Cell Biology: Cellular Organization, Division and Processes

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Lecture 27

Mitosis Part II

Hi, I am Shikha Laloraya, Professor of Biochemistry at IISc. Welcome to this lecture on mitosis, which is about the structure and the assembly of the mitotic spindle and the process of chromosome segregation, which are highly complex and dynamic events.

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There are two main cytoskeletal machines in the cell, which are important for mitosis. One is the mitotic spindle, which is important for chromosome segregation and the other is the contractile ring, shown here in purple, which is important for cytokinesis. The spindle is made up of tubulin filaments or microtubules, while the contractile ring is composed of actin and myosin. The spindle microtubules attach to the sister chromatids and help to pull them away to the spindle pole after they separate. The contractile ring constricts, dividing the cell into two equal daughter cells.

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This set of images shows human cells showing the stages of cell division starting with interphase and the cytoskeleton or tubulin is what is of interest to us in this lecture, it is depicted in green. We can see the changes in the organization of the cytoskeleton, as the cell changes and goes through different stages of the mitotic cell cycle. The stages shown early prophase, where here the centrosome is not yet separated. And late prophase where the centrosome is separated and DNA condensation has started to occur and also here the nuclear envelope breakdown has occurred. In prometaphase you can see some formation of the bipolar spindle beginning to form but all the chromosomes are not yet attached to the spindle and neither have they attained bi-orientation. In metaphase you can see that the assembly of the spindle appears to be complete, that is, a bipolar spindle has been established and most of the chromosomes appear to be attached and also are aligned at the equatorial plane. In anaphase, after the sister chromatids separate the spindle fibers pull the sister chromatids away from each other towards the pole. So, now you have two sets of

segregated sister chromatids. In telophase, there is a formation of the mid body and the cells begin to flatten out and also there is the disappearance of the organized spindle over here.

In early cytokinesis, the chromosomes are now getting decondensed and the nuclear envelope has reformed. And in late cytokinesis, the cells actually have divided and they move apart.

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The centrosome is a complex organelle, that is not membrane bound and it is very important for spindle assembly. The centrosome is a structure that modulates cellular processes such as proper spindle assembly and placement, and also cilium formation. A centrosome is composed of these two barrel shaped structures, which are referred to as centrioles; these are by the way two different depictions of centrosomes. The centrioles are approximately 450 to 550 nanometers in length and 250 nanometers in outer diameter, and they contain cylindrical arrays of a triplet repeat. So, there are triplet microtubules here, which are organized with a nine-fold radial symmetry. The centrioles are polarized along their proximal to the distal axis, that is the proximal and the distal ends are not identical, biochemically speaking.

In animal cells the centrioles are surrounded by a matrix, which consists of several large coiled-coil proteins of the pericentrin family, which also anchor other matrix proteins. The matrix surrounding the centrioles is referred to as the pericentriolar material or the PCM. The centriole as I already mentioned is not surrounded by the membrane. And we will also discuss about one of the components of the matrix, the gamma tubulin ring complex, in detail, which is important for the function of the centrosome in nucleating microtubules.

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Now each centrosome has a pair of centrioles, as I already mentioned, and one of these centrioles is older and it is the mother centriole and the other is the daughter. The centrioles are surrounded again by the pericentriolar matrix and this as I already mentioned has got many proteins, it is got hundreds of proteins in fact, cell cycle regulators, signalling molecules, microtubule organizers such as a gamma tubulin ring complex. And it functions as the microtubule organizing center or the MTOC thereby facilitating the establishment of the mitotic spindle. Now to establish a bipolar mitotic spindle, a single centrosome needs to be duplicated into two mitotic centrosomes and this happens during S phase during which the mother and the daughter centrioles template a new centriole, a pro-centriole, that forms orthogonally from the parent centriole. So, the new centriole over here is shown in yellow, just for clarity.

Now the steps in the centrosome duplication cycle are that in G_1 the two centrioles which are shown here very close to each other they separate by a few microns or so. In S phase, the daughter centrioles, these yellow things over here, begin to grow at the base of each centriole and these daughter centrioles are perpendicular to the original mother centriole.

In G_2 phase, the growth of the daughter centrioles has been completed and in M phase the centrosomes finally, that is the PCM part of it also, splits into two and it begins to separate and each part of this centrosome has received a pair of centrioles.

