Introduction to Biomaterials

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Module No. # 01

Lecture No. # 07

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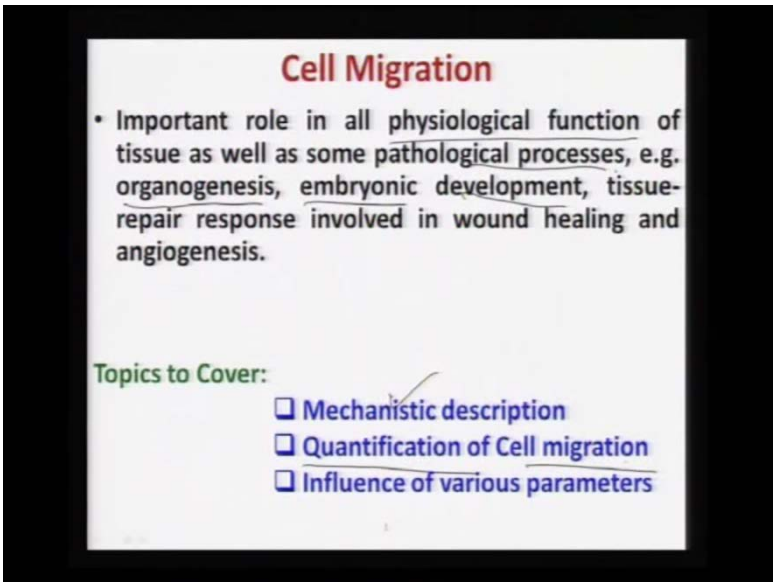

Now, coming to the next topic, that is the cell migration. Now, the cell migration is important in physiological, cell migration is important in all physiological function of tissue, as well as pathological processes like, you know organogenesis, embryonic development, etcetera. Now, the couple of things that I will cover under the cell migration, one is the mechanistic description, second one is the quantification of the cell migration. So, you will have some ideas about some values, like, what is the typical speed of the cell, with which cells move, what is the time scale and all those things and influence of various parameters.

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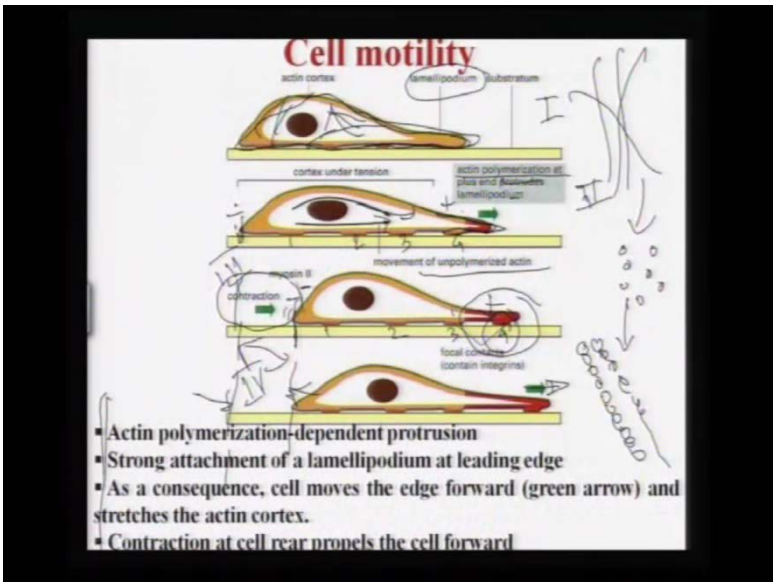

Now, this is the, this slide actually, clearly shows that, how this cell movement occurs on a substrate. From the earlier lectures, you know that, when a cell interacts with the matrix, then what will happen, initially, the cell starts changing its shape, right; from more spherical shape to the off-spherical type of shape; and, that is possible because of the cytoplasmic reorganization, or because of the actin filament polymerization and de-polymerization, disassembly and reassembly of the actin filament in the cytoplasm; actin filament means, these all are actin filaments, right, in the cytoplasm. Now, this is your nucleus, and so, this is like, more kind of, of this shape, shows larger departure from the typical spherical ((symmetry)). Now, why it happens? It happens because, cell wants, a biological cell wants to have more interfacial area, or more interaction area with a given substrate; and, that is possible, when it flattens out; when the cells surface flattens out, so that, more interaction takes place. So, that comes from a very basic physics.

Now, as I said also earlier that, like human being, cells also have a, its own hands and legs; here, it is showing that lamellipodium is the extended part of the cytoplasm which is producing more like a trunk of an elephant, if you see the trunk, So, trunk of an elephant; similarly, this lamellipodium is extended on the cells, on the substratum; substratum means substrate on which the cells want to move. Now, this is the stage one. In the stage two, what you see here that, this actin de-polymerization that takes place at the plus end here; so, there is polarity difference, plus and minus, and that protrudes

lamellipodium. So, here, you can see, so, at the leading edge of the cell, there is more actin polymerization take place.

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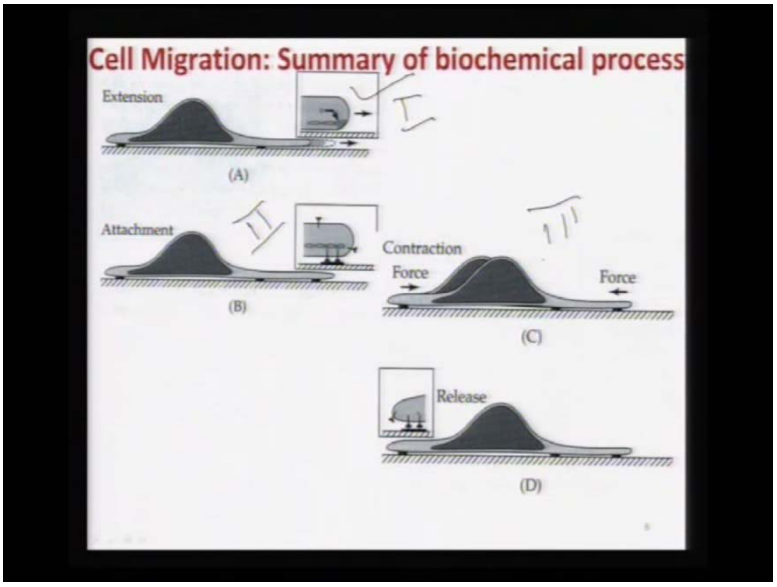

If you remember, this actin polymerization means, there are large number of actin filaments. When they de-polymerize, they form small, small monomer unit, and this monomer unit, again can come, and then, again can be, again can re-polymerize to make a larger kind of, larger kind of structure. And, that is what is happening exactly here, in these particularly lamellipodium area, that leading edge of the cell, actin re-polymerization occurs; that means, this macro molecular structure, again forms, from the actin monomers, ok. Now, therefore, the movement of the un-polymerized actin, that also take place here. This is just because, the space of accommodation and all. Now, in the third stage, what you see in the negative end here, there is some contraction. So, from this point to this point, you can see, here, there is some contraction. This contraction takes place, because here, the focal contacts are established. So, which are the focal contacts here, in this figure? This is the focal contact 1; this is the focal contact 2; this is the focal contact 3; this is focal contact 4. And, once this red shaded area, you, you will notice here, this is actin, new actin de-polymerized area. Here, again, you have this focal contact 1, 2, 3 and this is the, again that 4; number 4 focal contact has been established. If you remember, this focal contacts are the places, or contact points, through which cells interact with the exocellular matrix, or cells receive signals from the exocellular matrix for its migration, for its proliferation, for its replication, all these cellular activity, ok.

So, each time, there is polymerization of the actin filament at the plus end take place, what will happen? At the plus end, there are more number of focal contacts will be established. So, if there are focal contacts established, that means what, that the cells can move further. It is just like a child, who wants to, who just learns to crawl on the floor. So, what, what happens? The two hands, he wants, he wants to just push it further. So, the moment a child puts its another hand, little bit, one step ahead, child can progress. So, similar things here. This number four focal contact has been, so, if you call it as 4, this is 4 prime. So, this 4 prime focal contact, once it is established, cells can push it little farther. So, that is the way, cells will move on the substrate. Now, in the stage four, you can see that, the cells have be, have moved from this place to this place; it is kind of several microns and then again, the cells are growing, or cells are migrating along the arrow directions on the substrate. Now, there are a couple of things. Now, say, a particular cell, can move on a substrate in an unidirectional manner, in a straight line manner, or they can change the track and they can turn it and then, turn it 90 degree, or certain degree, and can also move on a zigzag kind of fashion. So, all those, actually, whatever I have explained, these are written in this text, for your better understanding.

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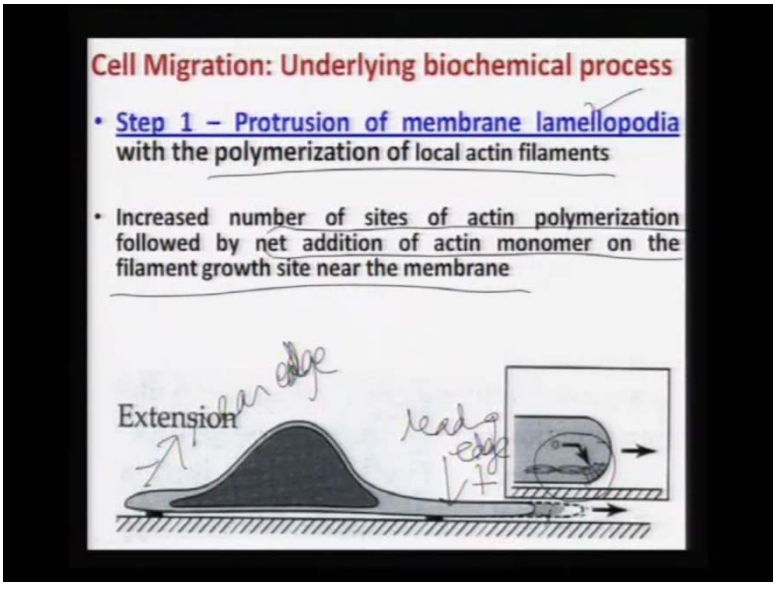

This is the more, kind of a description of individual steps here. So, step one is the protrusion of the membrane, lamellopodia, and that is taking place with the polymerization of the local actin filaments. If you notice here, what you notice that, here, it is a like monomer units of the actin filaments; this monomer units, they come together,

then, they can form the polymers of the leading edge. Now, this leading edge, these are like, shown here as a dotted line. So, dotted line means, that this actin polymerization has not yet taken place, but, they are going to take place in this particular area, and that will form another focal point here. So, increased number of sites of actin polymerization, that will be followed by the net addition of the actin monomer on the filament growth site, near the membrane. So, actin monomer means, these are the small monomeric molecule of the actin, which is formed at the leading edge. So, typically, for a cell, it is considered as the plus end; this is considered as the minus end; this is called as a leading edge and this is called as the rear edge, ok. So, this is called the leading edge, and this is the, other one called the rear edge.

