Introduction to Mechanobiology
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Week – 04
Lecture – 20
Actin dynamics during mesenchymal migration

Hello and welcome to our lecture on Introduction to Mechanobiology. So, over the last 2 3 lectures we have been discussing about acting dynamics in migration.

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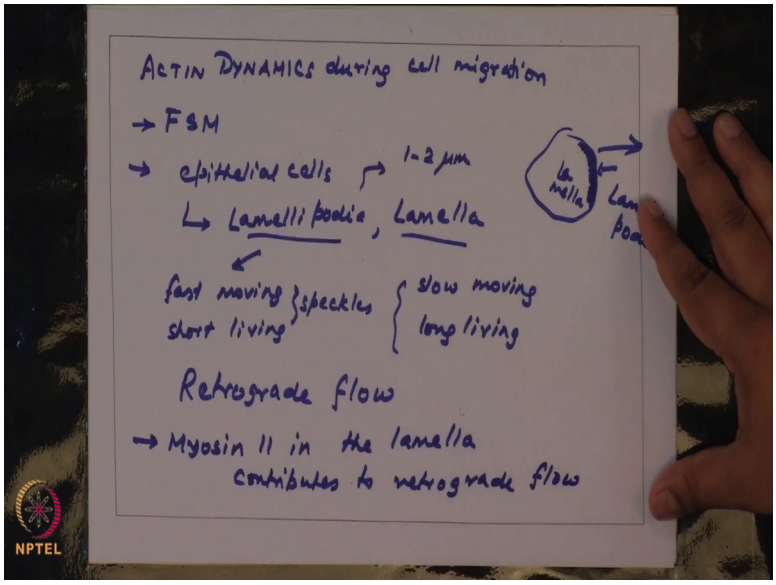

So, we have been I have introduced you the technique of fluorescent speckle microscopy for tracking dynamics and in this regard we were discussing a paper where fluorescent speckle microscopy was performed to track or quantify actin monomers movement during migration, based on that the authors for cells using epithelial cells. So, author showed the existence of two zones lamellipodia, lamellipodia and lamella. So, in lamellipodia you have speckles fast moving, fast moving short living speckles while in lamella you had slow moving long living spectacles this was one difference.

So, this, lamellipodia is within first one to two microns from the leading edge. So, if this is a cell you have the lamellipodia as this zone in the front. So, this is your lamellipodia region and then this is your lamellum this is your lamellipodia and this is the migration

direction. So, speckles all these speckles move backward. So, the system retrograde flow retrograde or backward.

What we also, I also told you was that these two networks lamellipodia and lamella are molecule indistinct, and using Blemish Statin experiments the authors had shown that myosin two in the lamella contributes to retrograde flow. So, if you inhibit if you treat cells with Blemish Statin which is the myosin 2 inhibitor then the retrograde flow in the lamellar region is eliminated, but the retrograde flow in the lamellipodia region still exists.

So, the last thing we discussed yesterday was by taking a cell age we wanted to know how actin dynamics is contributing to age displacement.

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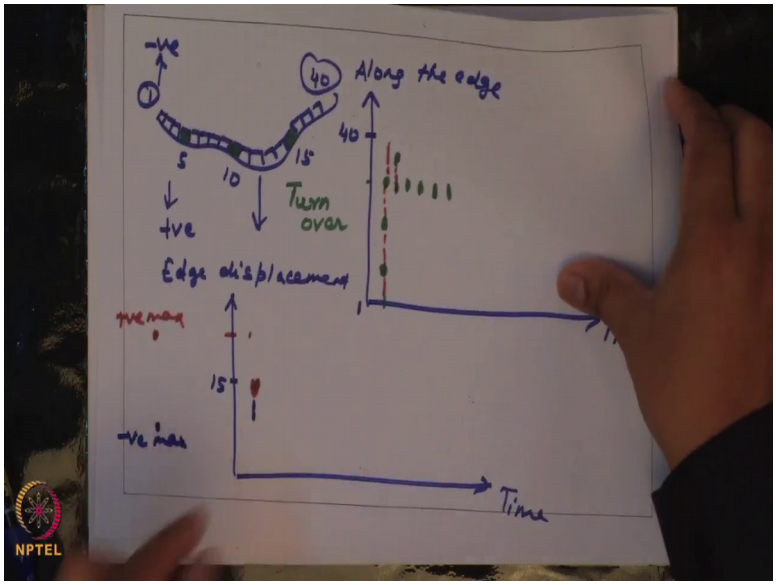

So, what the authors would do is, they would discretize this network. So, you would discretize this network into small small zones, you have these zones level from 1 at 1 and 2 whatever let us say forty at the other end and you would plot, your x axis is time and y axis is whatever you are talking, but along the edge.

