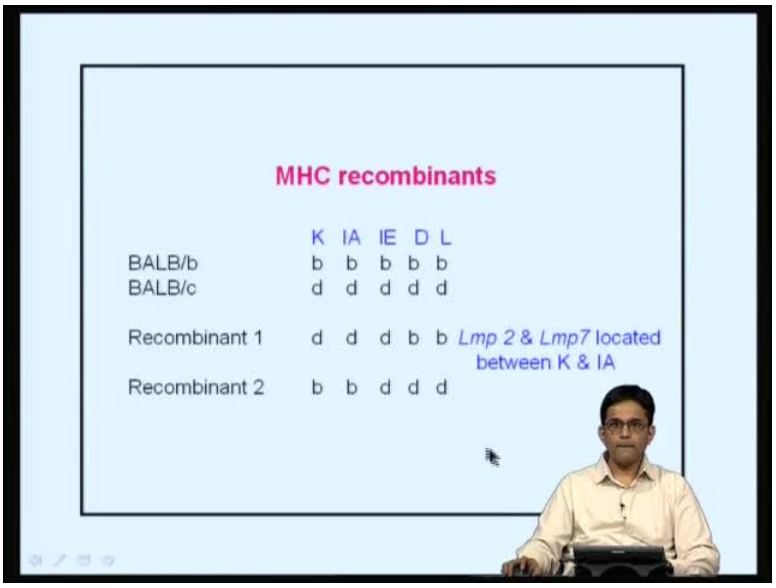
anti H 2 d because the BALB b generated antibodies that were different from BALB c, and the only place where the differences were, were over here. So, these were immunoprecipitated, and you perform 2D NEPHGE PAGE. This is long time and NEPHGE stands for non-equilibrium pH gradient gel electrophoresis. So, what the way… what this does, is that you have a ampholine gradient. You separate your immunoprecipitated proteins based on pI or approximate pI, and then you separate them based on molecular weight.

So, there are two ways of separating– one is the pI, and the other is the molecular weight. So, this is different from IEFs because it is non-equilibrium, because you are not reaching the pIs of all proteins, but the acidic proteins are primarily reached. So, what this done… what this did was, once it was… once this sort of experiments were done, what was found is you had the MHC, your typical MHC class 1 and class 2 molecules were found out. But, much to the surprise, what was found is that you had several low molecular mass peptides, polypeptides, being shown up.

Now, the fact that you generated this response against these this mouse, and the only difference was in the MHC, suggested the allosera was recognizing something that was there in the MHC, but was distinct or between BALB c and BALB b. So, and two proteins– two small or two low molecular mass peptides were identified. This is Lmp2 and Lmp7. They are polymorphic; they were linked to the MHC and the LMP complex, as such, are interferon gamma inducible. So, these are hall marks of something to do with the MHC, and it is clearly important because MHC plays such an important role.

What was interesting about this whole thing, was that it turned out, or at least gave the appearance at this point, that they were not typical MHC class 1, MHC class 2. They were some other proteins that were linked to the MHC. They were interferon gamma inducible; they were polymorphic, suggestive of something important.

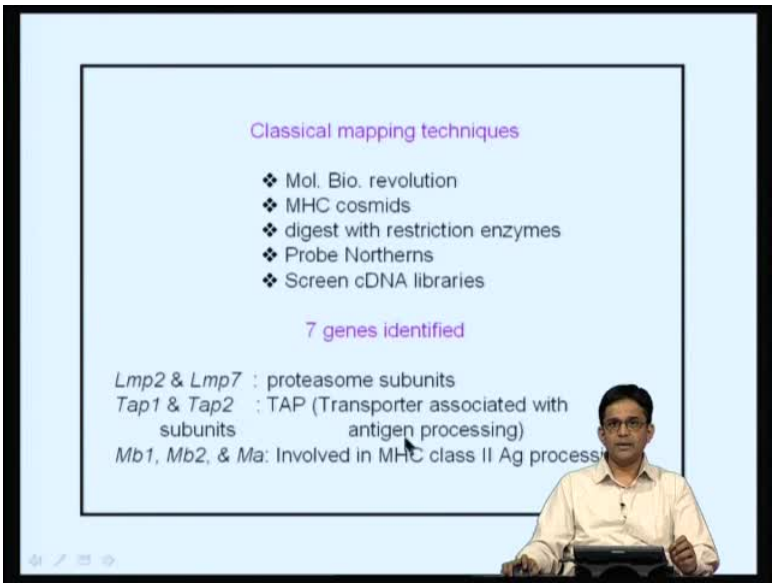
(Refer Slide Time: 24:20)



So, further experiments were done to fine-map it, and as I said, what if you look at BALB b? The haplotype that you get is this– is b b b, and this is… this is the MHC class, MHC regions. So, you have the K and then you have the MHC class 2 region, and then you have D and L, which are the… which is the MHC class 1, and you remember in mouse, I had mentioned that the K is centromeric to the MHC class 2, which is shown over here.

Whereas, in BALB b, you have, all this is d d d. Now, if you have recombinant between this part, this region and this, and you have another recombinant, that is recombinant between this and this, you had this different lines that were recombinants; you can actually map where the location is using this antisera, because the antisera specifically recognize the d and not the b parts.

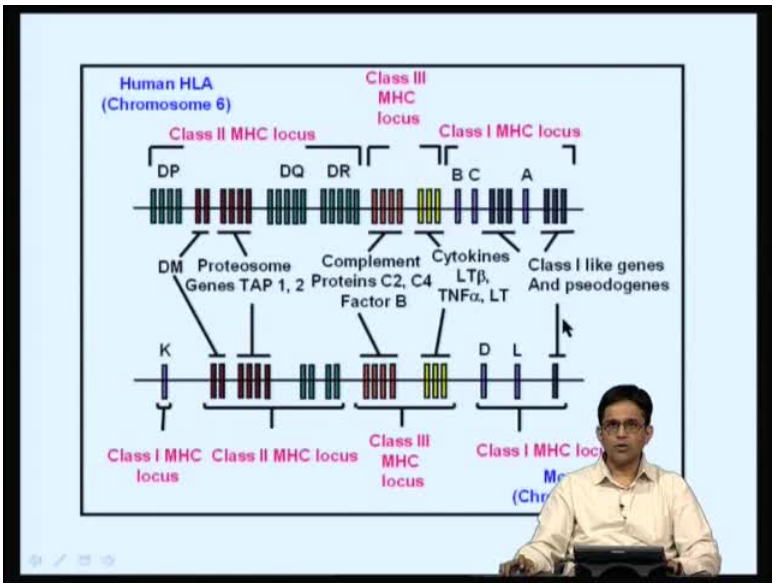
(Refer Slide Time: 25:30)



So, using these sorts of experiments, what was found is that those polymorphic proteins were located between this region, which is the K, and the IA region. So, they were centromeric with the MHC class 2 region, and so, the fine localization of these molecules where done. So, that was certainly very interesting, and then after the general gross localization, the genes had to be found out, and subsequently, the molecular biology revolution took over. You had cosmids– they were digested with restriction enzymes, northerns were done, which so to look over expression, and then cDNA libraries were screened and seven genes were identified.