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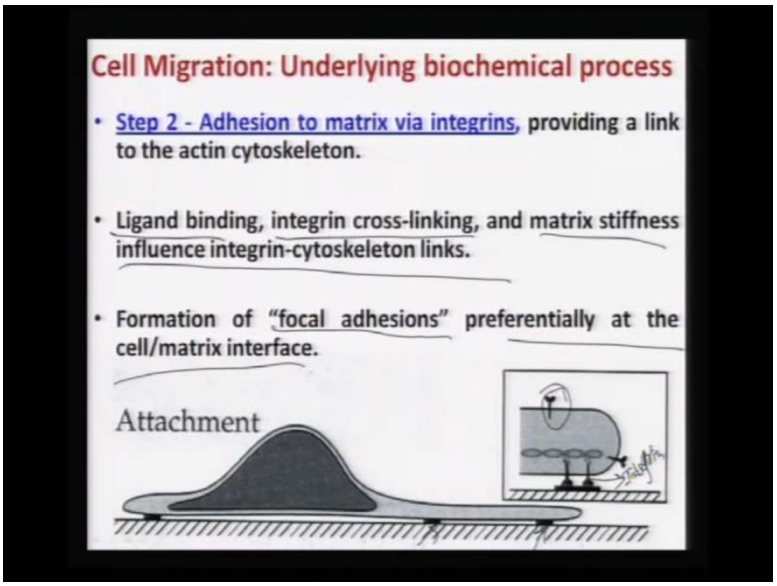

Step two is the adhesion to matrix via integrins. So, this is your integrin proteins, or another, one particular type of cells surface receptors. So, you can see, this integrin proteins, they directly interact with this actin filaments here, and providing a link to the actin cytoskeleton; there is ligand binding, integrin cross-linking and matrix stiffness, all influence the integrin-cytoskeleton links. And then, third point is the formation of the focal adhesion. So, these are the focal adhesion points. These are the focal adhesion points, preferentially at the cell matrix interface, that I have already mentioned to you.

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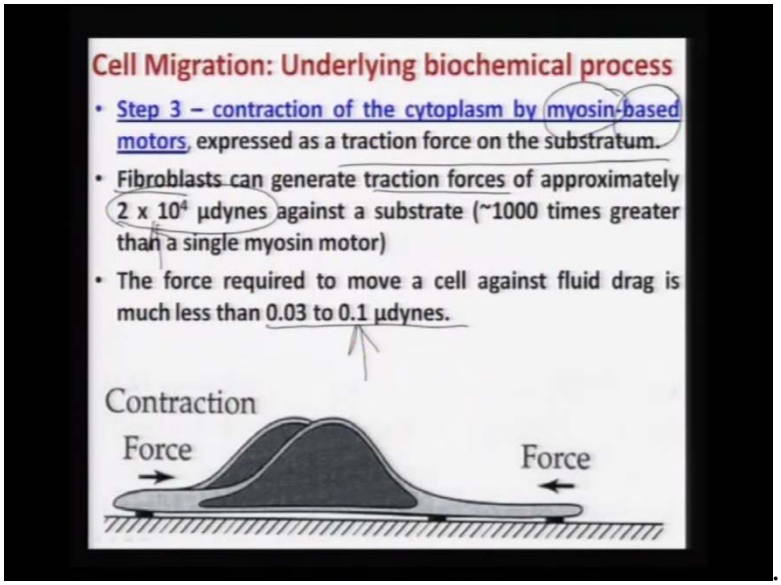

Now, step three, that is the contraction of the cytoplasm by myosin-based motors. Now, these are the certain kind of motor proteins, which are expressed as a traction force on the substratum. Now, what is this motor proteins? These are like one particular motor proteins, which are responsible for the cell migration, or cell motility process. Migration and motility they are synonymous word; they mean the same thing. Now, what you see here, that fibroblasts typically generate traction forces, approximately 2 into 10 to the power 4 microdynes. 2 into 10 to the power 4 microdyne means what? 2 into 10 to the power minus 2 dyne; dyne means, gram per centimeter square and 2 into 10 to the power of minus 2 dyne means, 0.02, right, 0.02 gram per centimeter square. So, you can understand, such a small amount of force you require, in order to move a cell on the substrate. And, this force required to move a cell against a fluid drag is much less; fluid drag means, for example, in the blood cells, these blood cells, they always move in a blood stream, right. So, that is in a fluid drag. So, there, you require even 0.03 to 0.1 micro dynes; that is even much, much less; that means, at least 1 into 10 to the power minus 7 dynes; that is, a force typically your, a cell would experience. Sometimes, as I said earlier, this feel for the numbers are important; that means, that you have to understand that, how small is the force, that is just required for cell migration to take place.

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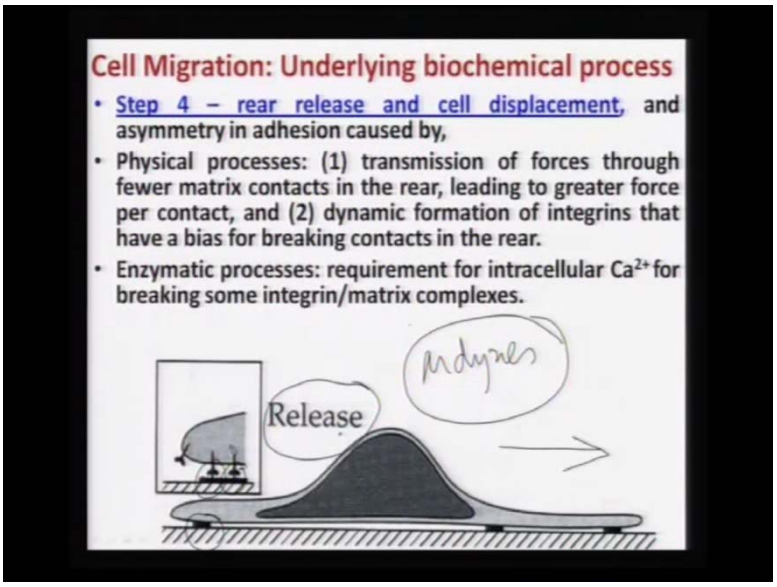

So, step four is the rear release and the cell displacement. So, that is causing the asymmetry of the adhesion. Now, what happens here is that, if you go back to the first slide here. So, what you see here, in this slide, there are 1, 2, 3, 4 focal contacts, in stage two. Now, if you go to stage three, what you see, this one focal contact is no more there, because, cells has moved little bit further. So, how it is possible? That is possible, if the focal contacts are dislodged from the substrate; that means, this focal contacts are moved away from the substrate and then, move to the next one, that is. So, these, this number 1 focal contacts was initially in between the number 1 and number 2. So, therefore, 1, 2, 3, 4, this state of focal contacts are completely gone here, and the newly established focal contacts is like, 1, 2, 3, 4 prime. Therefore, if you go back here, then, how this is taking place? This is taking place, by release of this focal contacts; so, that means, where there was these integrin proteins and then, actin filaments were interacting, so, these are broken; these small junctions have broken and in order to facilitate the movement of the cell towards this direction.

You understand what I am saying? What I am saying, this focal contacts can be established, the focal contacts can be broken, during the cell migration process. And, that takes place in a very dynamic manner. And, all these things are possible because, all these contacts are essentially mediated by the hydrogen bonding, or non-coherent bonding. So, therefore, with little bit of forces, this focal contacts can be broken and

these release is possible and typically, the forces which you require, is of the order of micro-dynes and this micro-dynes is extremely small amount of forces that you require.

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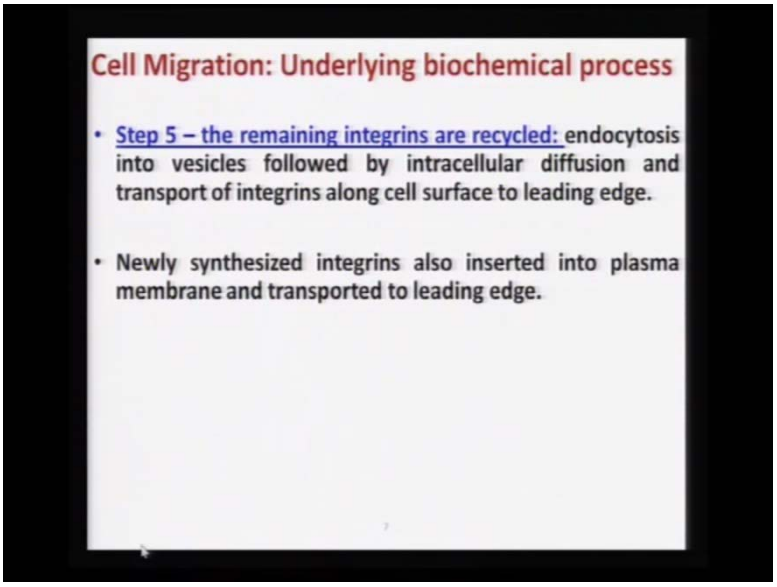

Now, step five is the remaining integrins are recycled; that is, endocytosis into vesicles, followed by intercellular diffusion, etcetera takes place; and, newly synthesized integrins also inserted into the plasma membrane, and transported to the leading edge. So, this is the summary of the biochemical forces, that is biochemical processes. So, first one is your extension, that is the stage one; stage two is the focal contact established; stage three is the contraction forces and stage four is the release.