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After the separation the centrosomes they of course move apart further, and they start nucleating individual asters of microtubules, as can be seen here in prophase where you can see this green coloured depiction of microtubules, which are radiating out from each centrosome. Then the bipolar spindle is assembled by metaphase and this of course helps in chromosome segregation.

So, what you can see is now the original centrosomes with the two centrioles each are present at the poles of the bipolar mitotic spindle. And of course, the spindle plays an important role in chromosome segregation. But after segregation the cell divides and each of the daughter cells inherits one centrosome and the spindle also gets disassembled. So, the microtubules of course are still there but they are not oriented in the fashion such as to form the spindle.

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In animal cells, the MTOC or the microtubule organizing center, which is also known as a centrosome we just discussed is located near the nucleus. It nucleates microtubules such that the plus ends point outwards. So, the minus ends are towards the centrosome whereas the plus ends of the microtubules are radiating and pointing outwards. The MTOC consists of a pair of centrioles and a fibrous centrosome matrix and it has many proteins as I already mentioned. And it may have more than 50 molecules of gamma tubulin ring complex and also various other regulators.

So the gamma tubulin ring complex is shown here; this is this deduced structure of the gamma tubulin ring complex. And originally biochemically it was found to be composed of eight polypeptides and it has a ring-like structure having a 25 nanometer diameter. The gamma tubulin ring complex, I will often refer to it as gamma-TuRC, nucleates microtubule assembly.

So, two subunits of gamma tubulin associate with two accessory subunits and they form what is known as the gamma tubulin small complex or gamma TuSC. Now seven copies of this gamma tubulin small complex associate to form a spiral structure; you can see the spiral organization here and it results in the formation of a spiral ring of 13 exposed subunits and the 14th gamma tubulin, which is located beneath the first one,so, it is overlapping. So, in the ring there are 13 exposed subunits.

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Shown here is the structure of the native human gamma tubulin ring complex, which was determined by electron microscopy. So, this is a very interesting structure; it has got an asymmetric cone shaped structure and the gamma tubulins are arranged in a helical geometry and they are

poised to nucleate microtubules. The native gamma tubulin ring complex presents at least 13 gamma tubulin molecules on the face of the cone. And there is also a structural bridge that includes an actin like protein and this spans the gamma-TuRC lumen; this was not expected but it was found in the structure, you can see it here I think. There is also diversity in the gamma-TuRC subunits and this introduces large surfaces that may allow for interactions with many different regulatory factors.

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The mitotic spindle consists of the centrosomes, all right, we have already discussed this most important part of the spindle. Microtubules are also very important part of the spindle and they are these green filaments shown here and we will discuss more about their structure in this lecture. And kinetochores, which are a large protein complex associated with the centromere region of the sister chromatids. The kinetochore actually bridges the microtubule to the chromosome.

There are three classes of microtubules, the astral microtubules shown here, which are projecting outwards as an aster from the centrosome, the kinetochore microtubules, which are attached to the kinetochore, and interpolar or overlap microtubules, which basically overlap with microtubules in an anti-parallel orientation. These overlapping microtubules are coming from opposite poles and their orientation of course is anti-parallel with their plus ends facing away from each other. So, the minus ends therefore of all the microtubules are focused at the spindle poles and the plus ends as you can see, they radiate outwards away from the poles, regardless of the type of microtubule it is.

And there are also a couple of additional interesting points about the spindle organization that I should mention here. That higher plant cells seem to nucleate microtubules at several sites, which are distributed along the nuclear envelope. So, you do not see a pole like structure there. So, there are no centrosomes in higher plants, and also the microtubule organizing center or MTOC in fungi and diatoms is embedded in the nuclear envelope and it is referred to as the spindle pole body (SPB or spindle pole body), which also does not have centrioles and therefore also no astral microtubules are present in these cases.

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Microtubules are filaments that extend throughout the cytoplasm and their function is mechanical, it has to provide support. Microtubules are composed of alpha and beta subunits of tubulin. Microtubules also provide tracks for the movement of two different types of protein motors, kinesins and dynein. Microtubules form the main part of the mitotic spindle; they attach to sister chromatids at the centromere via kinetochores and they are important for chromosome segregation.