So, if locally let us say, this position is 1 and this position is 40 let us assume at position ten you have a polymerization event at position 10 you have a polymerization event. So, if you are plotting, if you are plotting the network turn over. So, if you are plotting turn

over. So, let us assume at position 10 position 15, 15 and 5. In position 5, 10 and 15 you have polymerization events.

So, what you will have at this position 5 at this particular time T you would have a green signal here indicative of polymerization at position 10 also a green signal and position 15 green signal and at all other points you will have red signal. So, this allows you to capture what is happening at this particular time instant along the edge. So, this may vary in the, if this is the dynamics that is happening on the along the edge at this time the next instant you might see that. The neighboring point you might see a green here and red here.

So, suggesting that in the next point next time instant there is a depolarization event so on and so forth or you can have prolonged polymerization you can had have multiple time points along at this particular point let us say at this point you see polymerization happening continuous. So, this is turn over.

You can generate the same plot for let us say edge displacement. So, if the cell is migrating this way then this direction is positive and this direction is negative. So, this direction is negative this direction is positive. So, you can have a length scale where red is positive max blue is negative max, you have a continuous scale and just like you did this you can do the same thing. So, at this point let us say the cell is migrating fast. So, in if I want to go with this thing then at position 15, I would see max y there is a polymerization event I would see high displacement high edge displacement in the positive direction and where there has been deep polymerization I would see negative which means that the cell is retracting. So, these suggest that these three things are all correlated these three things are all correlated. So, you can target, you can track the coupling between these individual components by doing cross correlation.

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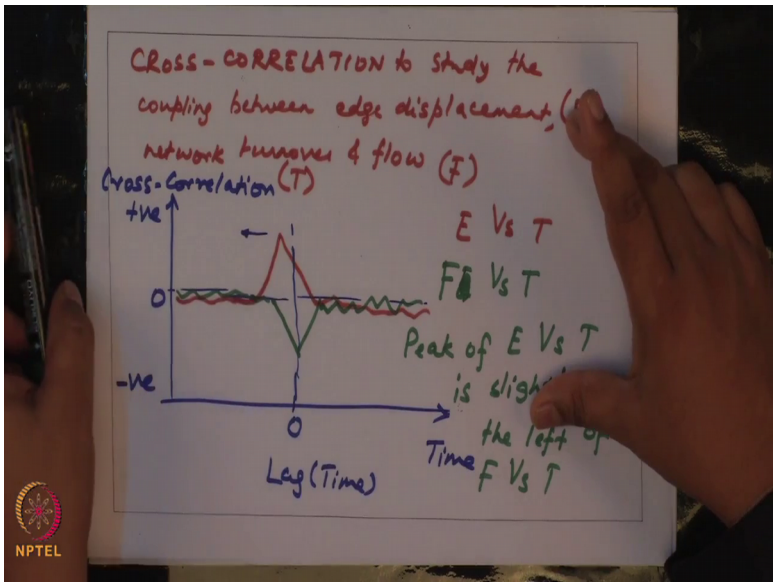

So, you can do cross correlation to study the coupling between edge displacement network turn over and flow. So, let me draw a sample plot as to how this cross correlation might look like. So, you have time here this is your cross correlation. Now the way cross correlation is defined, let us say this is my 0 and this is my 0, this is 0 or you can say lag time lag time and this is 0 cross correlation. So, you can have cross correlation of positive or cross correlation as negative. Cross correlation as positive would mean between any two quantities would mean that one guy if is positive the other unit also would be positive.