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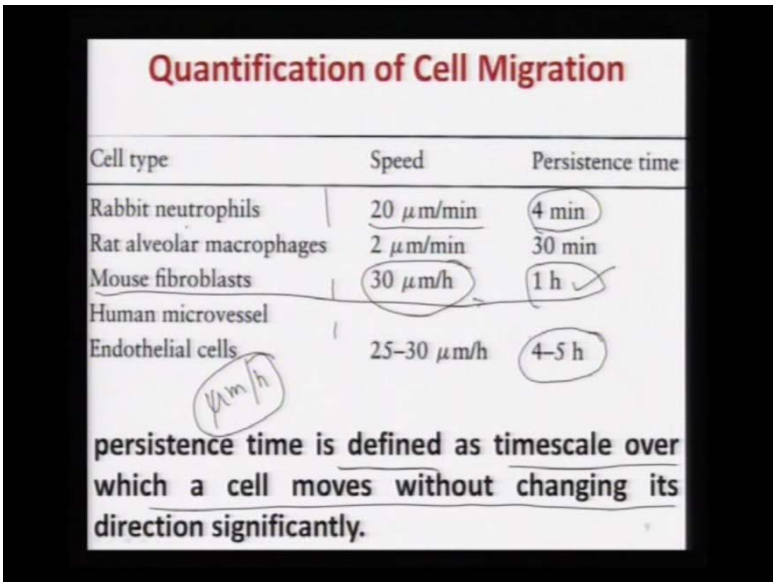

So, little bit of the quantification of the cell migration. Now, if you, this has been different type of cell types, what, what you, what has been provided in this table. Now, what is the typical values that you can see? For example, mouse fibroblasts. Mouse fibroblasts, what is the values you are getting? You are getting the speed of 30 micron per hour. 30 micron per hour means, in terms of minute, it is 0.5 micron per minute; that means, in 1 minute mouse fibroblast can move by 0.5 micron, that is half a micron; micron is 10 to the power minus 6 meters. So, with the naked eye, it is just next to impossible for you, to absorb the cell migration process, just because, it is moving at a, such a small and low speed. So, similarly, there is persistence time is, typically 1 hour. Now, the question is, what is the meaning of persistence time? It is defined as the timescale, over which a cell moves, without changing its direction significantly. So, what it means that, mouse fibroblasts cell can move in a straight line manner for 1 hour, before it changes its direction, maybe 90 degree, or 35 degree, maybe 45 degree and change his direction and go in a another direction. So, this also, if you see, right, rabbit neutro neutrophils and so on, there also the speed can be 20 micron per minute and persistence time can be 4 minute.

Let us continue with the cell migration. So, coming to the field for the values of that cell speed, as well as persistence time, what you can notice here that, typically, this depending on the cells speed, cell type, the cell speed typically varies over several micro meter per hour. So, that is the typical speed. Now, persistence time means that, time

scale over which the cells move without significantly changing its direction and that is again, in the, depends on the cell type and fibroblast, it is 1 hour; in the endothelial cells it is 4 to 5 hours. Endothelial cells, you can see that, you know, that, it can, it can move on a very integrated manner over a, for the longer time period, whereas, rabbit neutrophils, it can just change its direction even after 4 minutes. So, it can vary, you can see, 4 minutes to 4 hours. So, there is a timescale difference.

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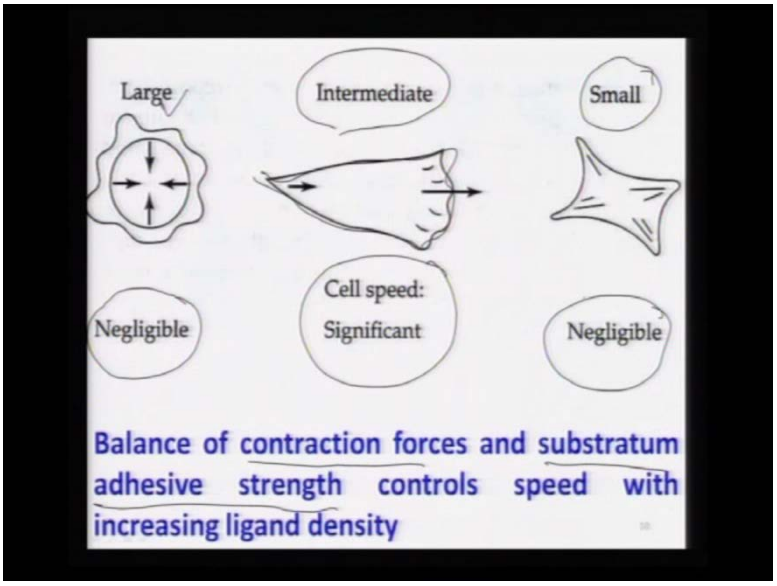

This is like, from the shape of the cell, many times one can essentially, explain, one can essentially make a comment, whether the cells are in migratory motion or not. So, here, this is the balance of the contraction forces and substratum adhesive strength; that means, there should be a balance between the contraction forces, like when myesin motors interacting with the integrins and the adhesive strength; if the adhesive strength is too much, then, you require more forces to break this substratum adhesion, right. So, that is very simple.

Now, if that balance is quite large, then, your cell migration is negligible; that means, cells will simply not move. Now, if the balance is in intermediate, then, the cell speed is also significant, as you can see that, cells are already changes its size, quite significant extent. If the balance is small, again, the cell, then, the cell movement is negligible. So, if the balance, or if the difference between the contraction for the substratum adhesion strength is large, or small, in both the cases, you do not realize much of the cell speed;

only when it is in the intermediate values, then, it should show some significant cell speed. Now, I will summarize it, all these cell (( )) processes; let us move to the…So, the way I am describing all these cell fate processes is that, first, I have show by, given a overview of the different signaling mechanism, signaling processes; then, started this cell division; then, it, I will show you that, the then, start with the cell migration, then, now, I will be discussing the cell division. It will be followed by differentiation; then, it will be followed by cell death, that is necrosis and apoptosis. So, in the cell division, I will first give an overview of the cell cycle; then, coming to the different description of the different stages of the cell cycle, mechanism involved and again, quantifying cell division, so, you have an idea of the feel for the numbers, like, you know how this cell division is taking place.

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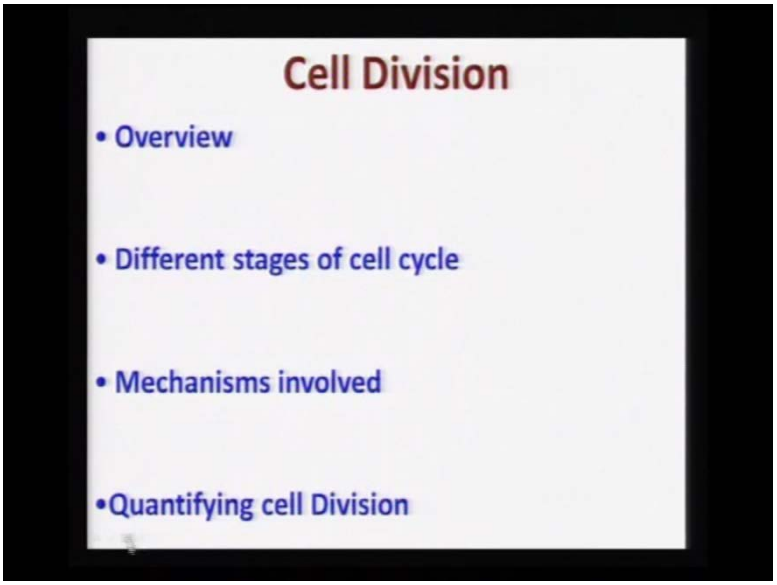

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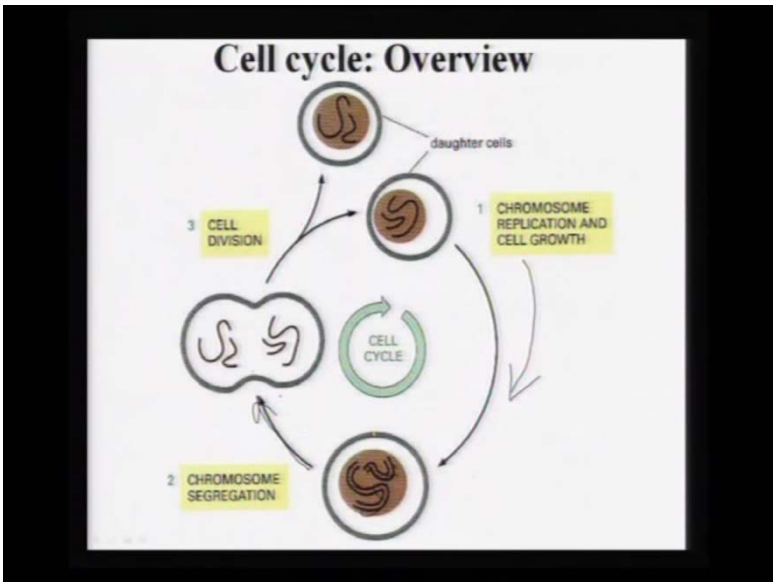

Now, this is the overview of the cell cycle. What you see here that, there is chromosome replication in the cell growth here and then, so, this is a chromosome replication cell growth taking place and there is also, here chromosome segregation, here. Now, this will be followed by the, this is called cytokinesis stage and then, that will be followed by the cell division to two daughter cells. So, this is, cytokinesis means, that is the division of the cytoplasm itself.