So, in a small part, seven genes were identified. All these seven play an important role in the MHC class 1 and class 2 antigen processing. This is the important part, because the presentation part were already found out. So, Lmp2 and Lmp7– these are proteasome subunits; TAP1 and TAP2– this is a transporter associated with antigen processing, and we will be discussing more about these, subsequently, and you have the Ma and Mb genes, which are involved in MHC class 2 antigen processing.

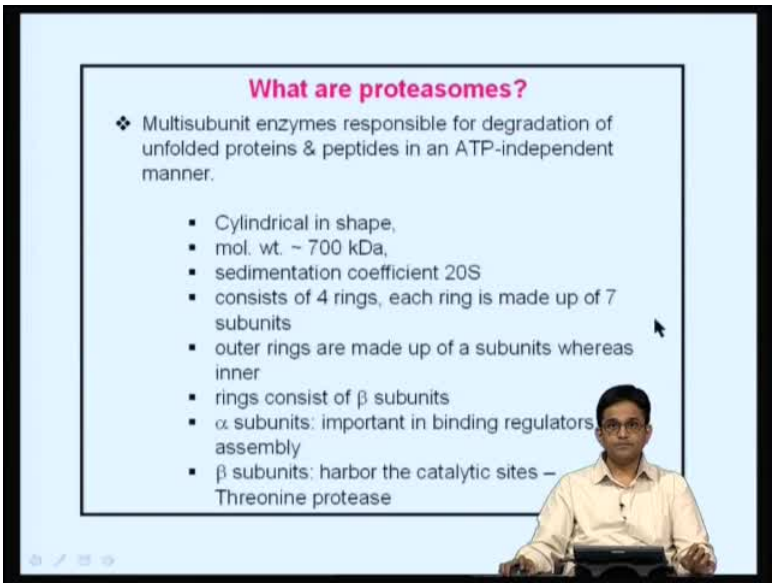
(Refer Slide Time: 26:48)



So, this is the amazing part. So, you have this particular region between the K and the IA, which encoded the seven genes, and all the seven plays such important role in antigen MHC class 1 and class 2 antigen processing and presentation. So, what are these proteins are encoded by this genes? So, this is what I am saying.

You have the K and you have the IA part over here, and these are with the genes. This is the DM and this is with the proteasome, and the Tap genes are and even in the human, it is in the MHC class 2 locus. You have the… between DP and the DQ. You have these genes sets, so it is very important that you know. So, it is actually in the MHC class 2 region, and but we are encoding genes that are important MHC class 1 antigen processing, as well as MHC class 2 antigen processing.

(Refer Slide Time: 27:22)

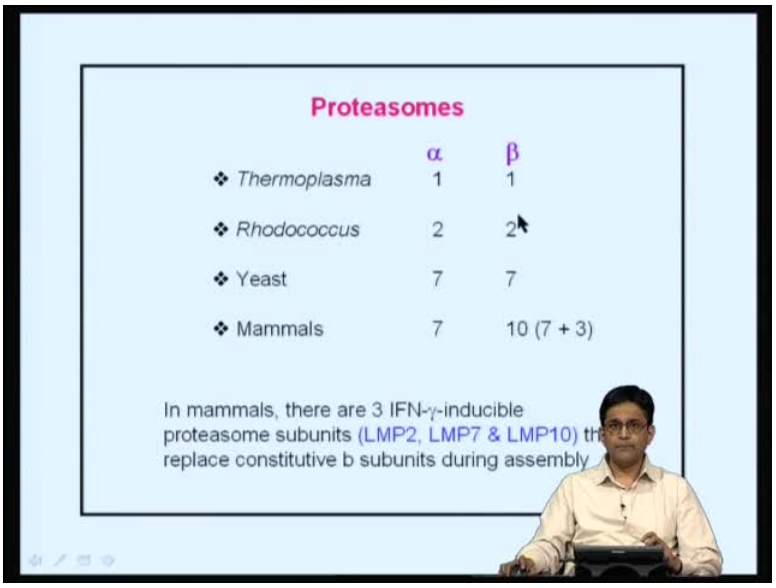


So, now I had said that there are two components which were…, which were subunits of proteasomes. Now, what are proteasomes? So, these are the enzymes that are primarily responsible for cleaving antigen proteins and then churning out peptides. So, these are the… I had previously said that you have antigen, and then we need to generate peptides.

So, the proteasomes are the ones that are responsible for that. Once these peptides are generated, they need to be translocated into the ER, and that is done by the transporter associated with the antigen processing, that is, the TAPs. So, you have two main components over here– the proteasomes and the TAPs. Right now, we will study a little bit more about the proteasomes.

So, they are cylindrical in shape; the molecular weight is about 700 kDa; the sedimentation coefficient is about 20S; more importantly, it I consists of four rings. Each ring is made about seven subunits. So, you have the alpha subunits on the outside, and the beta in the center.

(Refer Slide Time: 28:46)

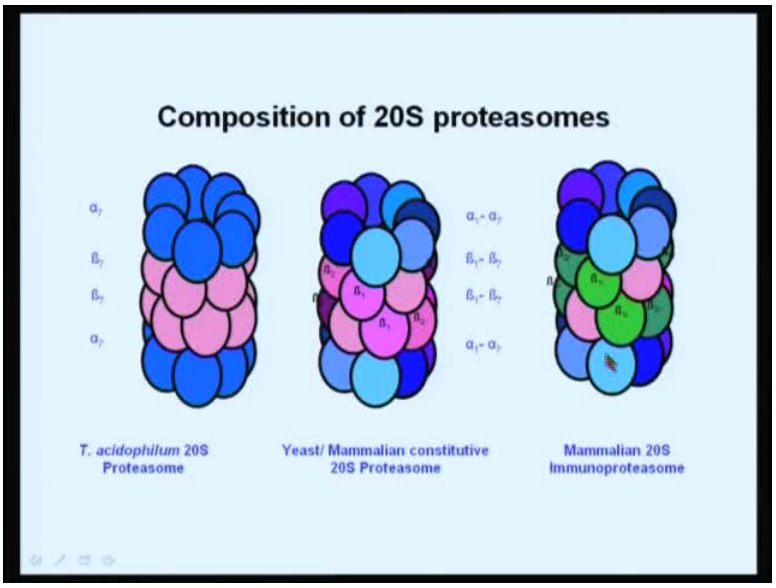


I will be showing the pictures of proteasomes, so you will have a better idea, but the alpha subunits are important assembly, whereas the beta are important for catalysis, and they are the catalytic threonine is the… or the N-terminal threonine is really the important residue over here.

Now, and this is just a little bit to inform you about proteasomes, proteasomes are present in thermoplasma, which is an archaebacteria, but you have a single alpha and a single beta subunit, but the quarternary structure of proteasome is conserved from archaea to mammals. So, even though there is a single subunit, what is happening is, you have seven subunits that make up the thermoplasma 20S proteasome. So, you have the seven alpha, seven beta, followed by the seven beta, and then seven alpha, because the alpha are the two ends, but it is the same subunits over here.

In Rhodococcus, you have two each; in yeast, you have seven distinct ones. So, the seven subunits are encoded by seven distinct genes– seven alpha and seven beta. In mammals you have seven alpha and ten beta. This is, this was a surprise, and this ten can be divided into seven that are constitutive and the three that are extra, and these three are the interferon gamma inducible ones.