So, what the authors found. So, they did cross correlation between three things. So, let us say that we call the edge displacement as E the network turn over as T and the flow as f. So, what they found let us first plot E versus T and what they found was this curve looked something like this. So, if you notice the way I drew have drawn the curve. So, suggest that there is at one point there is a positive correlation at other points is mostly 0 or baseline.

Now, let me draw the curve E versus turn over. So, what you find the E versus turn over gives you a peak here. So, this is E versus turn over and this is E versus this is a F versus this is not E versus turn over this is F versus turn over, F is flow. So, F and turn over are negatively correlated while E and turn over are positively correlated. What you also see that the peak the positive peak between E and turn over when they are related is slightly

to the left. So, peak of E versus T is slightly to the left of F versus T suggesting that if I want to imagine the sequence of events that are happening temporally there is a polymerization event, which would mean as turn over that polymerization event is first pushing the membrane or in other words edge displacement is positive, you have this positive correlation in the red curve.

Immediately, immediately after that there is a negative correlation between F versus T. So, the red curve, the turnover is positive. So, you have a polymerization event and flow is negative which means that there is retrograde flow.

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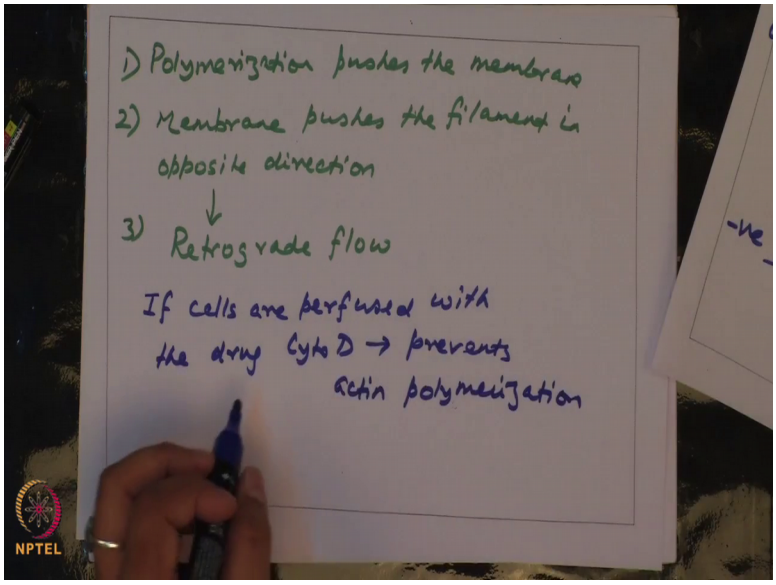

So, what is happening is that polymerization pushes the membrane, this is my first step and right after this. So, if I want to think of Newton's laws of motion to every action there is an equal and opposite reaction. So, if the actin filament the polymerizing acting filament pushes the membrane, the membrane will push the filament back. So, membrane pushes the filament in opposite direction. So, as a consequence and what will happen if the membrane pushes the filament back; then this should lead to retrograde flow.

So, this is, once again this is the time sequence of events in terms of cross correlation. You have a peak in age versus turn over and right after that positive peak when the edge moved ahead then the edge you know. So, your edge moved ahead and again the edge

your correlation dropped there was a corresponding flow and this flow was backward because the turnover is positive the flow is backwards we have a negative correlation.

Now, what happens to this curve if cells are treated perfused with Cyto D with the drug Cyto D right, Cytochalasin D and what the Cytochalasin do? Cytochalasin treatment actually inhibits the polymerization of filaments it prevents Cyto D describe prevents actin polymerization.