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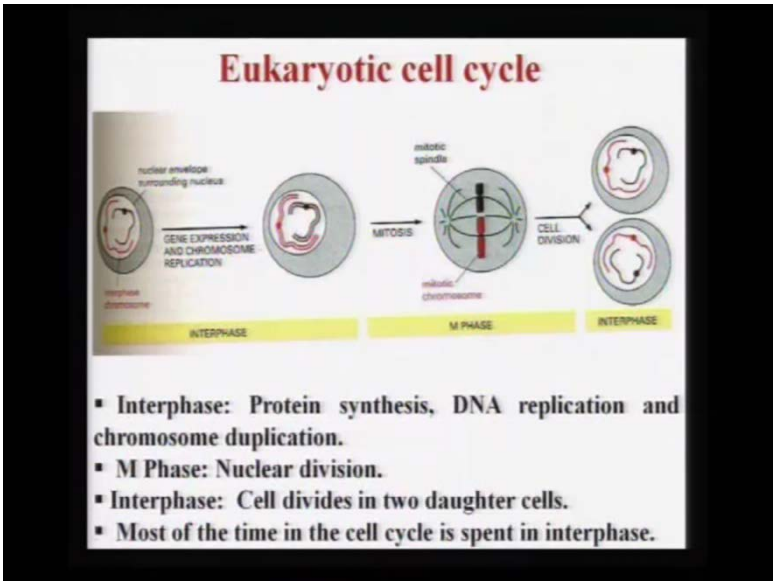

So, this is the eukaryotic cell cycle; that means, that is the nucleated cells, not the bacteria, that is prokaryotic. Now, what you see here that, in most of the cases, that cell cycle, the cell spends most of the time in the interphase stage and let us show first this slide.

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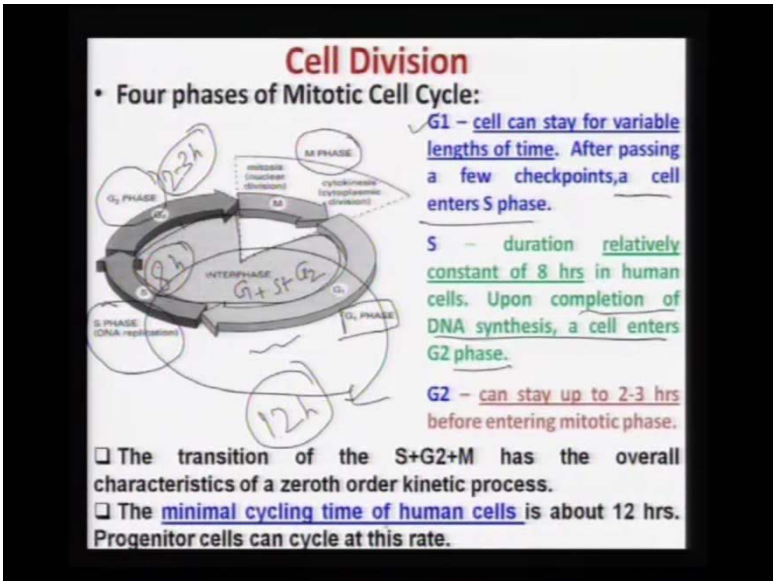

This slide, essentially shows you that, you know that, there are four distinct phases of the cell cycle. What are those phases? One is the G1 phase, that is the gap phase; S phase is the synthesis phase, that is, where the DNA replication take place; this G2 is the, that is the gap phase 2 and then, M phase is the mitotic phase; this is called a mitotic cell cycle. Now, what you see typically, in a total cell cycle, significant amount of time is spent in this inter phase region. And, what is this inter phase region? This is G1 plus S plus G2. So, the combination of this three phases, G1, S and G2, that constitute the interphase. This interphase is the period, when cell is getting ready to enter into the M phase; that means, after the G2, cell enters into the M phase, where there is nuclear division, and where there is cytokinesis, the cytoplasmic division, everything is taking place. G1, if you see G1, cells can stay for variable lengths of time; like this G1, it can, after passing a few check points, cell enters a S phase.

And, S phase, duration of relatively constant, that is 8 hours. So, typically, that is 8 hours, cells stay there; and, upon completion of the DNA synthesis, a cell enters a G2 phase. And, G2 phase, here again, cell can stay for 2 to 3 hours. So, G1 phase can be

variable; S phase, a cell can stay for 8 hours; G2 phase, the cells can stay for 2 to 3 hours, and the transition of the G, S plus G2 plus M has overall corrected of zeroth order kinetic process. Zeroth order kinetic process means that, any growth process is not dependent on the concentration, because, it is zeroth order; it is depends on certain constants. And, minimal cycling time for human cells, is like 12 hours, minimum. So, entire cycle time of the human cells is typically 12 hours, or more; that is the minimum cycling time. So, 8 hours. So, if you see, the typically, S phase is 8 hours, 8 hours here, plus 2 hours here, 10 hours; that means, G1 phase can be 1 or 1 and half and mitosis phase is very fast; so, half an hour, it completes; then total is 12 hours is the phase.

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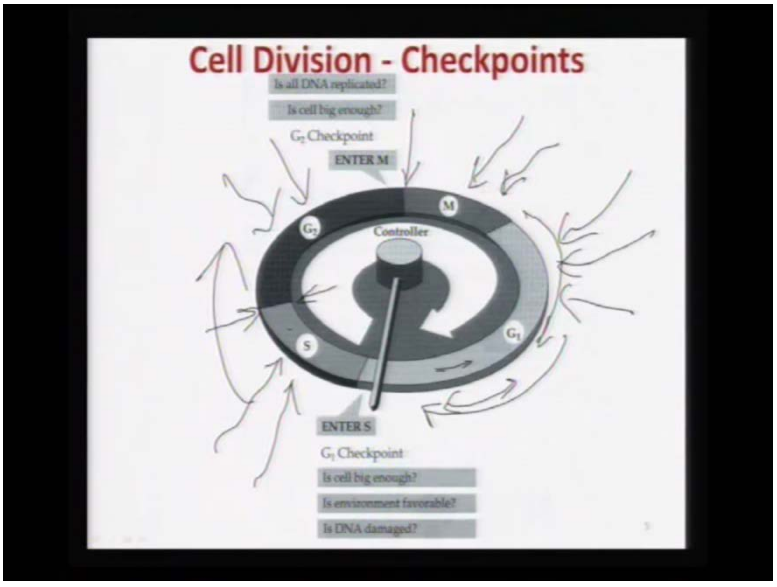

Now, this is very important in the cell cycle process; that is, it is controlled by the check points. Now, check points, if you see that, most of the Indian roads, at many points, you can see that, when you enter from one state to another, for example, from Uttar Pradesh to Delhi for example, so, there will be a check point at the border. So, then, they will check that, whether you are an authorized person, carrying some authorized goods and so on, and then, they will allow that, you can now enter to the another state, that is called Delhi; or similarly, between Bengal and Bihar. So, there will be check points. Now, in these checkpoints, similarly, in the cell cycle, what you notice here that, each time the cells enter from G1 to S, there is, there is a check point here. So, here, cells, there will be automatic checking for what? That is the G1checkpoint. Is the cell big enough? Is the environment favorable? Is DNA damaged? So, if the DNA is damaged, then, cells will

not even move to the S phase; the cell cycle will be stopped here, and cells will, cells will initiate its own suicidal program, which is known as the cell apoptosis. Are you getting my point? So, what I am, what I am trying to say here that, when cell is moving from one phase to another phase, G1to S, then, cells will check itself that, whether cells is big enough, for that it can be suitable, or whether the cell has correct environment; correct environment means, whether the cells are being constantly supplied by the nutrients, proteins, vitamins, etcetera, from the culture medium, that is where the cells are kept.

Now, if they are deprived of these, any of these things, or if the DNA of the cells are damaged, then, the cells will not simply move to the second stage; that is, the S phase. Now, after this S phase, then, again, there is a checkpoint here. So, similar checking will be done; after that, between G2 and M, again another checkpoint is there; again, this checkpoint that, the check, the checking would be there that, is all DNA replicated and is cell big enough, because, there is chromosome segregation and there is replication. Replication means, whether this is that, whether all DNAs all are replicated after the chromosome segregation, because cells, here, we have not indicated G1, S, G2 M, but, it is at particular stages of the cell cycle. So, what it shows, what this checking here, before you enter M, the cells will check itself that, whether its all DNA, which is available... So, suppose that, initial is DNA was small n. So, the amount of DNA would be now, 2n, right. So, if all the DNAs are replicated, so that, cells will simply enters mitosis phase and will go to the two daughter cells, simple, will divide into two daughter cells. So, this is the G2 checkpoint. So, all these checkpoints, again, I repeat, all these checkpoints are important, because, these checkpoints will control, or there will be policing; there will be policing here, at this checkpoint and if any of the criteria is not satisfied, then, simply cells will not move from that stage to the next consecutive stage, and cells will activate its own suicidal program. So, that cell will undergo apoptosis, or the death process. So, this is the important thing about the check points. Cell-cycle transitions are unidirectional and controlled by the checkpoints. What it means? Unidirectional means, it is always going like this; this is not a reversible process, remember that.

Once the cell will go from G1to S, it is never possible, just because cells are not enter into G2, cells will go back to G1; you understand what I am saying? So, it is all unidirectional process. So, if it is not able to enter from S to G2, from S phase itself, it

will activates a suicidal program, to undergo apoptosis. If it is goes to G2, then, if it is not able to enter M, it cannot come back to S simply; because, this is a unidirectional process, clear? The check points serve to order cellular biochemical events, that would not otherwise be biochemically linked. What it means that, all these biochemical events that are taking place at different stages of the cells, they are not linked to each other. Therefore, cells has to find out, whether certain biochemical process, which are supposed to take place in that particular stage has been completed or not.

