Human Physiology
Prof. Nishikant Subedar
IISER-Pune

Lecture – 05
Physiology of muscle - Part : 2

So, the aim of this slide is to understand a sarcomere. What are we now looking at? Sarcomere. Sorry, because if we have understood one sarcomere, we have understood the skeletal muscles. We have to understand the sarcomere. So, if I draw this line here, this greenish line here, which is a little crooked. and there is another disc. So, what do I have in front of me? A sarcomere. Now, if I see very carefully, I find that anchored on the sarcomere are certain green filaments, these are coded in green.

Those ones, are you with me? I am talking about what? I am talking of these green line going here, these green line, they are also going in both directions. So, if I am looking at, if I am standing on the Z membrane here, on this side is one unit, one sarcomere, on this side the another sarcomere. Extending like my hands are protein filaments which I will call as actin.

We will call as what? Actin. So, one actin filament goes in this direction and it goes how far does it go? Well, it goes so far, so far, so far and then it ends. It does not go all the way. So, it will go some distance. But there are along the length, a series of actin filaments, and they will all go to the same distance and they will stop.

So, where are the actin filaments anchored? They are anchored on the Z membrane. And who does the function of making sure that they are anchored firmly and they stay that way? The proteins. Very good. Tell me the name of the protein. Yes.

Very good. So, so actually here you have your membrane rich in proteins and then these are the actin filaments which go this way and they also go this way. Then if you, keep track of these filaments for a while, you will find that they overlap with another filament which is red, which is encoded as red in colour and that red filament is called as myosin. In routine terms, it is customary to call the red filament as thick filament and actin as the thin filament. So, when you read about physiology of muscles, these two terms will come very often.

What terms? Thick filament and thin filament. So, if I use the word thick filament, what do I really mean by that? Very good. And the thin one? Actin filaments. Actin filaments and you will notice that the thick filament, the red one there, which extends all that to the other end of the sarcomere and then for a while it overlaps the actin filament on the other side of the same sarcomere. Are you with me? So, it is actually the part where the myosin filament and actin filament form the dark band.

Remember we talked about the dark band? And this part where there is no myosin filament, but only Z membrane in the middle, part of the actin, part of the actin, if you go little ahead, you will meet myosin. Do not do that. Just restrict yourself. You are in the I band. So, it is actually the presence of the myosin that makes the band dark.

Hello, are you getting my language? So, this part, so this from here to here is the dark band, from here to here is the light band and at the center of the light band, there is Z membrane. Okay. And from Z membranes in both the directions, you will have the extension of the actin filaments. Good. Yeah, sure.

What am I doing right now? I am building the anatomy so that I can introduce you to the function.

Are you with me? Patience for 5 minutes. I will answer the question. Right now, we are sitting here to appreciate the anatomical beauty, the geometry. And to appreciate that, I will draw your attention to, a section through somewhere near the M line, where I will get only the thick bands. I will take a section through where the overlapping is.

So okay, so this is actin filament, hello, this is actin filament. I will take a section here, I will get the middle image. I will go further where only the thick filaments are there. I will get the image on the right. Now, I will take the section where the two filaments overlap one another. And then I will get this image.

And if you look at this image, now this image is the crux of everything. I will ask you a simple question. Look at any thick fiber, which is myosin, around that how many actin filaments can you count? 6. Do the other way round. How many filaments can you count? 3.

Absolute geometry, you will never get 5 or 7, that is the beauty. Around any actin filament you will always go anywhere, any actin filament 1, 2, 3 or go anywhere, always 6, not only that. This distance, the distance between actin filament and the nearest myosin is always absolutely constant. Have I answered your question as far as anatomy is concerned? Now just wait, I will answer your question with reference to function also. Why is this so?

So it is not a simple wall which goes like this and there it goes like this. This actin Did I make my point? Correct. Okay, so why this geometry is important, let us try to address that point. But before that, so we have seen that in every cell, there are fibrils and fibrils, okay. Now, these fibrils are jacketed, covered, by a very elaborate system of endoplasmic reticulum.