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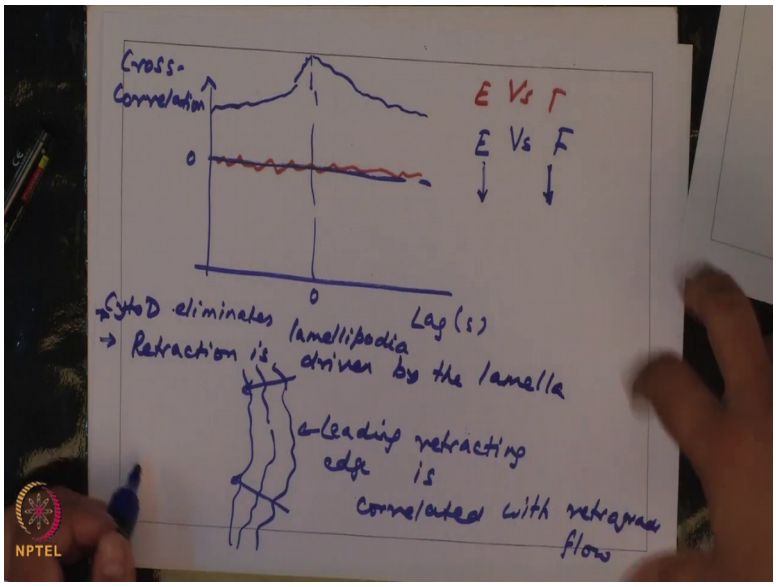

So, in terms of cross correlation you have the following - this is lag time and this is cross correlation. So, this is 0, 0, this is cross correlation. So, what you have - your red is the edge displacement there is no correlation between E versus T, but if you do the correlation between edge displacement versus flow what you will observe is a curve which is like this.

In other words edge, your flow network turnover is mostly negative because there is no new polymerization this is negative and, we are plotting E versus F, E versus flow edge displacement is backward. So, if I want to draw the curve what is happening here is after Cytochalasin D if this is your leading edge this leading edge at different time points which is retracting this coming, this response the leading edge.

So, leading edge is retracting and it is correlating with the retrograde flow is correlated with retrograde flow. So, what you have this suggest that Cytochalasin D eliminates the

lamellipodia. So, then in that case what drives the retraction, retraction is driven by the lamella you have myosin 2 in the lamella which is pulling the filaments back.

So, earlier you had two components at the leading edge that pushing back was provided by the membrane when you eliminate the lamellipodia using Cyto D what you are left with is the retraction of the cell edge that is driven by the lamella.

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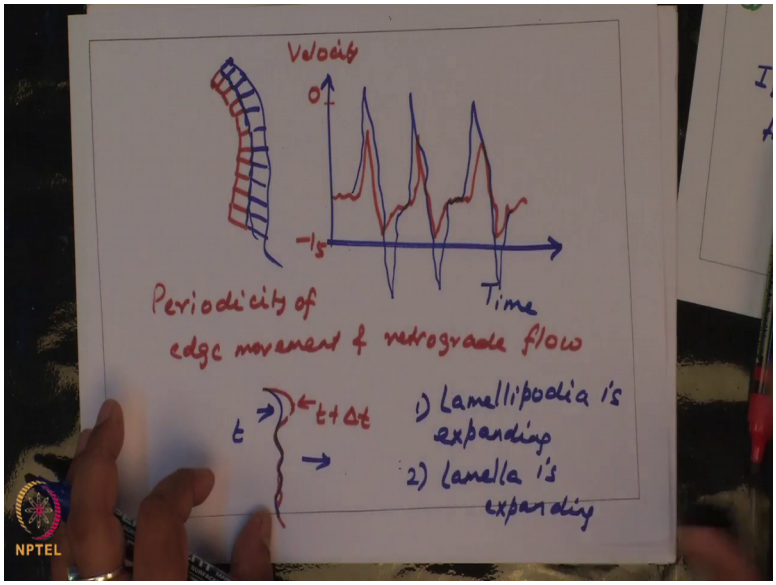

So, this would suggest that if I want to again once again draw the leading edge and I say this is my interface. So, if I have these discretized zones at the beginning in the lamellipodia and then I extend this to these discretized zones right behind the lamella right behind the lamellipodia along in the lamella region.

So, if I see that what how is velocity at this point correlated with velocity at this point at each point I can see the flow in the leading edge and see how is it correlated with the flow in the lamella. So, what we observe, once again this is time let us say every time the velocity is, because flow is backward your velocity this scale is actually negative it starts from 0 let us say this is minus 15 or whatever your velocity.

So, every time you have the flow at the leading edge what you see is, so I drew this wrong the flow in the lamellipodia. So, the way I have drawn is the flow at the leading edge seems to be exactly in sync with the flow at the lamella. So, there is a periodicity of edge movement and retrograde flow. So, this points out to a periodicity of edge

movement and retrograde flow in other words whenever the edge moves back the lamella also moves back.