If that has not been completed, what it will do, cells will stay in this particular stage for longer time period; and, even after longer time period, it is not able to fulfill its requirement, then, it will undergo cell death process. Now, before the cell allows entry into S phase, cell size, certain environmental conditions and DNA integrity to be checked. Similarly, before entering into M phase, biochemical mechanisms used to check whether DNA replication is completed. DNA replication is completed means, n number of DNA should go to 2n numbers. If the cell fails to pass these checkpoints, it may initiate apoptosis and die, that I have been mentioning several times now.

So, mitotic cell cycle is driven by a series of cell regulatory proteins and this is cyclin-dependant kinases and during cell cycle, a number of interacting cyclins and kinases goes through a highly orchestrated series of switches on and checkpoints. Switches on and switches off means, the meaning is that, this biochemical processes, which is occuring at G1, that is taking place only because, there are some intracellular signaling pathways are, activating of the cells to get into G1, ok. Similarly, you, it maybe, it may so happen that, other cellular signaling pathways are activating S phase; different set of signaling pathways are activating G2 phase, and different set of signaling pathways are activating the M phase. And, that depends on what kind of signaling pathways, that depends on what kind of cell type it is; whether it is fibroblast, whether it is neuroblast, (( )), etcetera, etcetera.

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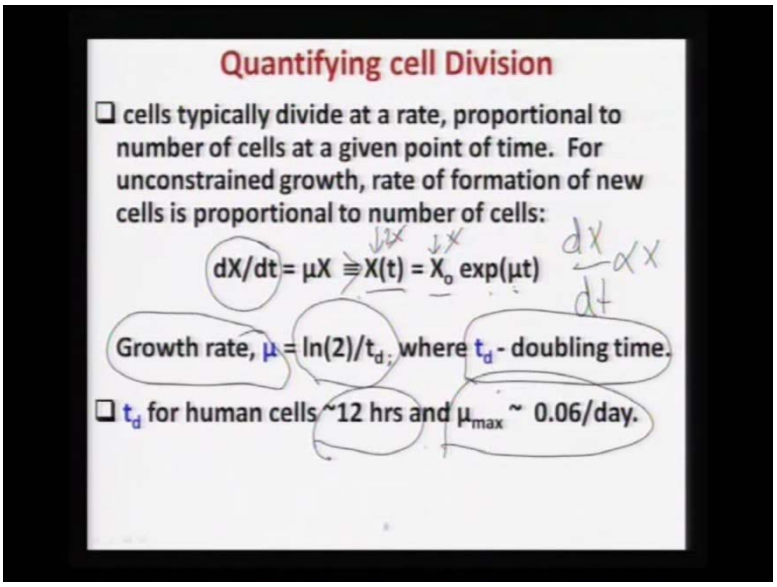

Now, this is the quantifying typical cell cycle. So, cells typically divide at a rate, which is proportional to the number of cells at a given timeframe; that means, at any given timeframe, let us say at point time t, the cell growth, or cell division rate d x by d t, where x is the number of cells at that point of time, is proportional to…So, d x by d t is proportional to x. So, that is, proportionality constant is mu. mu is nothing but, the growth rate, here. This is the growth rate is mu. So, from that, you can find out that, x t is nothing, but x naught exponential mu t. And typically, mu, you can find out ln 2 by t d, whereas, t d is the doubling time; doubling time means, you start with x, you go to the 2 x, ok.

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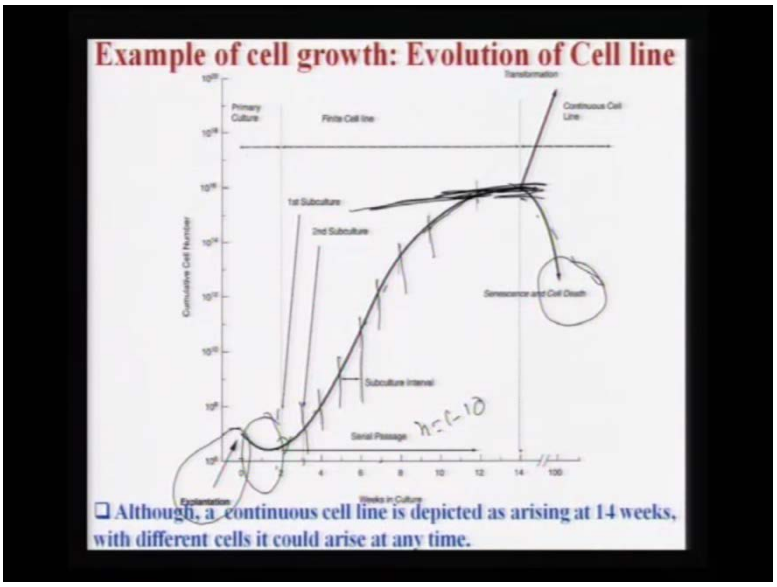

Now, if you, if you put these things here, you can immediately find out that, mu is nothing, would be ln 2 by t d; why, because, if you put here, this is 2 x here; it is, x, x, x canceled out; this is exponential; this is log. So, mu would be, that is, the t d. Now, t d, that is the doubling time for human cells, as I said, is 12 hours and mu max is the 0.06 per day. So, that is the typical growth rate. Now, this doubling time, again for the rat type of cells, for other type of cells is, maybe different; 12 hours to 24 hours, to different, 48 hours and so on. So, we do not know that, what is the…I mean, therefore, that is, doubling time for different type of cells are different.

Now, let us see that, how that human cells will evolve during the cell growth process. Now, in the cell culture, typically, cell culture, initially, what will happen? The cells are ex-planted from some organs, like, for example, liver cells; liver cells are ex-planted from the liver tissue, ok. So, tissue is nothing, but self-organized array of cells, with the exo-cellular matrix, which helps in the binding process. Now, if you have self-organized cells, these cells can be extracted from a tissue. Then, you have to do some primary culture. Primary culture means, initially, this culture, you have to culture in a particular solution, and this culture medium, you have to culture it for, maybe 1, upto 1 to 2 hours. Now, after to 1 to 2 hours, then, the cells will be cultured for several weeks, upto, sorry this is 1to 2 weeks is the primary culture.

Then, after that, you have to culture it for several weeks, upto 10 to 12 weeks, before the cells are ready for your use. Now, this kind, this stage, it is known as the subculture stage. Subculture stage means, when, what you have to do is that, you have to use the culture medium. Now, after the, after you go from one passage to another passage, you have to remove the culture medium; you have to add a fresh medium, ok. So, what you see that, a cell undergoes several subculture intervals here. Now, each interval can be one week, or can be less than a week. After several passage, so, what you see here, cumulative cell number increases. Cumulative cell number means, once you started with certain cell number; the total cell number at any given point of time, will continuously increase, because of the cell passages or subculture stages.

So, this is called serial passage, and this passage can be, n is equal to 1 to 10, or something like that; so, that means, you have to spend, in that subculture phase upto 10 to 12 weeks, and out of that, when it is reaching almost maximum, you can see yourself, if you are expert Biologist, you can see yourself that, when the cells are really in the congruent stage, or cells are really in the growing stage. And then, you stop, because, you know that, after that time, cell growth processes will almost reach a steady state. After that, cells will not grow any further, even if you continue passaging further. And, many stage, what will happen? Then, after the continuous cell cycle, if you keep on growing that cells, then cells also undergo certain death processes, or cells will undergo apoptosis process.

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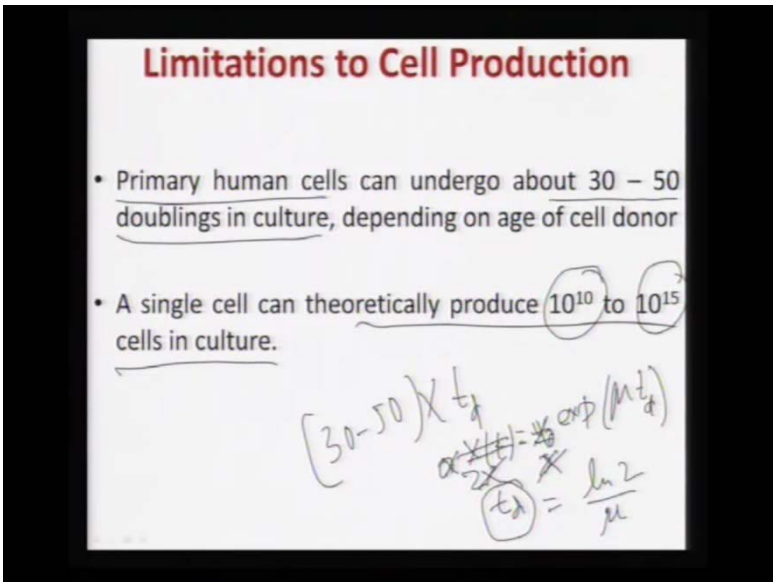

Limitation of the cell production, typically, primary human cells that can undergo 30 to 50 doublings in the culture. 30 to 50 doublings means, it you will undergo 30 to 50 times multiplied by t d. And, what is t d? That you comes from what? So, x t is equal to x naught exponential mu t, right. So, at t is equal to t d, what will happen? This will be 2 x; this will be small x, capital x. Therefore, t d would be ln 2 by mu, right. So, t d is equal to ln 2 by mu, and if you know the growth rate, then, you can find out what is the doubling time. Now, here, it has been mentioned that, 30 to 50 times doubling time is the total, actually can undergo depending on the kind of primary human cells. And, a single cell can theoretically produce 10 to the power 10 to 10 to the power 15 cells in culture; that is huge number of cells, that in single cell can produce; but that is theoretically, because many times, this numbers may not be achieved in the real situation, depending on the culture medium.