(Refer Slide Time: 30:30)

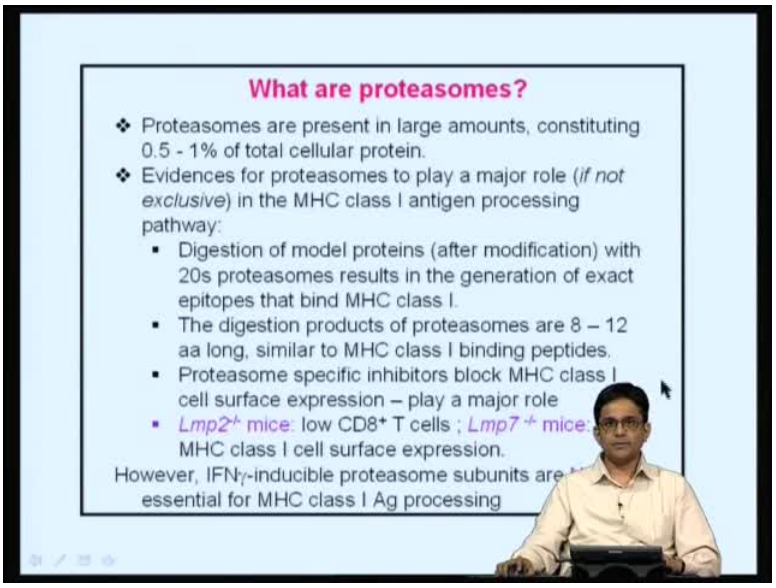


So, in the 20S proteasome subunit composition, there are three of them which are interferon gamma inducible. What is interesting is, two of those are MHC encoded, which is Lmp2 and Lmp7, and so why do you think that this should be interferon gamma inducible, and how would it be important in making up the assembly and part of the… and the importance of it in MHC class 1 antigen processing. You should be thinking a little bit about this, and also the fact that they are polymorphic may give some clues. This is coming back to what I meant about the structure of the 20S proteasome.

So, these are the seven– this is the thermoplasma one, which has the same identical alpha subunits of the ends and the beta subunits. You can see that there are seven alpha, seven beta, over here. If you just look at a glance, you can see three proteasomes. The quarternary structure is conserved from archaea to the mammalian ones.

This is the yeast one, which has seven different alpha subunits and the seven different beta, and if you see, the way the subunits are oriented, they are slightly off each other. So, there is a slight difference in their… and this is the mammalian 20S proteasomes. They are also different. What was shown is that the three… for the three interferon gamma inducible ones, you have 3 constitutive ones. So, it is… the subunits can go in only and take in, only replace particular constitutive subunits. So, they cannot just move in and replace any and get incorporated with any place; they can be incorporated only at a particular place. So, that is an important aspect.

(Refer Slide Time: 31:43)



This is a rather busy slide, but proteasomes are present in large amounts. They are important for protein degradation, protein homeostasis, and I will also show that proteasomes are part of the machinery which is known as the ubiquitin proteasome pathway.

So, proteins that are distinct for degradation, they get ubiquitinated. So, that is a tag that put on. These ubiquitin proteins are recognized by proteasome complexes, by 26S proteasome complexes, and these are degraded into peptides. So, you have amino acids that are recycled. It is a way by which protein homeostasis takes place. Older proteins get turned over, so on and so forth, and so, what the immune system has done, it has taken an existing pathway and incorporated it for its own needs. So that…So, you know it has not come up with the original pathway to develop peptides, and it is come up with the preexisting pathway that… evolutionary pathway that is used for protein homeostasis.

So, what are the…, but since this class is mainly on MHC, we will be restricting our understanding of proteasomes to the immune response. So, if you take model proteins after modification, give them to 20S proteasome, they generate the exact epitopes that bind MHC class 1, and the digestion products of proteasomes are 8 to 12 amino acids, which is similar to MHC class 1 antigen peptides.

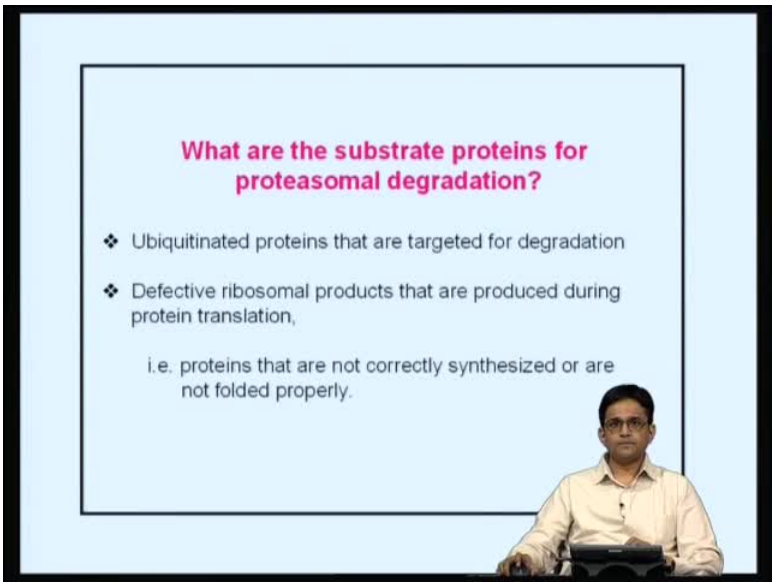
So, this is important. Now, what proteasomes do is they do not chew up the peptides into single amino acids. So, they generate peptides, and if these peptides are able to bind

MHC class 1 molecules, then they are stabilized. However, if they do not bind, then these smaller peptides are degraded by aminopeptidases and carboxypeptidases within this cell. So after this peptides are generated, if they bind MHC class 1 only, they are stabilized. Now, proteasome-specific inhibitors block MHC class 1, and they block MHC class 1 because the MHC molecules need peptide binding. After they bind to the peptide, their structure is made more stable and it can now egress.

So, in the absence of peptides, MHC molecules are unstable and they, sort of, tend to remain in the ER Golgi, and subsequently, specific knockout mice were generated. So, you have the Lmp2 knockout mice, and these express low CD8. So, low CD8 would mean that there is some problem with selection with MHC class 1, and the MHC 7 knockout, you have low MHC class 1 cell surface expression.

Now, what is important, over here, these mice are available, and the interferon gamma inducible producing subunits are not essential. If they are not essential, because you have constituted subunits, what these interferon gamma inducible subunits do is when an immune reaction is taking place, you have more of these subunits, and so then by law of mass action, you have more of these. So, the chances that they get incorporated into proteasomes as they are being assembled are higher.