So, this suggests, there are two possibilities that is it that if these were perfectly correlated is it that the protrusion. So, you have the leading edge you have the leading edge let us say at a given time step from this you had this local protrusion. So, overlapping the membrane reading edge membrane at two different configurations this is T plus delta T and this is T, is at T the cell is moving in this direction.

So, for this protrusion to happen locally one possibility one possibility is the lamellipodia is expands, one possibility is the lamellipodia is expanding or the other possibility is the lamella is expanding. So, what is the difference between these two possibilities? If the lamellipodia is expanding and if this protrusion is very local this would mean that the lamellipodial thickness will increase.

But since if this was true that the lamella is expanding then whenever you have a protrusion event independent of the protrusion event the thickness of the lamellipodia will remain the same. So, what the authors found was the lamellipodial thickness remains unchanged. So, this would mean that the lamellipodia is expanding and any local deformation is associated. So, edge displacement followed by expansion of the lamella, so how is it working.

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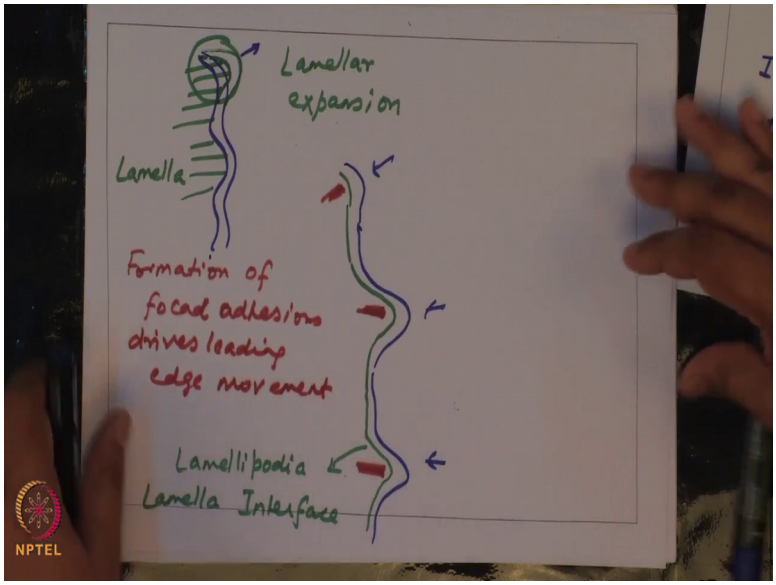

So, in other words, what you see if this is the leading edge at one time point this is you have the let us say you have a local increase you have a protrusion in this direction and this is associated, so I am drawing the initial position. So, this was the initial position of the lamellipodia and it extended to here.

So, it is not that the lamella this position of the lamellipodia remains the same rather the position of the lamellum increases. So, now, in the case of protrusion this whole thing will represent the lamella. So, in other words at this site you have a lamellar expansion. So, how come at you know this you have this continuous retrograde flow you have something flowing ahead.

So, actin filaments put polymerizing pushing the leading edge forward the membrane pushing it back and it is retracting. So, if that would happen then the overall position of the cell will not change it will it is like a curtain which is blowing in the air every time the wind hits it the curtain moves once the wind is gone the curtain goes back to its original position. But every once in a while you have this lamellar expansion which leads to stabilization what is happening, what is the difference between leading edge movement happening at some points and at other positions you have this fluctuation of the leading edge, so what the authors found.

So, I have drawn three points let us say where the leading edge extended. So, what the authors found. So, let us say this is the position of the interface. So, what the authors found wherever this protrusion was stabilized the authors found that focal adhesions were formed right behind the protrusion in the lamellar region. So, this suggests that formation of focal adhesions drives leading edge movement.