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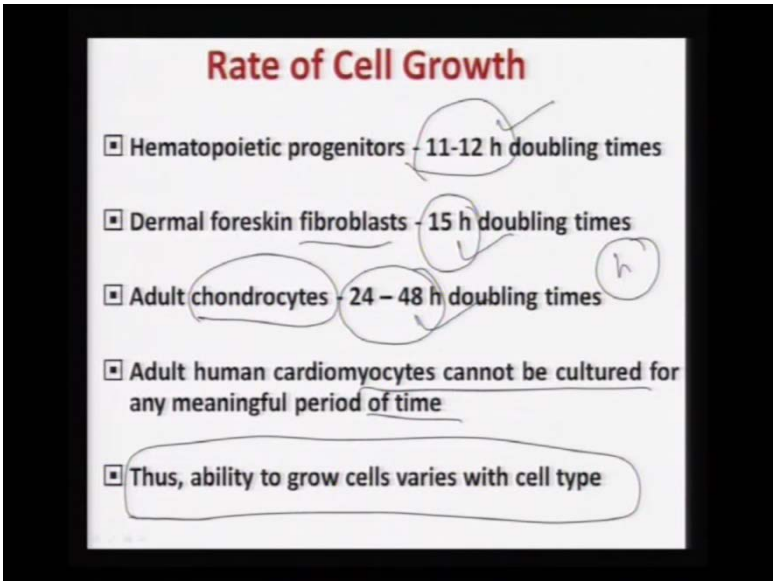

Now, just few feeling for the numbers, as I have given you for the cell migration. Now, hematopoietic, the progenitor cells, this is 11 to 12 hour doubling time, whereas, dermal foreskin fibroblasts, this is typically 15 hour doubling time adult chondrocytes, that is cartilage tissue cells, typically it is 24 to 48 hour doubling time, and adult human cardiomyocytes cannot be be cultured for any meaningful period. So, what you see here, that the ability to grow cells also vary with the cell type. Typically, it is of the order of few hours, or sometimes, 1 or 2 days. So, this is the 12 to 15 hours is the fibroblast cells, whereas, 24 to 48 hours is the chondrocyte cells' typical doubling times.

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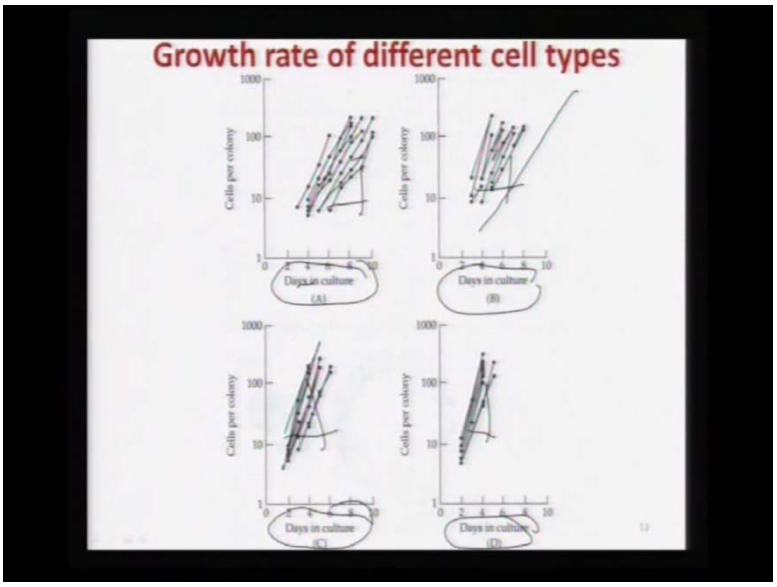

Now, this is again, is the, just to show you that, you know that, if you, if you cultured the cells in a particular culture medium, their growth rate also different, depending on the type of cells. So, I have not put that different cell name here. So, what it shows here, a, b, c, d different graphs; they are obtained with 4 different cell types and you can see qualitatively, the graphs are similar, because, cells per colony increases with time in the culture. But, however, the slopes, if you see, these slopes, these slopes are different. So, these slopes are different means, their growth rate is also different; that means, that mu value is different, for all 4 different cell lines.

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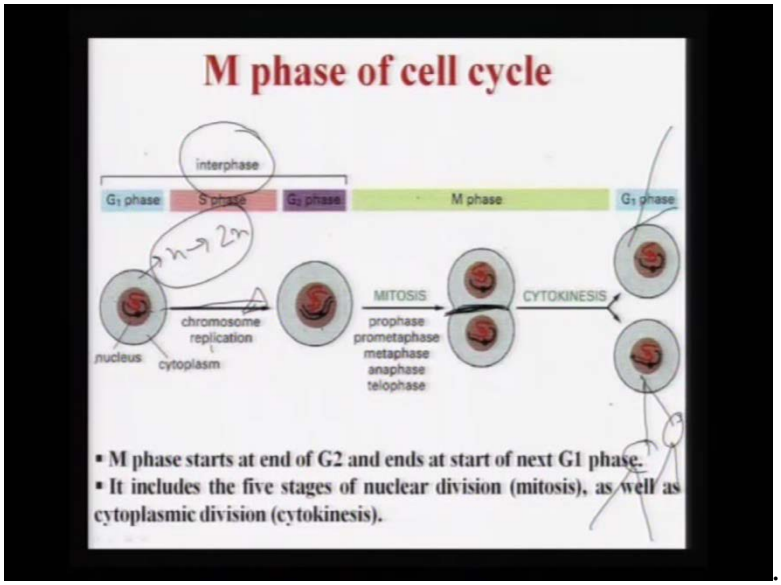

Now, again, coming back to the cell cycle, the description of the cell cycle. What I have shown you earlier that, typically interphase is the longest time period, over which a cell stays in a typical cell cycle. An interphase contains G1, S, G2, this three phase and your M phase is the mitosis phase, ok. So, G1, S, G2 phase also, at individual phase boundary, you have the checkpoints. And again, that strictly the checkpoint is maintained when the cell will go from G2 to M phase. Now, here, it is nucleus and this is cytoplasm, then, there is chromosome replication here. Now, when it will go to the G2 phase, and when G2 to M phase, it will check whether this is the n chromosome has been gone to 2n chromosomes here. So, the number is doubled, so that, in M phase it can simply go to the mitosis and cytokinesis; that means, there is a cytoplasm division and it goes to 2 daughter cells. Now, 2 daughter cells can give rise to another 2 daughter cells, when individually they will go to the cell cycle. This daughter cell also give rise to another 2 daughter cells and individually, these 2 daughter cells again, it will go rise 2 another daughter cells. So, you, what you can see that, one, in one doubling time t d, 1 cell to 2 cells, 2 cells to 4 cells, 4 cells to 8 cells, like that it will go on, during the cell death, I, sorry, cell cycle processes, in order to increase the cell number.

So, again, coming back to the real life processes, you have to, this 1 to 2, 2 to 4, 4 to 8, all these processes, in between at certain time, you have to change your media, you have to use the fresh media; because why, when a number of cell population increases, then, they will consume the food and nutrient from the media. So, there may not be fresh

nutrients, which are available in the medium for the newly growing cells. Therefore, if you change the medium, in biological language, it is called passaging; passaging, means, n is equal to 1 passage, n is equal to 2 passage means that, at every time interval, you change the medium; you add fresh medium; and then, this way, you have to do like, 5 to 6 times, 5 to 6 passaging, so that, cells will continuously grow in the medium. And then, cell numbers will also, will grow with time and as a result, that cells will slowly reach the steady state growth process.

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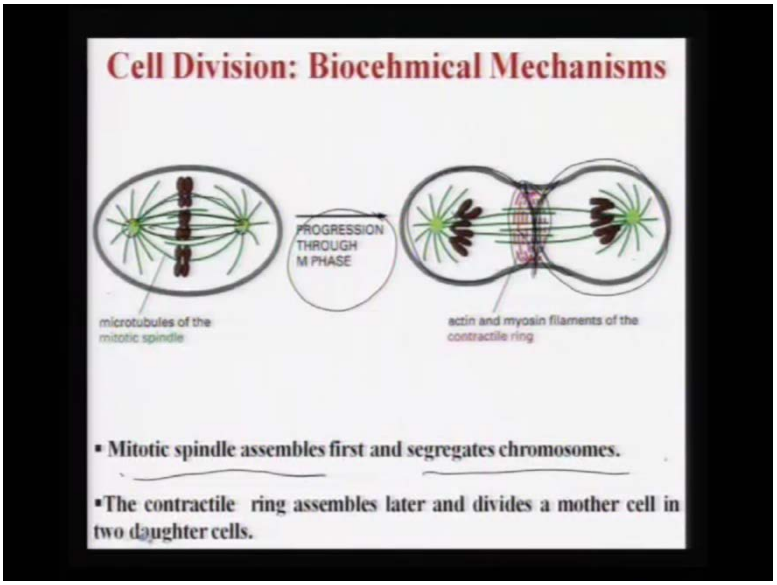

This is the biochemical mechanisms, what it shows here that, during the progression through M phase, this is some myosin motor proteins that are activated; this is your, green ones is what, that is the actin filaments, right. Now, actin and myosin filaments here, here this is that actin and myosin filaments are the contractile ring; now mitotic spindle is, is essentially assembles first, and segregates chromosome here, and the contractile ring assembles later and divides a mother cell to a 2 daughter cells. So, here is this, 1, 1, from 1 parent cell, you are producing 2 daughter cells, and what you see here that, this contractile ring here, it is producing 1 daughter cell here and here, it is another daughter cell.