The mitotic spindle is bipolar and we already discussed that the spindle microtubules are of three types. The interpolar microtubules, the plus ends of these particular microtubules overlap with the plus ends of microtubules coming from the opposite pole and they form an anti-parallel array in the spindle mid-zone and they can slide over each other. Kinetochore microtubules, in this case, the plus ends of these microtubules are attached to the kinetochores, which are large protein complexes that are assembled on the centromere of each of the sister chromatid. Astral microtubules are the

microtubules that radiate out from the pole and they are in contact with the cell cortex, and they help in spindle positioning within the cell.

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Shown here is the structure of a microtubule. Microtubules are composed of a cylindrical tubule, which is made up of the protein tubulin. Microtubules have got a hole in the middle, which is referred to as a lumen as can be seen here in the transverse section of the cartoon of the microtubule. Tubulin itself is a heterodimer of alpha and beta tubulin, and these tubulin dimers they stack side by side to form the cylindrical microtubule filament. Within the microtubule, lateral as well as end on interactions among the subunits stabilize the structure. And in this assembly, a single filament is referred to as a protofilament; 13 such protofilaments make up the cylinder that has an outer diameter of 25 nanometers.

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Now the structure of alpha and beta tubulin dimer is shown here and it reveals some interesting insights. What you can see here is that the alpha tubulin binds to GTP tightly such that it is an integral part of the protein and actually it is located at the interface of the alpha and the beta tubulin. The beta tubulin binds to the GTP less tightly and this end is exposed at the plus end of the microtubule. GTP hydrolysis therefore only occurs in the beta tubulin subunit and so, you can have a GTP bound beta subunit or a GDP bound beta tubulin subunit. They both have different conformations such that the GDP tubulin has a somewhat curved or different conformation that tends to depolymerise. And the GTP tubulin tends to polymerize or the polymerized state is more stable. It can form more stable interactions with the other subunits.

A growing microtubule has GTP tubulin subunits at the plus end forming a GTP cap, and there are certain drugs that bind to different tubulin subunits, a drug known as Taxotere is shown here in this structure and we will discuss more about these drugs in a minute.

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So, as I mentioned there are certain chemicals, which could be either synthetically derived or derived from natural sources such as plants that can bind and inhibit microtubule assembly or dynamics. For example, there is a drug known as taxol or paclitaxel, it binds along the filaments and it stabilizes the filaments, in fact. And of course, you know the spindle is a dynamic structure and spindle dynamics is important for its function and activity. So, having a very strongly stabilized spindle and microtubules is detrimental. Another drug is the nocodazole, which we commonly use in the laboratory for synchronizing cells in the M phase of the cell cycle. So, this binds tubulin subunits and it actually depolymerizes microtubules. Another drug commonly used is colchicine, which caps the filament ends and it also depolymerizes microtubules.

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The nucleotide GTP is bound to the beta tubulin and it affects its structure and properties, particularly with regard to the stability of the microtubule. The subunits as I mentioned can either be GTP or GDP bound and the GDP bound form has got a curved conformation of each individual subunit and this is inconsistent with stable assembly of a microtubule. So, a protofilament having these GDP bound subunits tends to be curved as is shown in this cartoon.

In an intact microtubule, the protofilaments which are made from GDP containing subunits, if they were to be there internally, are forced into a linear conformation by the many lateral bonds within the microtubule wall and particularly when a stable cap of GTP containing subunits is also present. However, loss of the GTP cap causes the GDP containing protofilaments to change into their more curved conformation and this leads to destabilization of the end and disruption of the microtubules can be seen here by loss of the subunits.

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Microtubules are highly dynamic structures; they exhibit dynamic instability. This is a property, which is measured in vitro and what it means is that an individual microtubule can go through growth as well as disassembly with respect to time. So, in this graph a single microtubule end is being tracked that is its length or the variation in its length with respect to time has been plotted.

So, the same microtubule can go through four different stages or events that you can see in this graph. One of them is assembly or growth depicted by the slanted blue line over here, this is when the microtubule end is growing, it is acquiring more subunits and adding them and growing in length. Another event that can happen is referred to as a catastrophe and that is a rapid transition from the growth phase of the microtubule to the shrinkage phase of the microtubule. So, the microtubule that was growing, suddenly starts shrinking at that same end, and this phase is known as disassembly or it involves shrinkage of the microtubule. And this transition here is referred to as a catastrophe. This disassembling microtubule can also be rescued that is it can change from shrinking again to growing, and this event is referred to as a rescue event. And this all depends upon the local availability of GTP or GDP bound subunits, where this microtubule is present.