(Refer Slide Time: 35:12)

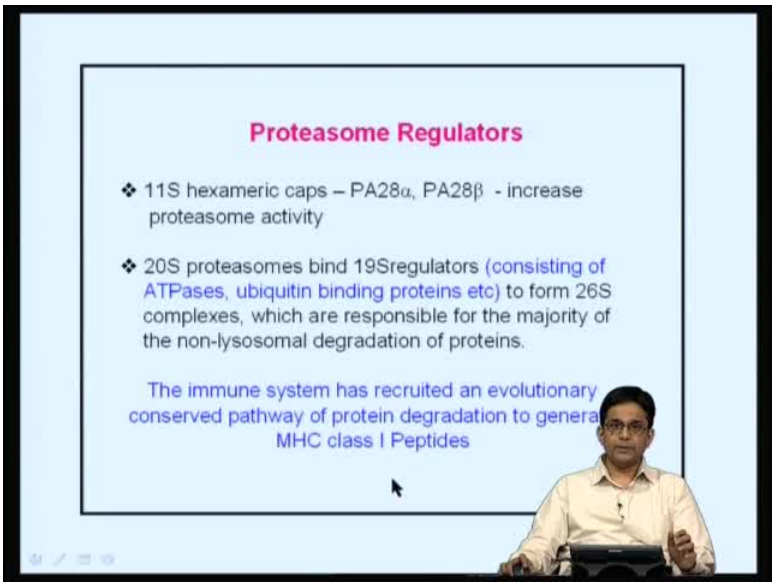


So, one needs to understand that aspect. So, what are the substrate proteins for proteasomal degradation? First is, as I mentioned, ubiquitinated proteins that is part of

the normal pathway. So, as for what the cell biology the method is if you want to target proteins for degradation, you… the proteins often get ubiquitinated.

The other is– while proteins are being translated, you have these products known as the proteins that are not properly formed, and those are defective ribosomal products or DRiPs, and DRiPs– D R i P s– these are distinct for degradation. And again, the proteasomes play an important role in this. So, what often happens is the MHC molecules end up showing peptides that arise from drips. So, again, an important point.

(Refer Slide Time: 36:01)



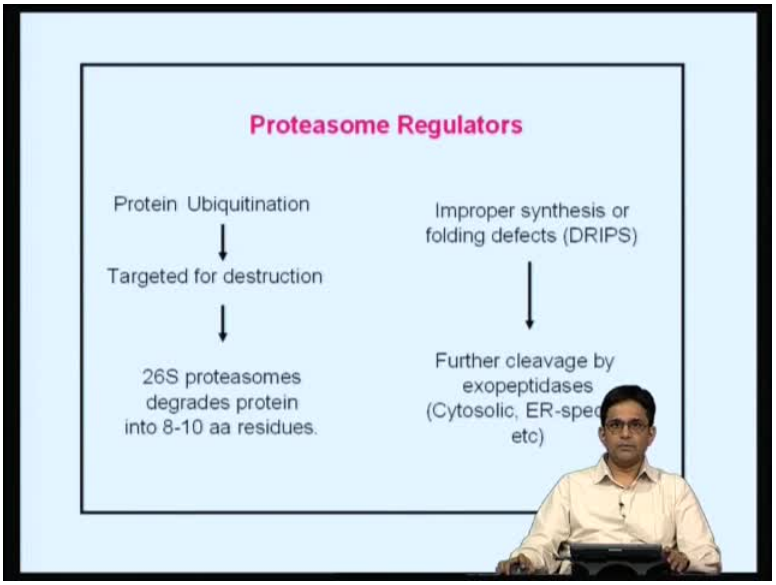
Now, you have proteasomes, which I showed you were consist of the four rings. Now, these proteasomes are regulated by other molecules. So, you have the PA28, and now, what these are the activators? What PA28 alpha and beta does is that it increases proteasome activity, and so, as the consequence, the… you know proteasomes will cleave peptides, or will cleave proteins lot faster.

The other more physiologically important one is the ubiquitination process, and the 20S proteasomes binding to 19S regulators. Now, these 19S regulators consists of ATPases, ubiquitin binding proteins, to form 26S complexes, and it is the 26S complexes, which are responsible for majority of the non-lysosomal degradation of proteins.

So, the important point for students is that the immune system has recruited and evolutionary conserved of pathway protein degradation to generate MHC class 1

peptides. So, this is an important point– they have made some changes in it. They have some genes that are involved. Newer subunits that are evolved along with this, but otherwise, they have used a common pathway. That is an important path point.

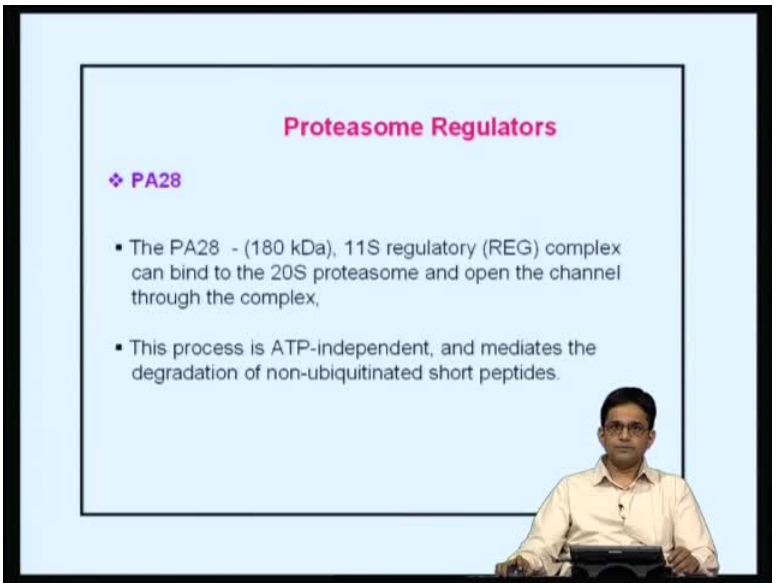
(Refer Slide Time: 37:24)



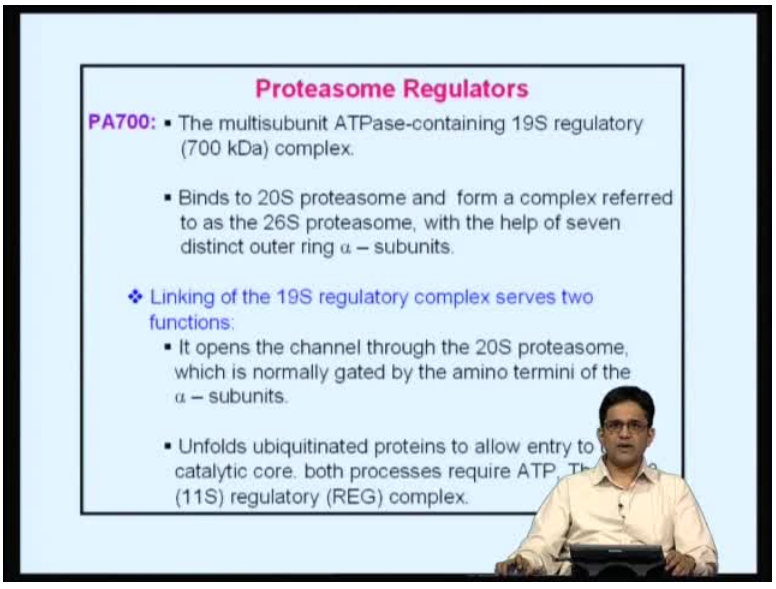
So, this is what is shown over here– there are two parts you have protein ubiquitination the targeted for destruction and 26S proteasomes degrade proteins to 8 to 6. Now, only these peptides bind to MHC. Then, they become stable because what peptide binding to MHC does is rescue it from degradation.

The other way as I mentioned was the DRiPs– the defective ribosomal products, and so, these are further cleaved by exopeptidases and all, and then, they are put up on… they are shown and they bind to MHC molecules.