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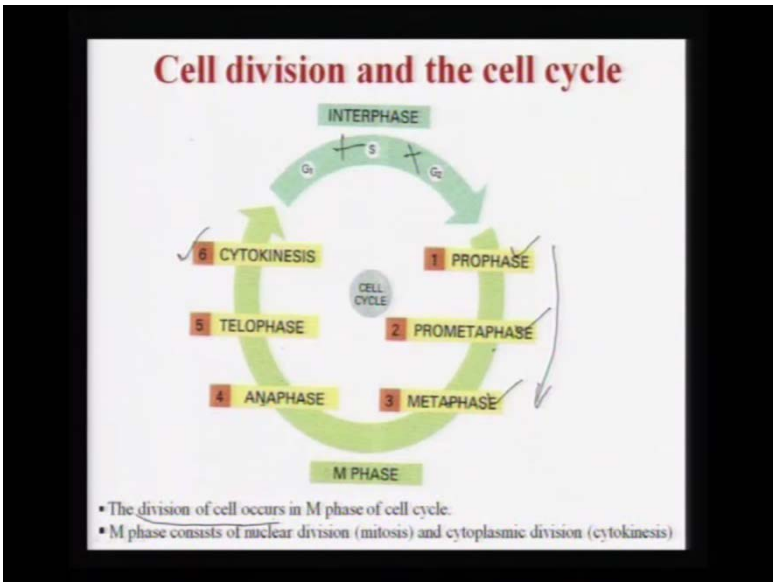

So, cell division and the cell cycle, once it goes from G1, S, G2, that is interphase, what will happen? It goes through the M phase. In the M phase again, in the microscale, it can be divided into several stages. Remember, what I am saying. What I am saying is that, you know already, interphase , G1, plus S, plus G2, G1, plus S, plus G 2. Once it goes into the M phase, although the M phase in the total cell cycle time, it shows only lower fraction, much lower than the total interphase timescale, but, this M phase also, biologically, it can be subdivided into 6 different phases. And, what is the 6 different phases? One is the prophase; one is prometaphase, and third one is metaphase. Again, you can remember, if you apply some logic, prophase, prometaphase; prometaphase means, something before the metaphase.

So, therefore, next phase would be metaphase. Then, there would be anaphase; there will be telophase, and there is cytokinesis. So, in this prophase, prometaphase, metaphase, anaphase and telophase, now chromosome are segregated into 2, 2 would be daughter cells; not yet, they had become, they have daughter cells; only when cytokinesis is completed, then, cytoplasm is exactly divided into 2 daughter cells, and then, that is 2 daughter cells are now complete, ok. Now, these 2 daughter cells can now enter G1, plus S, plus G2 phase and then, you will produce again 4 daughter cells; then, 4 to 8, 8 to 16, like that, it will go. Essentially, division of cell size cell occurs in the M phase but, G1, S, G2 phase is the interphase process, when cell actually prepares itself for the, for undergoing into the mitosis phase, and therefore, this preparation phase, this G1, plus S,

plus G2, is important for the cell cycle. If the cell cannot pass through this G1, S, G2, then, cells cannot simply enter into the M phase and cells cannot undergo the cell division phase.

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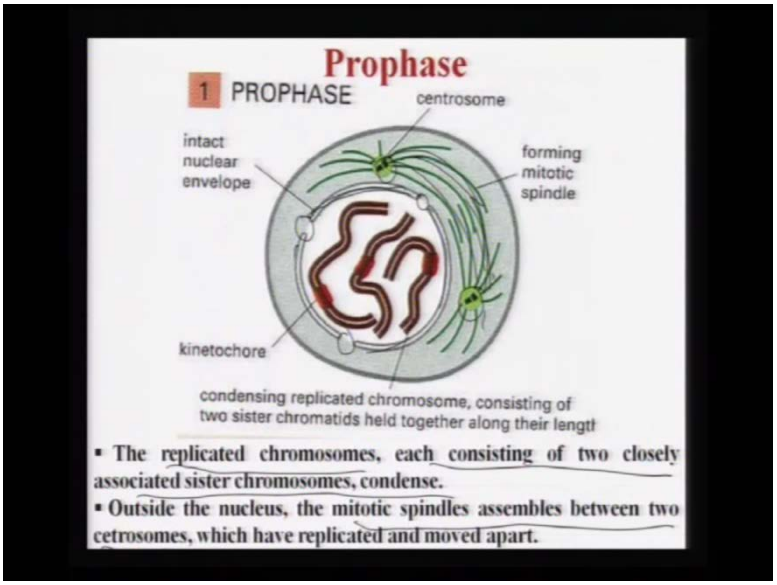

So, what happens in the prophase, that is the phase number 1, inside the M phase. So, phase number 1 means, here it is the centrosome and you have the intact cell membrane here. Now, there is, this is the mitotic spindles and myosin motors are here, and this is your actin filaments. The replicated chromosomes, each consisting of 2 closely associated sister chromosomes, they are in the condensed form in the prophase and outside the nucleus, mitotic spindles assemble between 2 centrosomes. So, this is 1 centrosome; this is another centrosome; between 2 centrosomes, mitotic spindles start assembling and chromosomes is more in the condense form. Let us see, what happens in the prometaphase. In the prometaphase, what happens, it starts abruptly with the, with the breakdown of the nuclear envelope. Here, if you go back to the prophase, here, nuclear pore complex are there; this is the nuclear pore complex. So, remember, and nuclear membrane are also intact. Now, in order to produce 2 daughter cells, you have to produce 2 nucleus also, right; but now, there is only 1 nucleus. So, the formation of 2 nucleus is possible, unless your nuclear membrane is also destructed, or fragmented, right. This is comes from a basic physical logic. So, what you see here that, these fragments of the nuclear envelope that you see and the myosin motors, then interact with this DNA chromosome, and here, it is becoming, slowly, you can see that, there will be

segregation of the chromosomes at two different regions, and chromosomes is now attached to spindle microtubules via kinetochores and undergo active movement.

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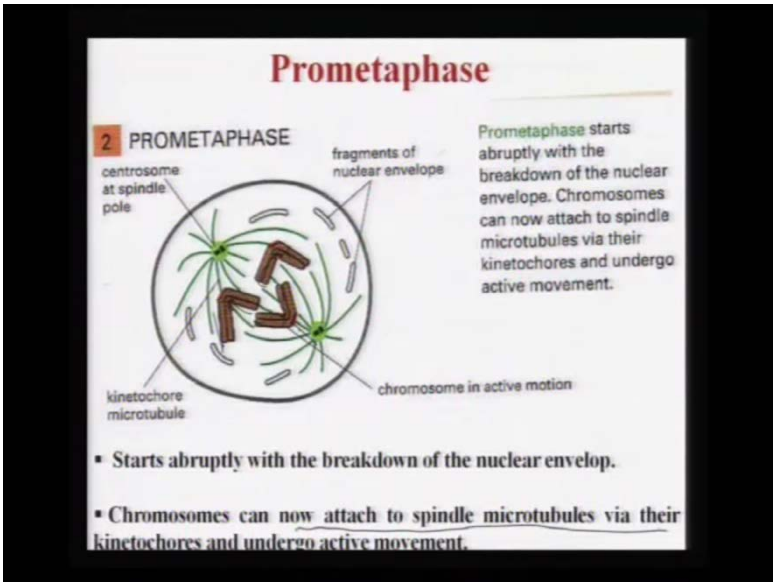

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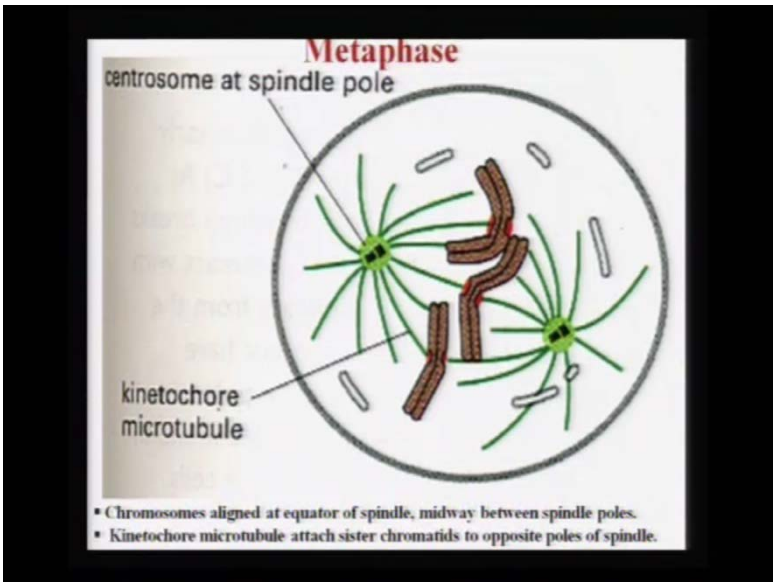

Now, in the metaphase, what you see very clearly that, this chromosome, this is the mitotic spindles here; this is your chromosomes, which are being segregated and which is out, which is still in the very loosely bound nucleus here, and there is no intact nuclear membrane, which is surrounding these chromosomes.

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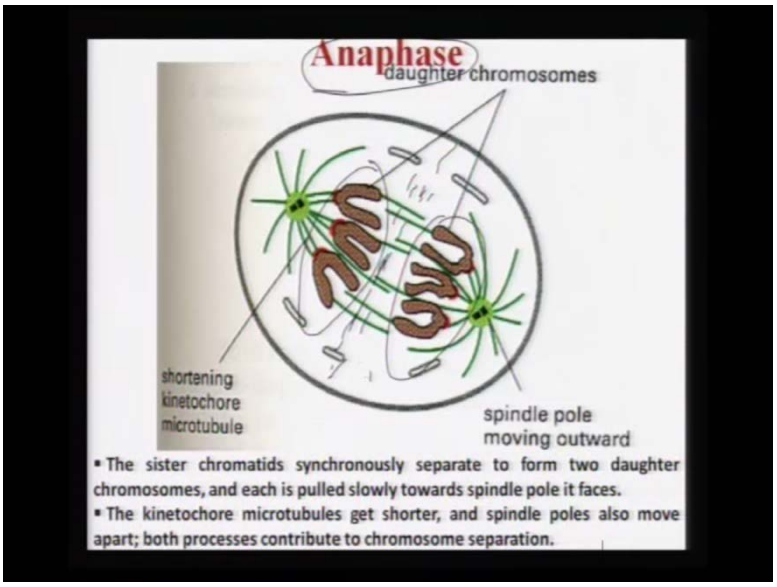

And eventually, they are trying to segregate, from this chromosome and this is the one end, this is another end, ok. So, in the anaphase, it starts this splitting very clearly; splitting into the nucleus into, from 1 nucleus to 2 nucleus and in the, after the telophase, it is exactly very clear, as you can see here, there is contractile ring also to starts forming, and there is overlapping microtubules.