And the aim of this slide is to show, I am absolutely sure you can identify the myofibrils here. So you can see the red ones and the blue ones, you know the actin myosin filaments, there is a fibril, there is a fibril, each is a myofibril and going around that is a very dense work of endoplasmic reticulum.

Point number 1, this endoplasmic reticulum is the very resource for calcium ions and they will release calcium ions when they are told to do so or when they are excited to do so, how that happens we will see shortly. And the calcium ions will play a very important role in sliding, I will come to that. Now, this is very interesting.

The problem is, you see its cell is thick, it is very long. So from the point of view of extracellular medium, how do you reach inside? I want to go inside, I want to send calcium ions inside, I want to send a current inside, how do I do that? Therefore, the anatomy of a cell has taken a very interesting evolutionary turn. What it has done is, it has in a very raw language, I will say that it has constructed tunnels, constructed what? Tunnels. And what is a tunnel? Tunnel is, now I will draw in front of it, this is a single cell going from one end to another end, are you okay so far? Along the length I will drill holes, which will go right through the cell and I will call each hole as a T-tubule, transverse tubule, what do I call it as? T-tubule. So I will ask you a simple question.

So if I am on the cells, if I am on the walking on the plasma membrane of a muscle cell, so I walk a few steps and then I find a hole there. What does that hole represent? It is a tunnel. And where will the T-tubule? The T-tubule will open on the other end. So if I can enter the T-tubule, I can get out of the cell at the other end, but I am going to ask you a very simple question. If you are standing somewhere within the tunnel, you are extracellular or intracellular? You are extracellular.

What are you? You are extracellular. What are you? You are extracellular. So if you are extracellular, I am going to ask you a very simple question. What will be the sodium ion concentration within the lumen of the tubule in the light of what we studied yesterday, you need to tell me the exact number. Say that everybody, 142 milli-equivalents per liter outside sodium ion.

So here, what is the sodium ion concentration? Here, but just if you cross the plasma membrane and what is the sodium ion concentration you are going to encounter? 10. What is it? 10, loudly. 10. Outside is how much? 142.

Inside is how much? 10. What is the difference like? 14.2 times, 14.2 times. There is a tremendous pressure on the sodium ions to go in. It is not going in because they have a charge and plasma membrane would not let them go in.

Remember this. So now we have, because of the series of transverse tubules and again look at the image and tell me with reference to what we have learnt so far, look at the image and tell me at what level of the fine structure of the skeletal muscle do you get the co-occurrence of the T-tubule? It is at the level of Z-membrane. It is at the level of what? Z-membrane. So, this tubule, can you see the author has given the Z-membrane. So at the level of Z-membrane the T-tubule will go from one end to another end and it will provide extracellular medium which is very, very close to the, so here you can see the T-tubules. Now, so we are still talking of the same thing.

We are talking about the Z-membrane. From the Z-membrane you have the actin filament. Are you with me? Actin filament and the red ones are the myosin filament and from the myosin filament you have some extremely interesting structure which we call as a cross-bridge. Now cross-bridge people always compare with, you know, you see people playing golf. Golf, the golf has a handle and there is a club at the end.

So something like that is there and we call it as a cross-bridge. So, who is, what is in fact, what is in fact the golf, the golf stick or the golf club? It is the myosin. It is what? The myosin. So here you have myosin. Listen to this, about 200 to 400 myosin molecules, individual myosin molecules they come together and form the myofibril.

You are working on a test tube in vitro. And then what you do, you provide them with proper buffer etc everything so that the biological structures remain inside and just leave them on their own and they will show you the amazing phenomena of self-assembly. Now what do I mean by self-assembly? You will find that, are you getting the message here? What is this part? This is the axis or this is the tail of the fibril. What is this? This is the cross bridge. They will all organize in such a way that you will never get an arrangement like this. Are you with me? And these protein molecules are self-assembling.

You are not doing anything. So when you leave the protein molecules on them, they will interact with one another and self-assemble. So individual 200 to 400 individual molecules will come together and self-assemble to form a myofibril and the myofibril is such that it is something like this that they will all have their heads either in this or in this direction and they are ready to interact with the actin filament. Did I stress on your mind the beauty of the protein molecule that constitutes the myosin? So it is organized in this way. Now, so I have talked of how many proteins so far? Actin, the thin filament, and the third one? Yes, ma'am. Okay, and I am going to talk about the fourth protein.