(Refer Slide Time: 38:04)



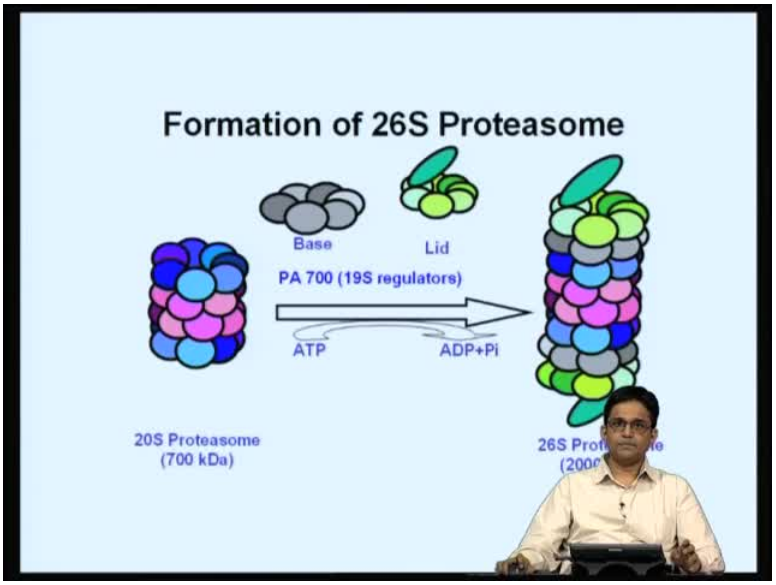
(Refer Slide Time: 38:13)



PA28, as I said, is a regulated in increases the activity of proteasomes. So, the cleavage is lot faster. So, this is PA700, which is the 19S regulator. Now, what it is important to understand that because what ones the 19S regulator bind, it forms a 26S complex, and what it does, it opens up the channel binding of the 19S regulators to the 20S complex opens up the channels, so that which is normally gated, so that you can have, now, proteins that enter into the proteasomes. Remember, these proteins need to be… cannot be folded, so they need to be unfolded, and unfolding requires ATP, and then it enters the proteasome from where they are cleaved.

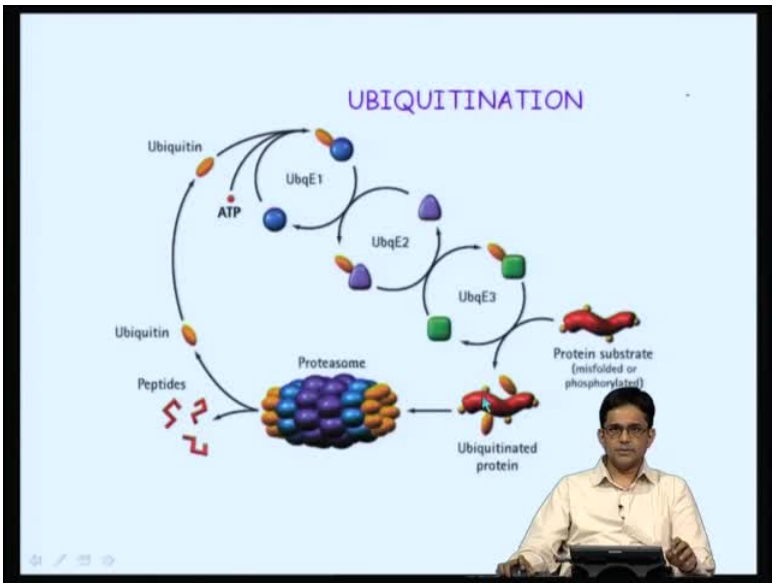
And so, this is an important aspect, and the unfolding of proteins require ATP. Also, the ubiquitination process by which you know proteins get tagged on to ubiquitin, needs ATP. So, this process needs ATP. Per se, peptide or peptide generation, that is the breakdown of proteins into peptides, does not need ATP. For example, DRiPs cleaves without ATP, but however, because of the unfolding process that is involved and the ubiquitination process, those need ATP, and therefore, it is a ATP dependent process.

(Refer Slide Time: 39:37)



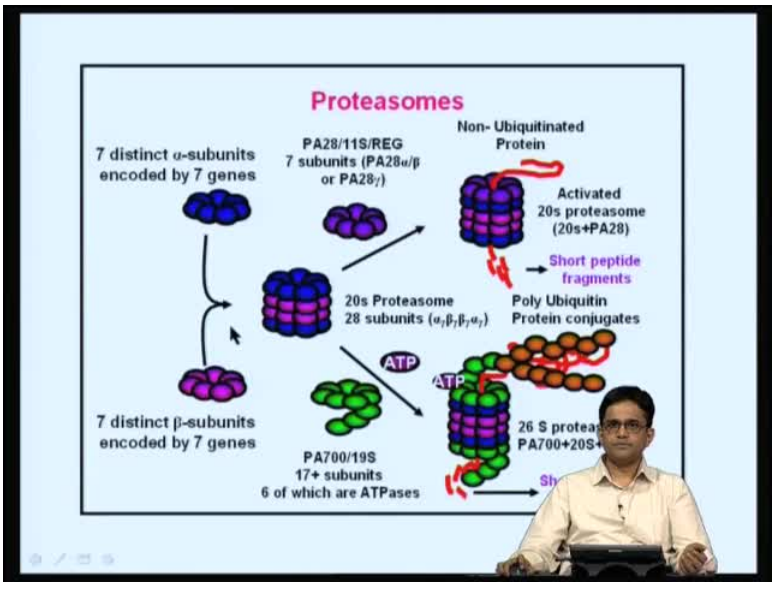
So, this is a cartoon just to show this is the 20S proteasome. As we mentioned, and this is the 19S base, and this is the lid, and you have this in the presence of ATP. You have the 26S proteasome, and it is the 26S the proteasome which is responsible for the ATP and ubiquitin dependent degradation of proteins.

(Refer Slide Time: 39:59)



So, this is what is shown with respect to ubiquitination. You have ubiquitin which gets linked up with different enzymes– the E 1, E 2, E 3. Ultimately, you have this is the protein substrate, which is misfolded or phosphorylated, which needs to be targeted. And so, this gets ubiquitinated, and this is shown over here. It gets poly ubiquitinated. This poly ubiquitinated protein is recognized by the 19S and it is unfolded, and then the proteasome then cleaves it into peptides, and these peptides can either bind MHC class 1 molecules, or they can be degraded by aminopeptidases and carboxypeptidases into amino acids, and that can be recycled back for protein synthesis.