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It has also been observed that mitotic microtubules are more dynamic than interphase microtubules. In this experiment a technique known as FRAP was used. FRAP stands for Fluorescence Recovery After Photo Bleaching. So, in this type of experiment, a laser beam is used to bleach microtubules, which are labelled with fluorescent tubulin and then the recovery of fluorescence in the bleached region is monitored as a function of time.

So, it can be observed here in this experiment that the recovery of fluorescence happens very quickly in microtubules, which are in metaphase relative to microtubules which are in interphase cells. And

this means that the mitotic microtubules are more dynamic, they are losing and gaining subunits, their subunits are getting replaced. And this could be important for the dynamic events that occur in mitosis such as spindle assembly, attachment of sister chromatids, and chromosome segregation, for all of which, microtubules are very important.

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Dynamic microtubules are also found within the assembled spindle, as shown by this interesting experiment from the Mitchison lab. Now in this experiment, the *Xenopus* egg extract system was used; we have discussed the *Xenopus* egg extracts earlier. So, when sperm DNA is added to the *Xenopus* egg extract then it results in the formation of a metaphase spindle in vitro. And this is because these eggs have accumulated lot of proteins and factors required for cell division, they are poised to enter cell division. And you know that is where the MPF was first discovered in this system. So, in this experiment the DNA is being detected by a DNA binding dye DAPI and it gives a light blue fluorescence. And in this experiment, while taking the *Xenopus* egg extract, mixing it with the sperm, in this reaction two different types of tubulin were added, these are fluorescently labelled tubulin. So, one was labelled with rhodamine. So, there is rhodamine tubulin, which fluoresces red in colour upon UV excitation and also there was caged fluorescein tubulin. Now this means that it was invisible it is not fluorescing when it was initially added in the reaction. But its fluorescence can be uncaged by UV irradiation. So, UV activation of the caged fluorescein tubulin results in the formation of uncaged fluorescein tubulin, which gives a yellow fluorescence, which can be detected.

So, when this yellow fluorescence was uncaged by using UV to illuminate a stripe to one side of the DNA mass in the spindle and then the signal was monitored as a function of time, it was observed that it was dynamic. So, this is already a preformed spindle, which was formed in this experiment and we can observe the DNA. So, this is a metaphase and they went and UV irradiated just a band to one side of the DNA on one half of the spindle, and thereby activated the fluorescence of the caged fluorescein tubulin. And then did a time course analysis and what was observed was after 1.5 minute and 2.5 minute, fairly quickly, the tubulin within this overall assembled spindle, which does not seem to be changing much in structure, seems to be moving poleward. So, it does not remain where it originally was but this band of tubulin it appears to be moving towards its respective pole but overall the spindle remains largely unchanged.

And another interesting thing to note is that the signal of this activated fluorescein tubulin appears to be reducing or diminished and what this implied was that individual microtubules are constantly depolymerizing and being replaced within the spindle, which overall looks like you know there is nothing much happening there. So, there are two conclusions, that there is poleward flux of tubulin within the mitotic spindle. And also that individual microtubules are also constantly depolymerizing and being replaced within the spindle. So, this experiment demonstrates that the microtubules within the assembled mitotic spindle are also quite dynamic.

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Now how do the microtubules attach to the sister chromatids? We have discussed this a little bit but we can look into this more closely now. So, this happens via the kinetochore which is a large protein complex that assembles at the centromere of a sister chromatid; the kinetochore is shown here. So, here in this figure there is a metacentric chromosome diagrammatically depicted and it shows the centromere, which is the primary constriction. The chromosomal arms are shown here, which are paired to each other and not shown but of course at the ends you have telomeres, which are the protective repetitive ends. And as you know by now that each sister chromatid is paired to the other one, and so in a metaphase chromosome that looks like this, there are actually two DNA molecules. Now here at the top is shown the centromere DNA and in many cases it is recognizably different from other flanking DNA, and a centromere specific histone binds to this region. So, the nucleosomes in the centromeric region are different in composition and also in their arrangement; generally close to the centromere there is a more heterochromatin-like or more compact organization. Now, centromere specific binding proteins bind and they help in assembling the kinetochore at this site. Now the kinetochore, it is a very large multi-subunit protein complex, and under the EM which you can look up in some images in books etc. I just depicted it diagrammatically there is an inner plate and an outer plate. So, of course some of the proteins in the inner plate they contact the CEN chromatin and the outer plate proteins, some of them actually associate with the spindle microtubules. So, this is a simple way to look at this organization.