And the fourth protein is interesting because it starts at the level of the Z membrane and goes, goes, goes, goes, goes, goes, all the way talks to M line and it comes from the other side also. Okay. And this protein is called as titin and can somebody please tell me why do you call it as titin? Big, big, big, very large. So if anybody asks you a question anytime tell me the name of one of the largest proteins. Okay. Yes, you can use the word titin. Okay. I do not remember I think it is 300 kilo Daltons or so is the weight of this huge molecule.

Okay. Titin. Okay. Okay. Then you will also find that there is another molecule I was talking about. The another molecule is called as nebulin. Now this is very interesting molecule. Nebulin. Okay, tell me what is the name? Nebulin. Nebulin you will always find here. Nebulin is this red guy here and this is intertwined and runs along with the actin filament. Okay, there is another protein, which I call as nebulin.

It starts with actin and ends with actin. Okay. Now its function. If I ask you to draw a line on your piece of paper, which is 12 inches long, simple, we did it in fifth standard or earlier than that, we will take a paper, we will put a scale and draw a line, which will start from one end and go to another end. Are you with me? Now the line you are drawing, hello, the line you are drawing is the actin and the ruler that you have used is the nebulin. Got the point? So it is the nebulin, which is determining how long the actin should be. Hello, it is beautiful.

I mean, just imagine the beauty of biology. I mean, just to make sure that when the actin molecule is being synthesized, you make sure that you do not want to go beyond or you do not want to make a little short. There is another regulatory product in nebulin which ensures that the actin go so, so, so, so, so far and stop. Everybody, you are okay with this? Good.

It is a very, very interesting question. I do not know the answer. We are still struggling to find out. We are still struggling to establish what it does. We are still struggling to establish that nebulin determines the length of actin. There could be some other genetic factor which determines the length of the actin, but we still do not know.

The question is correct. We do not know the answer. It is a polymer. They are all polymers. They are all, they are all polymers.

They are all polymers. And let us talk about actin as a polymer now. Are we okay so far? Hello. I will move on to this image. Now, we will spend some time and trying to find out as to what is actin and what is myosin. I am sure myosin we have already done. So, this is the tail of the myosin molecule or the tick molecule and this is what you call as the club of the golf club or let us use the right word, we will call it as the cross bridge.

So, if I have 200 molecules of myosin and I will let it allow to stay in a bath, they will organize themselves in like this. And, and, and, and in the middle somewhere is the M line.

Hello. M line, you do not forget M line. Good. Now, let us talk about the actin filament. Actin filament is polymer. It is made up of the unit is a globular protein, I will call it as a G actin.

Make sense? G actin and it is here. Can you see 1, 2, 3, 4, the G actin and the G actin molecules can stick to one another and they form a filament. Filament is actin, F actin. So, G actin is the unit that comes together and forms a F actin. Now, 2 F actin strands can come together. So, there is one strand and another strand and once they come together, okay, instinctively we know that they will form a helix.

So, here you get, so what you see here is a helix of 2 filamentous G proteins making a helix. Are you

with me so far? Yes or no? Good. Now, you can see this is a globular protein. This is an actin filament forming a helix. On each can you see this tiny circle? The tiny circle is the site on that protein, on the G protein that is the site where the cross bridge of the myosin filament is going to bind.

So, obviously this protein molecule has structure, fine structure here where it can combine with the head of the myosin molecule. So, interaction between the 2 proteins is going to happen at this site. How it is going to happen I will show shortly. Okay. Then there is another protein which is shown as in a purplish colour and called as tropomyosin.

Say that again. Tropomyosin. It is a form of a double chain, double chain that goes round like this along the actin filament and there are two. So, can you see here, there is one here and there is one here. So, one goes like this and now this one, this one goes on the other side and comes out here. The aim of the tropomyosin, get this point simple and straight, the aim of the tropomyosin is to sit on the site in the G actin site where myosin is going to combine.