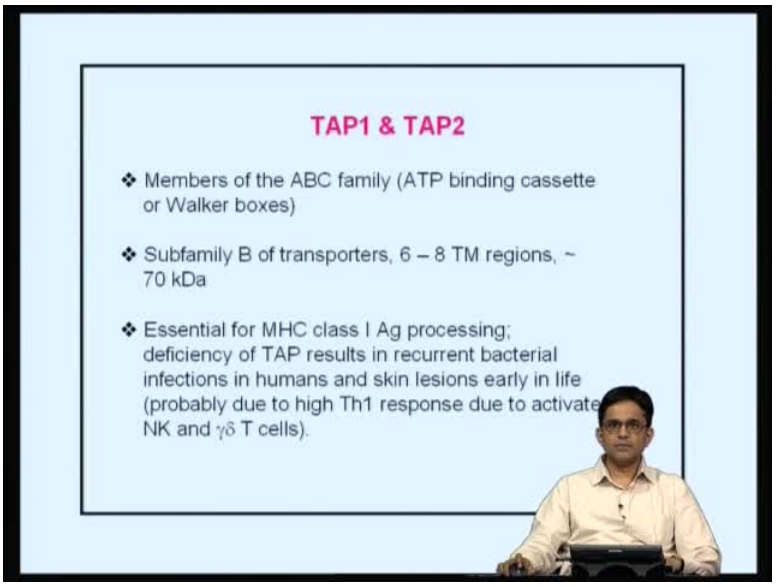
(Refer Slide Time: 40:42)



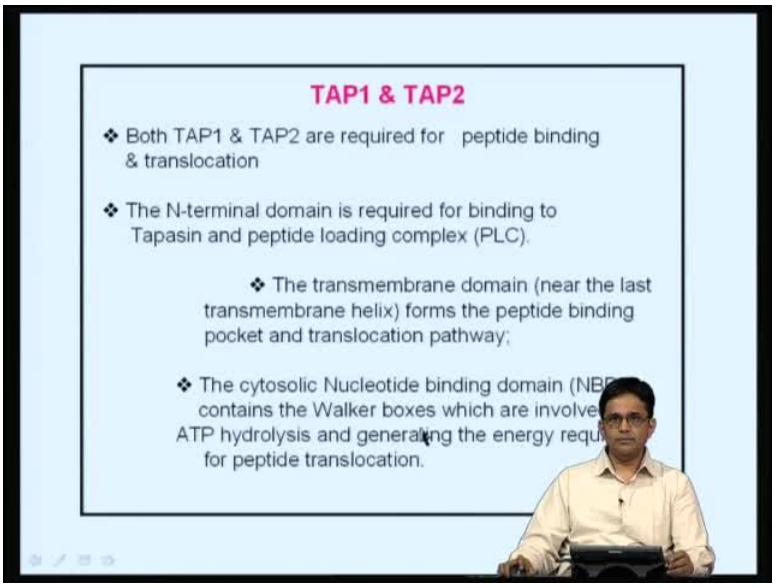
This is more of that different cartoon, again, to show proteasome assembly. You have the alpha subunits, and you have the beta subunits. They come together to give, to form the 20S proteasome. This 20S proteasome can now bind to the regulators known as the PA28, and so, once these bind to it, you will have generation of more peptides, because what PA28 does is, it is a positive regulator of proteasome activity, and then, this is the other one which is more important, which is the 19S regulators. What 19S regulators do is to bind the poly ubiquitinated proteins, and those poly ubiquitinated proteins are unfolded. Ubiquitin is removed from them; they are passed on to proteasomes, and they are… Now, these proteins are now degraded into peptides.

So, this part refers to the generation of peptides for MHC class 1 antigen processing. Now, students should be familiar with the way an antigen is taken up, and how it is broken down into peptides, and some of these peptides can bind MHC class 1 molecules.

(Refer Slide Time: 41:49)



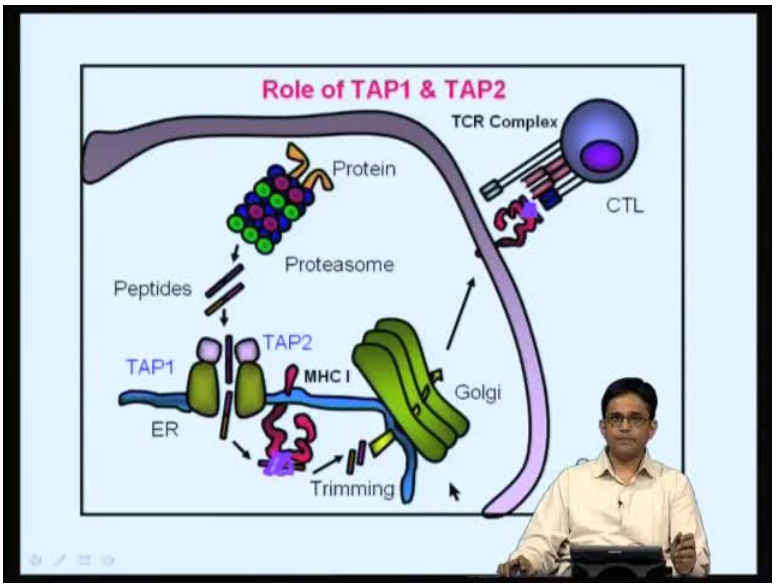
(Refer Slide Time: 42:39)



So, once peptides are generated, they need to be translocated from the cytosol into the ER, because the MHC class 1 molecules are present in the ER, and so, this is done by members of the ABC family or the ATP binding cassette family, and there are… So, they are about 70 kDa protein, and these are essential for MHC class 1 antigen processing. So, if there are peptides that are generated, but the peptides cannot be translocated into the ER, then you will have… then they will not be able to bind the MHC class 1 molecules, and they will be stuck over there.

So, they are absolutely essential that peptides that are generated are translocated into the lumen of the ER. There, it binds to MHC class 1 molecules now both TAP1 and TAP2 are required for peptide binding and translocation, and what is shown is the N-terminal domain is required for binding to tapasin, which is a sort of a linker molecule between the TAP and MHC class 1 molecules, and part of the larger complex of proteins known as the peptide loading complex.

(Refer Slide Time: 43:19)



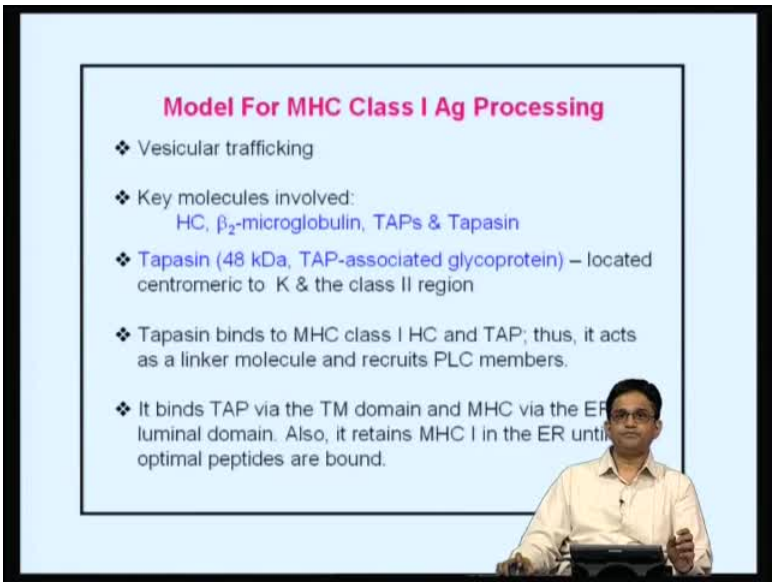
The cytosolic… The nucleotide binding domain containing the walker boxes are involved in ATP hydrolysis. Now, for peptide translocation, you need ATP, and that is why you need the walker boxes that are an important part of this. And so, this is a cartoon to show how this is all done. So, let us say, this is a particular protein, and you can see, this particular protein– it is degraded by proteasomes to generate the peptides. Now, obviously, students need to understand this is not done to scale. The peptides are then translocated from the cytosol. They go through the TAPs, transporter associated, and over here, they undergo some trimming also by some other molecules, by some other peptides, the ER specific peptides that are present, and then and bind on to MHC class 1 molecules.