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Now shown here is a model of the yeast kinetochore, the budding yeast kinetochore, and this is interesting because the budding yeast kinetochore binds only one microtubule. The centromeric region is also very small, it is often referred to as a point centromere. The attachment of the kinetochore depends on this protein complex, multiple NDC80 complexes. So, these complexes you can see, they anchor the kinetochore on the one end, to the sides of the microtubule, on the other end. So, this linkage is there but it is being made in such a way so that there can still be addition and removal of tubulin subunits at this plus end of the microtubule. There are also many other centromere proximal proteins that are shown here, there is this CEN specific nucleosome, a protein that binds specifically to the *CEN* known as MIF2, and also one of the complexes is the CTF-19 complex. And of course, there are various other kinetochore proteins, as I already mentioned, the kinetochore is a very complex and large structure. So, we cannot really look into its full composition in detail here.

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Now the attached sister chromatids must attain bi-orientation prior to segregation. The sister chromatids in a pair, they must attach to the opposite poles of the spindle to ensure that after the cohesion is dissolved, they move to the opposite poles in order to achieve equal segregation. You do not want both of the sister chromatids moving to the same pole, because that will cause mis-segregation, one cell may get an extra chromosome whereas another may get one less.

So, to facilitate this, the sister kinetochores are made or assembled in a back to back orientation, that reduces the chance that both of the sister kinetochores will face the same pole. And the cohesion at the centromere also makes the paired sister chromatids at the CEN and pericentric regions more rigid to maintain this opposing orientation of the kinetochores. This bi-orientation is important for generating tension at the kinetochore after both the kinetochores are attached to spindle microtubules from opposite poles. And this increases the microtubule attachment to the kinetochore, the generation of the right amount of tension stabilizes the microtubule kinetochore attachment or interaction. Note the bipolar spindle, which is shown here with the paired sister chromatids at the equatorial plane.

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The kinetochore also senses tension. So if there was to be monopolar attachment of sister chromatids, it results in a low tension and when under such a situation the kinetochore sends an inhibitory signal that loosens its microtubule attachment. Upon bi-orientation, that is when both the kinetochores of a sister chromatid pair are attached to microtubules coming from opposite poles high level of tension is generated. And this shuts off the inhibitory signal, and when this happens, the microtubule kinetochore attachment is strengthened, it becomes stronger or more stable. Tension sensing resulting from bi-orientation depends on the Aurora B kinase enzyme, which is associated with the kinetochore. It generates the inhibitory signal by phosphorylating several components of the microtubule attachment site.

The Aurora B is shown here in green; you can see it is actually present at the place that we are thinking and seeing that it is actually present there. So, it can act on these proteins, which are components of the kinetochore. Aurora B kinase can generate this inhibitory signal by phosphorylating various components of this complex, such as the NDC80 complex, which we already discussed. It is the complex that bridges the kinetochore with the microtubule and it increases its affinity for the microtubule plus end. Upon biorientation, the resulting tension reduces the Aurora B mediated phosphorylation and it increases the affinity of the attachment side.

So, in this bipolar spindle shown here with a paired sister chromatids, there is a protein CENP-F which is a centromere binding protein, which is shown in red. And the Aurora B kinase is shown in green; you can see they are actually present here at the metaphase plate.

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Now mitotic checkpoints monitor the completion of spindle assembly and they also determine when the cell should enter the metaphase to anaphase transition. The spindle assembly checkpoint is an important checkpoint, it ensures that cells do not enter anaphase until all the chromosomes are bi-oriented at the spindle and all the kinetochores are attached to spindle microtubules.

This checkpoint monitors the strength of microtubule attachments to the kinetochore, perhaps by sensing the tension. Unattached kinetochores send out a negative or inhibitory signal that blocks the activation of APC by Cdc20 and therefore it blocks the metaphase to anaphase transition. The unattached kinetochore changes conformation of the Mad2 protein, remember the mitotic arrest defective protein, so, that it can bind in complex to the Cdc20 APC and inhibit it. The metaphase to anaphase transition, after a bipolar spindle assembly and attachment of all the kinetochores to the spindle microtubules to achieve bi-orientation of all the sister chromatid pairs has occurred and their alignment at the metaphase plate has occurred. There is a signal which is generated that triggers the sister chromatid separation.