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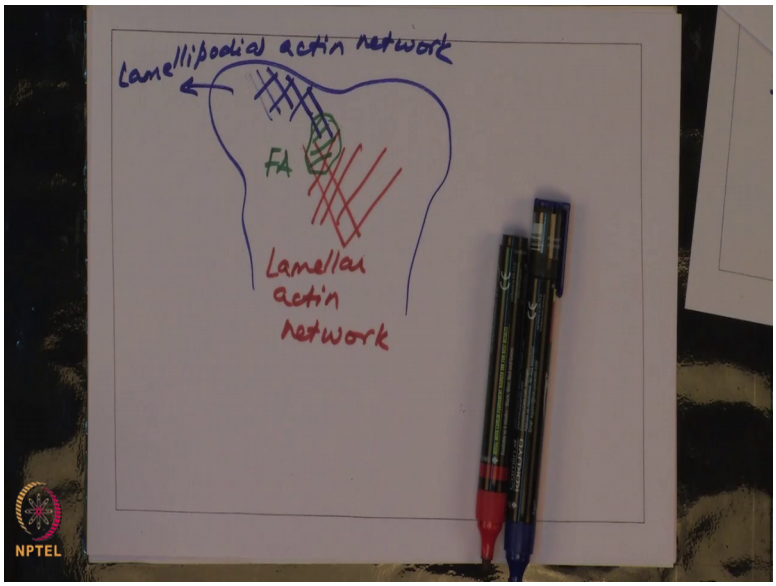

So, to summarize, these observations so based on this you can we can summarize the data; as if this was a cell membrane you have two networks of actin filaments one is here the other network is here and then, there is some overlap and at these positions of overlap you have the formation of focal adhesions. So, this is your lamellipodial network, lamellipodial actin network this is your lamellar actin network and this is your focal adhesion. So, suggesting that in cells migration is driven by the existence of two kinetically and kinematically distinct acting networks.

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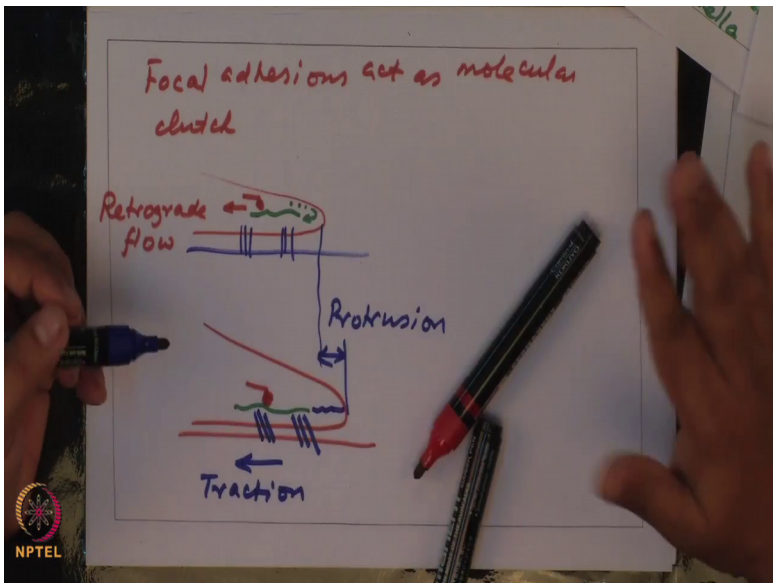

So, these observations also lead to or understanding of migration saying that focal adhesions act as molecular clutch, if I want to draw the membrane of the cell you have these integrins let us say these are my focal adhesions. So, if you have an actin filament this is an actin filament and you have monomers which get added to the front edge of the cell right. So, in the absence of this connection when, in this case when these actin monomers get engaged, at the rear end in this filament you have myosin which pulls on this, causing this myosin pulling on it causes retrograde flow. So, if the actin filament is not attached to the integrin or in the disengaged configuration you will not have any edge displacement but you will only have retrograde flow.

However, if the focal adhesions are engaged, if the focal adhesions are engaged, if the focal adhesions are engaged to the actin, this is my actin network now you can have filament polymerization at the leading edge, this is your protrusion and the myosin motors pulling on the filaments will cause traction at the base. So, this is called a molecular clutch molecular clutch.

It is similar to our cars that if your clutch is not engaged then there no matter what gear you put there will be no motion of the car only when the clutch gets engaged is motion generated. With that I stop here for today and I thank you for your attention.