And then, these are then… MHC class 1 molecules then egress ER Golgi, and then they move on to the cell surface. Now here, it is at the cell surface. What is shown over here, now, the MHC class 1 molecules have peptide on them and they are free to present peptides to cytotoxic T cells or CTLs, as shown over here, and again, the recognition that is shown is you have the T cell receptor complex binding to MHC peptide. So, this has overview of the pathway by which MHC class 1 molecules… the MHC class 1 pathway works.

First is, the proteins is need to be generated into peptides. The peptides in the cytosol need to be translocated via TAPs, so proteasomes are important, transporters are

important. Over here, there is trimming involved by ER-specific aminopeptidases and known as ERAP, and then you have the peptide binding to MHC class 1, and then, the MHC class 1 molecules then egress and travel to the cell surface. It is on the cell surface that you have the MHC molecules that can present, that show these peptides to T cells, and you can get a T cell activation pathway being initiated.

(Refer Slide Time: 45:22)

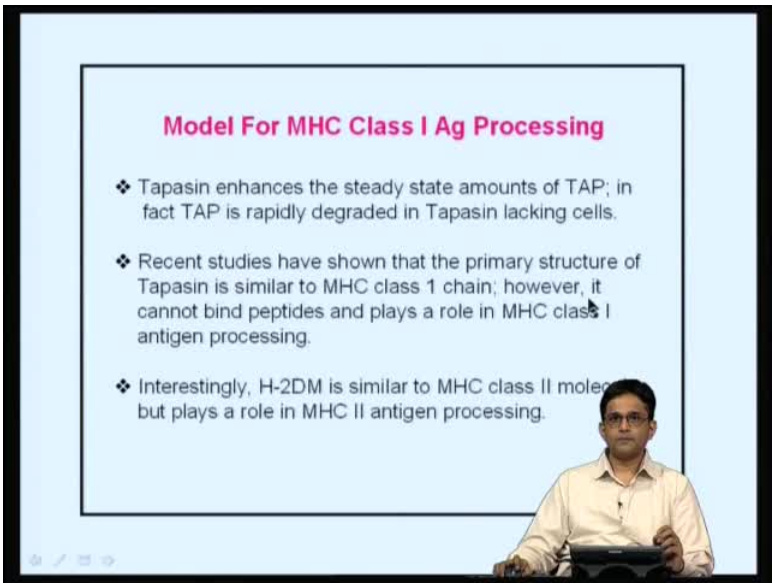


So, let us go over the model for MHC class 1 processing– vesicular trafficking. So, what do, we define by vesicles. So, MHC molecules are present in ER, and they travel from the ER Golgi to the cell surface as part of vesicles. So, that is the vesicle a pathway that we are talking about.

The key molecules involved are heavy chains, beta 2 microglobulin, because those two are form the MHC class 1 molecules. You have transporters associated with antigen processing, and you have tapasin– tapasin is the linker molecule. It is a 48 kDa linker molecule, and again, what is interesting is the location of tapasin, is that it is centromeric to the K region, into the H 2 K in the MHC.

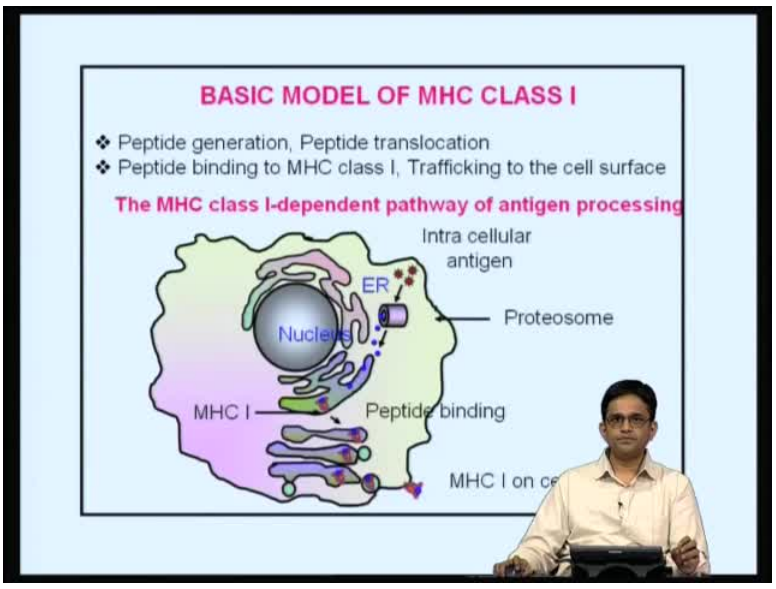
So, centromeric means it is further, it is away from the MHC, and it is on the other side. So, tapasin binds to MHC or class 1 heavy chain and TAPs. That is why I said is a linker, and it binds to TAP via the transmembrane domain, and MHC via the ER luminal domain. It retains MHC class 1 in the ER until optimal peptides are bound.

(Refer Slide Time: 46:43)



Tapasin enhances the steady state amounts of TAP, so the TAP amounts are increased in the presence of tapasin, and what is shown is that tapasin is sort of simulative MHC class 1, but it cannot bind to peptides.

(Refer Slide Time: 47:22)

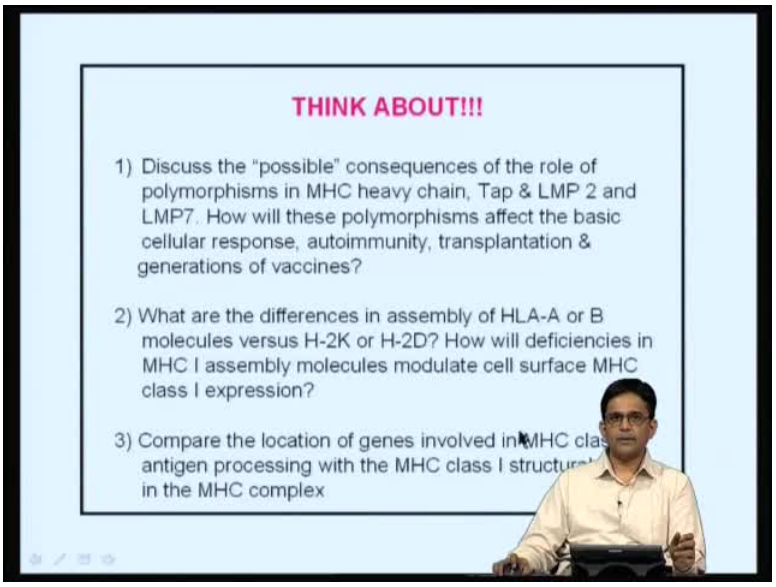


H-2DM is also somewhat similar to the MHC class 2 molecules, but again, it cannot function as MHC class 2. So, this is the basic model of MHC class 1. So, you have, first, the peptide generation and the peptide translocation. Peptides need to be generated; they are generated by proteasomes. Peptide translocation– they are generated by TAPs–

transporters associated with antigen processing, then peptide binding to MHC class 1, and then, you have trafficking to this cell surface.