And E3 ligase, APC, the anaphase promoting complex is activated by binding to Cdc20. Cdc20 synthesis increases the cells approach mitosis and APC is phosphorylated by M-Cdk, which also allows Cdc20 to recognize and bind APC. APC, this E3 ubiquitin ligase, targets securin also known as Pds1 in budding yeast, for ubiquitin-mediated proteolysis. Securin was present in a complex with an enzyme separase. So, when securin is degraded, separase that was sequestered in this inactive securin-separase complex is released and is active. And separase now can cleave the cohesin kleisin subunit Mcd1 or Scc1, and the paired sister chromatids are released from each other and they can now separate. Another role of APC also is to target the S and M cyclins for destruction resulting in Cdk inactivation. And after this, phosphatases can dephosphorylate CDK targets helping in the completion of mitosis and cytokinesis.

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So, the mechanism of dissolution of cohesion and chromosome segregation at the metaphase to anaphase transition is shown here, which we have already described. So, this activating subunit Cdc20 it associates with APC and it can cleave this securing, which is shown here in orange. By doing so, it releases the separase enzyme from its complex with securing, and this enzyme separase, which is also activated now. This of course only happens after all the chromosomes have aligned at the equatorial plane and are attached to the microtubules it goes ahead and cleaves this cohesin subunit which bridges the two head domains of Smc1 and 3 and when it does so, then the two entrapped DNA molecules, which are held together by this cohesin complex can separate from each other. So, they can be released from this complex. These chromosomes or sister chromatids have now separated and for segregation they are now pulled away from each other by the spindle microtubules in anaphase.

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Now coming to molecular motors which are a fascinating class of proteins, which are often associated with the cytoskeletal filaments, these motors are proteins that bind to a cytoskeletal filament and they use the energy derived from repeated cycles of ATP hydrolysis to move along these filaments. Molecular motors can be classified based on the type of filament they bind to, whether it is actin or a microtubule, the direction in which they move along the filament, that is either they may be either plus or minus end directed, and the cargo they carry with them such as other cytoskeletal

filaments or other proteins or membrane-bound organelles. So, molecular motors are absolutely fascinating proteins and they use the energy derived from repeated cycles of ATP hydrolysis to move along a polarized cytoskeletal filament track. These proteins generate force by coupling ATP hydrolysis to conformational changes.

Motor proteins bind to their tracks through a head region. You can see, I have shown one of the motor proteins in blue over here walking on a microtubule track. So, the head region is the one which is contacting the filament and it is referred to as the motor domain. So, this domain binds and hydrolyzes ATP and coordinating with the cycle of nucleotide hydrolysis and conformational change, these proteins can cycle between the bound and the unbound states.

So, this is referred to as a mechanochemical cycle of filament binding, then conformational change and filament release, which happens sequentially for each of the heads. And then again there is a conformational relaxation and the head domain reaches its original conformation and it can re-initiate another cycle. So, in this way with this cycle of filament binding and then conformational change, release of the filament then relaxation of the conformation and then re-binding of the filament, the motor proteins and their cargo can move along or walk along the track one step at a time. Each step may be a few nanometers or so, depending on the type of motor protein that you are studying. So, this is depicted in this cartoon where this particular motor protein is walking along the microtubule filament and it has it is carrying on its other end a very large cargo; it is some kind of bound vesicle.

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The microtubule-dependent motor proteins are quite important for spindle assembly and for its function in mitosis. The cytoskeletal motor proteins, they move unidirectionally along this oriented polymer track such as an actin or a microtubule filament. We know that the spindle is made up of microtubules. The motor proteins that move on actin filaments are members of the myosin super family whereas the motor proteins that move on microtubules are members of either the kinesin superfamily or the dynein family. Both the microtubule based motors operate near the ends of the spindle microtubules. Kinesins usually walk towards the plus end of the microtubule and they are referred to as plus end directed motors whereas dyneins move towards the minus end of the microtubule and they are referred to as minus end directed motors.