Hello. So, actually tropomyosin has blocked the site. What has it done? Blocked the site. Just blocked the site. Do not make it available.
What do I mean by that. I will tell you shortly. You need to make sure that there is no random, okay, unauthorized interaction between the head of the myosin and the site. So, the typical picture is on the G protein, there is active site and the active site is covered by the tropomyosin. We are okay so far? Great, great. Now, there comes another very interesting player and that is called as titin. The advantage of titin is, if you stretch a muscle too much, it has elasticity, it will go back to the same original position and that happens because of titin.

So, what is titin? Titin is like a spring in the muscle. So, if you stretch it, it does not go back to its original position because of action-myosin. No, it goes back to the original position because titin does it. And therefore, the author has used, can you see this spiral spring like design to indicate that titin is spring? Okay. Well, we have been, it is good to talk about the arrangement using diagrammatic figures.

But the real biology does not work that way. So, to find out how and which way the real biology works, we take a section through the muscle and try to see the electron photomicrograph, try to with the transmission electron photomicrograph. If you see this image, very carefully can you see that vertically there are some thin lines and alternating thick lines. Hello.

Can you see or you cannot? You are there, they are very clear. Okay, the thin ones. Obviously, the thick ones are myosin and the thin ones are actin. Are you okay, are you good so far? And then you can also see that from the thick lines, certain cross bridges come and they go and join the actin molecule. So, on the myosin molecule, you have the cross bridges. So, this image is really interesting

because in the electron photomicrograph, we are able to see the occurrence of cross bridges that go from myosin molecule and they communicate with the actin molecule, great. So, we are doing the entire series, we have the muscle from the muscle, the muscle fiber is enclosed in the endoplasmic reticulum, sarcoplasmic reticulum.

Okay, so now let us see, now let us come to your point, sliding. Okay, so here we have the actin filaments, the myosin filaments here. Are you okay so far? And when the cell is stimulated, how it is stimulated, we will see a little later. Okay, all that myosin has to do is to slide on actin. It was in about 1950-54 that the basic concept of how a muscle contracts was revealed and it was absolutely certain by then that the contraction happens because of the sliding of membrane, I made a mistake, sliding of filaments. Okay, sliding of filaments. What filament over? The actin myosin filaments - slide on one another and thereby they bring about the contraction of the muscle.

When the contraction of the muscles as you can see in the image, hello, take a look at the image, the upper image and the lower image and you can see the two Z membranes are coming closer to one another. You can see, so if this is, I am the myosin filament, okay, I can pull the actin filament in this way, I can pull the actin filament in this way and the two actin filaments are anchored on the Z membrane here, the two Z membrane here and the Z membrane will be pulled towards me. Okay, and if all these, and the distance between the two Z membranes is reduced, then the entire muscle is going to contract. Have you followed through the flow of thought? Everybody, hello, are you okay so far? Great, great. So, here you have the actin filaments, myosin filaments, actin filaments and then here you can find, so you can, if you can see the degree of overlap, there is only so much, if you see that the degree of overlap is so much, that is because the myosin filaments have pulled the actin filaments towards them and as a result of that the two Z membranes have come nearer to one another.

Okay, we are looking at the same thing, but actually you can see the work being done here. Okay, the, you can see the two sarcomeres here. Okay, and then there is overlap and then as the sliding happens, you can do the work and you can can do the work, that is what it is. Okay, obviously, you can appreciate Andrew Huxley and Nedder Gerk, when muscle fibres contract, the thick and thin filaments do not shorten, do not shorten, but instead they slide on one another. These are the investigations of 1950 and so you can read. So, we have what you call as the sliding filament theory that explains how the muscles contract. Now, we let us, having read something about actin filament, it is time for us to take a quick look at what, at the myosin filament.

What are we doing now? Hello, take a closer look at myosin filament. No, please. This is a single myosin molecule, this is a molecule, this is what? This is a molecule. 200 to 400 such molecules can form by virtue of self-assembly, they can come together and form a myofilament. Are you with me? And that is the myofilament there.