So, this… that is again, sort of, shown. You have your intracellular antigen– it gets cleaved by proteasomes. You have peptides being generated, so they enter these peptides, are translocated into the ER Golgi, and then, you have the MHC class 1 molecules. These end up by vesicular trafficking on the cell surface, and now you have MHC on the cell surface.

(Refer Slide Time: 48:14)



So, now this is some parts that I would like you to think about, and discuss what are the possible consequences of the role of polymorphisms in the MHC heavy chain– TAP, Lmp2, and Lmp7. Now, what was shown, or what was discussed, is that the MHC heavy chain is highly polymorphic.

In mouse and humans, the TAP and the proteasomal subunits are not polymorphic. So, there are some polymorphisms, but they are not incredibly polymorphic, the way MHC heavy chain is, but how would the polymorphisms in the TAP and the proteasome subunits affect our ability to respond to an immune response?

If you just think about it, MHC molecules will bind to peptides– different kinds of peptides, and then, ability to bind different peptides is because of the polymorphisms that are present in the MHC. So, these different polymorphisms give it the ability to bind

different types of peptides. Now, if you have some polymorphisms in the TAP and in the proteasome subunits, how would that affect?

So, first is– polymorphisms in the proteasomes subunits might result in alterations in the kinds of peptides that are being generated. Now, in a cell, if you have polymorphic 20S proteasome molecules, you have different types of peptides being generated. So, that allows for that.

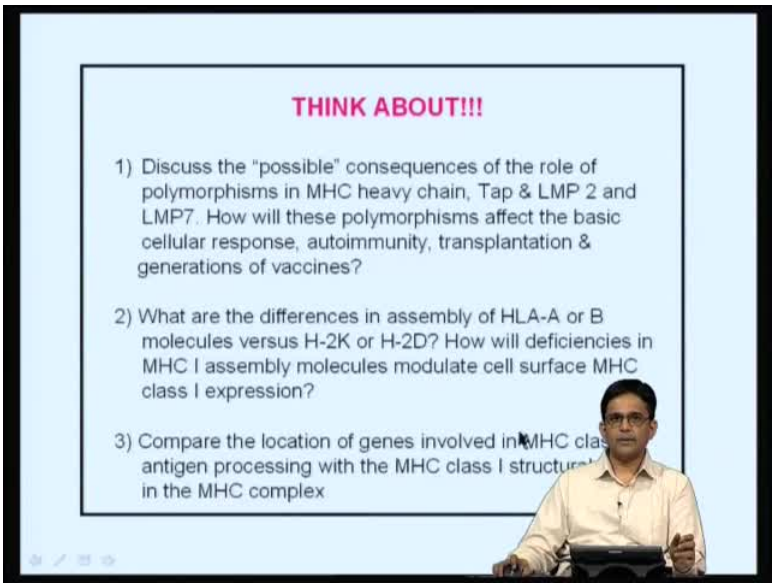
So, you have different types of peptides, and now, if you have TAP being polymorphic too, you… so the different polymorphisms may allow for different set of peptides being… to be translocated. So, some TAP molecules with the particular polymorphism may not allow some peptides translocated, whereas others may allow it. So, this allows for it, and then, finally, you have different MHC molecule, where… which has the capacity to bind to different peptides that are generated, based on what type of peptides are generated and what type of peptides are translocated.

Remember, antigenic proteins need to be degraded first, and so, proteasomes play an important role over there. Subsequently, they need to be translocated. So, if peptides are not generated or are being generated, the peptides are not being translocated or are being translocated, they will, finally, affect the ability of the different types of peptides to bind to MHC molecules, and that is something that you should be certainly aware of.

Now, and obviously, this would affect our ability to respond to different scenarios. So, that is why you have some people who are highly susceptible to certain organisms, whereas others are not susceptible. Perhaps, you know the different polymorphisms allow us with these huge variations in our responses. So, that is one very important aspect that students should be thinking about.

The second is the fact that the MHC molecules are… there is a difference between the trafficking of human MHC molecules and mouse MHC molecules. By and large, mouse MHC molecules, even if they do not have peptide bound to MHC molecules, they will traffic to the cell surface, they will traffic, but they will unstable. They will be quickly internalized, and that is why, in mutants of TAP, you have low cell surface expression, but it is not absolutely absent.

(Refer Slide Time: 48:14)



So, you have low because the MHC molecule egress to the cell surface are internalized rapidly because they are unstable, but they will go there. By and large, human MHC molecule are somewhat different, in that in the absence of proper peptides, they will be stuck in the ER Golgi, and they will not even go over there, by and large. And since, again, both human and mouse molecules are both polygenic and polymorphic, you have differences, but what I am saying is, by and large, a scenario.

So, now you can see that there are, obviously, apart from TAP, apart from proteasome, you have other players in this, especially in the peptide loading complex, and that is some of the other players like calnexin and calreticulin are the ones that I will talk about a little bit in the next class, but certainly, you should be somewhat aware of this, this broad generalization, by which MHC molecules– mouse MHC molecules, will egress to the cell surface. Even the absence of peptides and…, but they will be unstable because… and that leads to low cell surface expression. The human ones– they would not even egress there, because they become stuck in the absence of it. By and large, HLA molecules will not go up.

What you should do is to compare the localization of the HLA and the H2, and look at the different genes in the MHC little bit more closely, and then try and see where the TAPs, the DMs, and the proteasome subunit genes are localized in the human versus the mouse MHC, also look at the differences in… difference in genetic organization of these

two, and I think that will be something very useful. But most importantly, what you should be thinking about is the way we have evolved, so that you have at least human and mice, where you have, primarily, you have differences in the MHC molecules, and you have polymorphic MHC molecules, so that they can bind different types of peptides.

Now, apart from this, you have also polymorphisms in both in the proteasome subunits and as well as in the TAPs. So, you can see variations of these molecules. You can understand the role of these variations– the genetic variations– which will affect function of these different proteins, and it will affect function, in the sense that certain peptides may be generated or may not be generated, and if these are generated, they may be translocated or not be translocated.

So, you can see, ultimate peptide binding to MHC molecules is a consequence of different processes. First is antigen presented… antigen processing, by which they are broken down into peptides; then translocation; and then, final binding. Remember, all the peptides that are generated or all the peptides that are translocated may not bind, because again, once peptide binds, some may be very low affinity peptide, which will be competed out by the higher affinity peptides. So, ultimately, binding will depend, and also the binding of the MHC binding groove with the particular peptide.

So, you have different processes over here. Ultimately, the highest, or the ones with the greatest affinity, will bind into MHC molecules; will go to the cell surface and be presented, and what… Over there, we will need to have some sort of T cells that can recognize and peruse it, and if you are able to have a particular T cell specific for this, and especially, the peptide comes from pathogen, you will be able to generate a T cell activation and generate a T cell response.

So, I hope this class has given students an idea about the MHC class 1 pathway of antigen processing, and then, ultimately, presentation, and processing involves proteasomes, then generation of peptides take place, the transporter associated with antigen processing presentation where translocation takes place, and final binding of the peptides with the MHC molecules.

Thank you.