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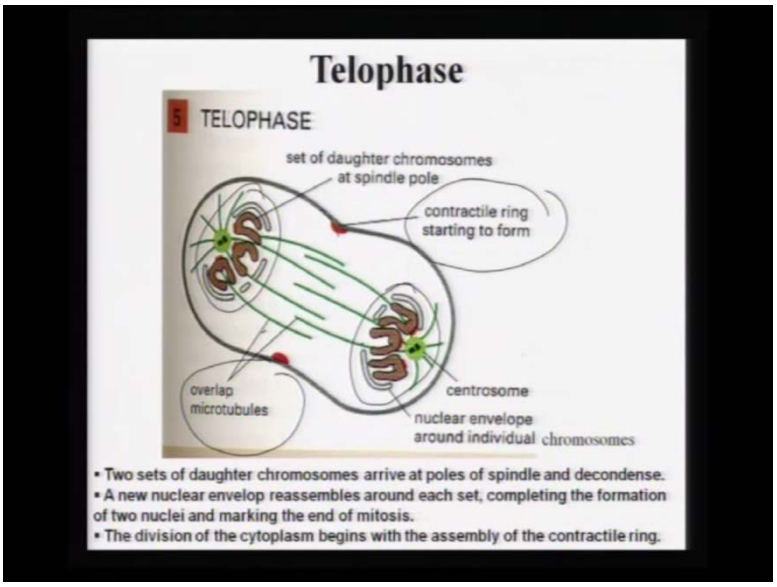

So, this 1 nucleus is starts forming at one end of the cell; another nucleus starts forming at the another end of the cell and after sometimes, what will happen at the, after the

telophase, then, it comes your cytokinesis. So, before the cytokinesis, this is the last phase, telophase. Telo means, end, almost, right and telophase means, here, this is the, cells are almost prepared to undergo, now cytokinesis process and in the cytokinesis process, here, you can see that, it contracts here at the contractile ring.

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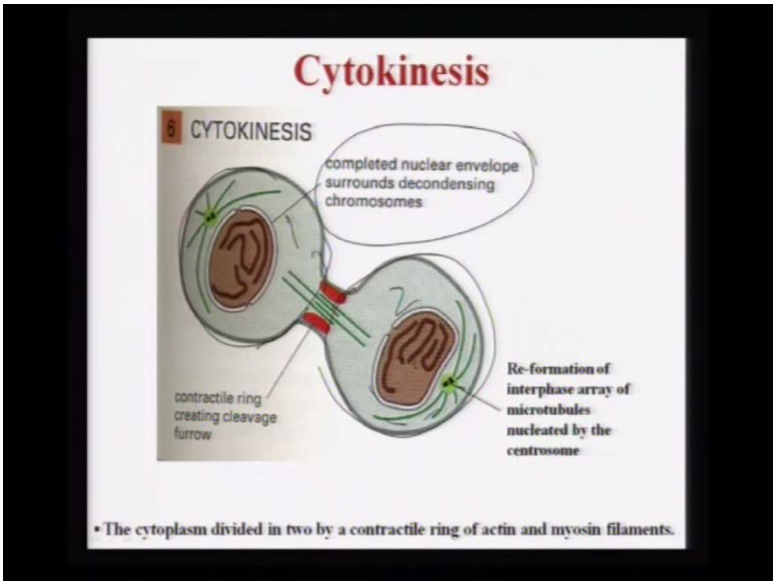

Contractile ring means, something it helps in the contraction; exactly at the middle of the parent cell, and once it contracts, then, these cells, this is cell number 1, daughter cell, this is cell number 2, another daughter cells. So, this is one cell, this is 2, cell number 2. And then, again, there is reformation of the nuclear envelope. So, completed nuclear envelope surrounds the decondensing chromosome. So, these, this is the decondensing chromosomes and then, again, the nuclear pore complex and nuclear membrane is, formation is possible. So, all this biological membranes, if required, they can be broken and they can be reformed. And, one of the example I have shown you here that, how nuclear envelope, when time is required, that becomes fragmented and when at the end of the telophase, it becomes again reformed, and cytokinesis is formed in the 2 distinct sister cells, or daughter cells. So, this is the interphase; again it starts.

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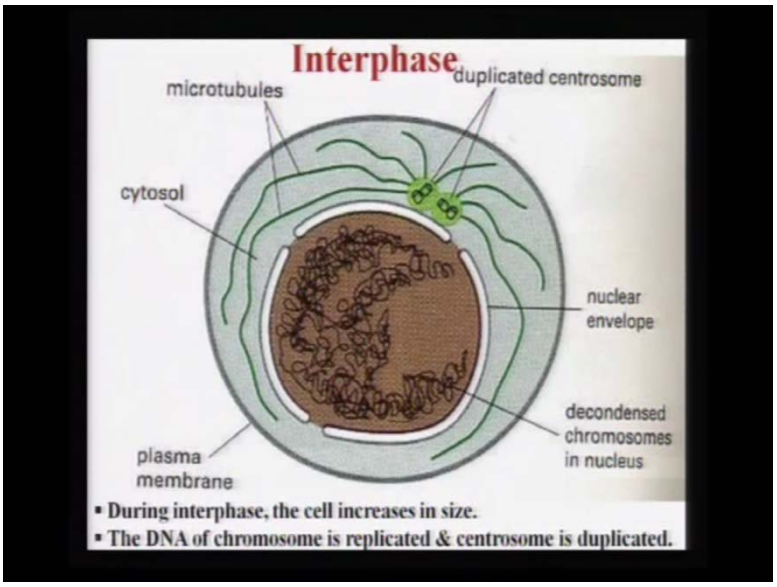

In the interphase, you can see that, chromosomes, DNA are getting replicated and it is becoming more and more condensed, and during interphase, cell increases its in size also. And, this increasing size is possible, because of the more protein synthesis, or more metabolic activity has to be there inside the cells, because cells can undergo atrophy and hypertrophy. Atrophy means, increase in size, or decrease in size; hypertrophy means, increase in size. The, all this cellular adaptation process is possible, because, that increase and decrease in size means, when cells will decrease in size, cells start an intercellular, the constituents has to come out; when cell wants to increase in size, that means, more proteins has to be synthesized in the cell and therefore, it will undergo hypertrophy. The DNA of chromosome is replicated and centrosome is duplicated.

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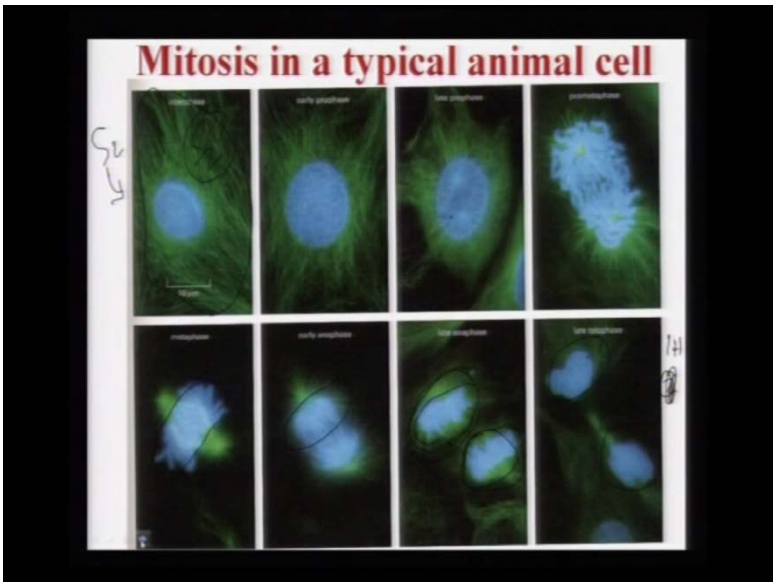

And, this is the typical mitosis in a typical animal cell. What you can see, this is a very nice fluorescence scenarios, right, and this fluorescence scenarios, this is a kind of sequential processes; it shows that, how this nucleus is being also duplicated. You start from the top left, it is the interphase, green stain region is that your cytoplasmic area, actin filaments; blue stain region is your nucleus area. Typical cell cycle is 20 to 25 micron, and that is why, if you see that micron bar here is a 10 micron, ok.

Now, in the interphase area, cells have increased in size; you can see that large cells. In the early prophase, late prophase, prometaphase, that means, it is a, in the M phase here; it is already in M phase. And here, this is the end of the S1, G, S2. So, that is the end of the S2 phase here. So, the cells have passed the check point; it is entering the M phase; it is the early prophase; it is the late prophase; this is the prometaphase; this is the metaphase. Metaphase, you can see here that, the, in the nucleus itself, there is mitotic spindle and they are actually attaching to the chromosomes. Early anaphase here and late anaphase here; this is their 1 daughter cell; this is 1daughter cell. But still, these daughter cells are not completed. At the late telophase, you can see, there is cytokinesis and there is, this daughter cells would be 2 daughter cells. So, this is like, 2 daughter cells here, ok.

So, you started with 1 cell; you end up with the 2 cells, one plus one. So, this is, this is what a series of events, that take place in a typical mitosis cycle, typical mitosis phase, M phase; but, remember if a total cycle time for the human cell is 12 hours, this mitosis

phase, actually takes place sometimes, in 1 hour or half an hour, because your maximum period, that is cell, stay in a cell cycle is your interphase. As I mentioned earlier that, G1can be variable phase; S can be typically 7 or 8 hours and G2 can be 1 or 2, 1 to 2 hours. So, 8 plus 1 plus 2 means, almost like 10 to 11 hours; rest time, the cells spends in the mitosis, M phase. And therefore, you can understand that, mitosis is a small timeframe, over the, when you consider the longer timeframe of the cell cycle.