Okay, so from the myofilament, I take a single molecule and blow it up here. Okay, so far. And

when I do that, I find that the molecule, the myosin molecule is made up of actually 6 protein units. How many? 6. Two are called as heavy and four are called as light. Now, let us see what is heavy.

Now, this is a pair, okay, this is the head, this head is actually the cross bridge. Hello, this is what? Cross bridge. From the cross bridge, but this heavy protein has a tail and this guy has a tail and then the two tails form a helix, okay, and they will go as long as it overlaps. So these are the two heavy proteins. Now, four light proteins are here, 1, 2, 3, 4, they sit on the neck, you get my language, they sit on the neck of the cross bridge of the myosin filament.

Now, these are regulatory proteins. These are regulatory proteins we will talk about a little later. Now, just focus on the head, just focus on the head. In the head, the protein, the author is drawing our structure to number one, the green part which symbolizes what, can you please read for me? Actin binding site. So, you know that, we know that if, calcium is available and if tropomycin is not there, hello, because if tropomycin is there, it will block the site on the G protein. So if tropomycin is not there and if calcium ions are available, they are all interlinked, then this part of the protein will combine with the actin filament and then it will go through a process of sliding.

How that will happen? That I will show you. Then point number one. Point number 2, then on the same protein, there is another part. What is that protein? Head, head of what? Myosin filament, okay, which is what? Cross bridge, okay. That part is nothing but ATPase. Hey, what did I say? ATPase, so it can bind to ATP, hydrolyze it, remove inorganic phosphate, take energy, it is still chemical energy, it is still what? Chemical energy. Now this is the beauty, use that chemical energy to convert it into mechanical energy so that the myosin filament can pull the actin filament towards it. Because here you have a protein that uses the chemical energy and converts it into mechanical energy, therefore the protein qualifies to be called as a motor protein.

Hello, are you good so far? So is myosin a motor protein, yeah, it is a motor protein. Why is it a motor protein? Because, chemical energy - it is able to convert into mechanical energy. Since there are only 6 actin filaments around one thick filament, is it only facing in 6 directions, the head or are there in the middle and… It is facing only in 6 directions.

Yes, there is a great geometry. His question is correct. Just see the geometry here. Can you see a geometry here? So if you take a molecule and it will look around, and exactly at a point where on the other side there will be an actin molecule with which it can bind.

I think that answers your question. Yeah, anything? Yeah, please. What is the purpose of M-line? Okay, okay, that is a very good question, I forgot. She is asking as to why is M-line there? The purpose of M-line is to make sure that the myosin filaments, they stay in place. Look, what is so important and what is so critical for the entire organization is the geometry.

You need to have a protein there. You see, you are going to make one protein interact with one another. So when the myosin filament throws a cross bridge to the actin filament, actin filament better be there. Are you with me? So just to make sure that every molecule is where it is supposed to be. You do not want the density of myofilaments more here and a little sparer here, no? No? If you look at the M-line, there is a complete geometry of where every M-line is passing through or M-line is anchored on. And what do I mean by where it should be? It should be, if it is a myosin, it must have actin there, if it has actin there, it must have myosin there and the two can interact.

No, all that has all happened during development. We study about that assembly because it helps us to understand the property of a biological molecule, okay. So you do the experiment in vitro and you find the self-assembly, so then you start asking the question, what is it in the individual molecule that it forms a self-assembly? If I want to understand the phenomena of self-assembly, I have a very good model here, okay, alright. So, Which one? When you want to study the self-assembly of myofilaments, it will happen even without the cells, just myosin filaments alone.

The directionality of the actin is also clear. You made the point. I will make a statement, let us see if we can catch it. Whether you are talking of actin filament or whether you are talking of myosin filament, these molecules are polarized. Hello, polarized means if the molecule is organized this way, you cannot do it this way, got it? Okay, so and actually if I am standing on a Z line, okay, and I have actin filament here and I have actin filament here, are you okay so far? Listen to this. So, this actin filament is a mirror image of this, got it? And if I am standing on the M line, okay, then this my filament, okay, which has the cross bridges that way, will have a cross bridges that way. So this is the mirror image of that. Does it answer the question? Okay, good, good, good.