For spindle assembly, there are four types of motors that are important for the spindle assembly and function. One is kinesin 5, this has 2 motor domains that the plus ends of inter-polar overlapping microtubules in the spindle mid zone. So, each of these they are bound to two different inter polar microtubules. Then there is kinesin 14 which is a single motor domain and it can also cross link into polar microtubules in the mid zone. But interestingly this is a minus and directed kinesin motor; it is an exception and it tends to push the spindle poles together. Kinesin 4/10, these are chromokinesins, which associate with chromosome arms and they push the chromosome away from the pole. Dynein is a very large motor protein; it is a minus end-directed motor and it operates in various locations within the cell. One of the important activities is it links the plus ends of the astral microtubules to parts of the actin cytoskeleton in the cortex of the cell. And therefore it pulls the poles towards the cortex, that is, away from each other.

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Now loss of cohesion results in separation of the sister chromatids. However, segregation of the sister chromatids occurs as the mitotic spindle pulls the chromosomes to opposite poles. This occurs in anaphase. Anaphase has two phases: anaphase A and anaphase B. In anaphase A there is the movement of sister chromatids away from each other by the shortening of the spindle microtubules. And there is microtubule depolymerization at the kinetochore, loss of subunits at the plus end of the microtubule, and the kinetochore moves towards the pole. There is also poleward movement of the microtubules towards the pole, that is the minus end depolymerization is also going on and this was referred to as microtubule flux.

In anaphase B, there is movement of the spindle poles further away from each other. And this depends on the motor protein activity. The plus end directed motors such as kinesin 5 that cross link the plus ends of overlapping microtubules, push the poles apart. So, their activity is such, their plus end directed activity is such, at these overlapping microtubules that the net result is an outward force, which is pushing the spindle poles away from each other. And the minus end directed dynein motors which are attached to the plus ends of astral microtubules, by their activity, their minus and directed motor activity pull the spindle poles apart. So, they exert a pulling force via the astral micro tubules on the spindle poles.

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There is an additional interesting regulatory checkpoint that operates at the end of M phase. This is the spindle position checkpoint. This checkpoint provides a fascinating example of spatial regulation of the exit from mitosis. It consists of the proteins of the mitotic exit network or MEN. In this case, it is the position of the spindle pole in budding yeast, that determines the exit from mitosis.

MEN is activated when one of the spindle pole bodies moves into the daughter bud and there is the protein Tem1, a GTPase that has a critical role in sensing the spindle pole body position. A protein known as Lte1 is the GDP-GTP exchange factor for Tem1 and its localization is restricted to the bud. So, when the spindle pole body happens to enter the bud, the Tem1-GDP is brought in proximity with Lte1, which is the GDP-GTP exchange factor. And therefore, after this event has occurred Tem1 is converted into the Tem1-GTP, that is the GTP bound form of Tem1. So, this then activates a kinase cascade and one of the outcomes of that is the release of the protein Cdc14, which was sequestered inside the nucleolus by Net1, which is another nucleolar protein. So, Cdc14 now is released from the nucleolus, it comes out of the nucleolus, and being a phosphatase it can dephosphorylate a number of proteins thus helping in the exit from mitosis.

Cdc14 dephosphorylates Cdh1 and it enhances the APC-Cdh1 activity resulting in Clb2 inactivation, Clb2 is a cyclin. And M-Cdk substrates are also targets of this phosphatase and therefore it antagonizes the effect of M-Cdk, and Sic1, a cyclin-dependent kinase inhibitor, and it activates it. So, in this way the cell can now exit mitosis and undergo cytokinesis that has been discussed in a separate lecture.

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Now let us look at some of the dynamic mitotic events and complex assemblies that we have discussed today, which are depicted in this fascinating animation by Drew Berry and Etsuko Uno that also includes a time lapse of mitosis by Jeremy Pickett-Heaps. So, here we are looking at mitotic chromosomes and in fact we are looking at or focusing our attention on one isolated chromosome in mitosis.

(Video Starts: 1:01:10)

You can see it is got two DNA molecules, each of them are packaged into a sausage shaped chromatid and there are these filaments sticking out from either side, these are the microtubules and they are important for orienting the chromosomes in the spindle, the red is the kinetochore that forms the interface between the microtubules and the chromosomes. The kinetochore is a very complex and large macromolecular assembly that performs many different functions in the cell. It is crucial for chromosome movement and can attach to dynamic ends of microtubules. Note the subunits here that are attached to the sides of the plus end of the microtubules. You can see some parts of the kinetochore, they are attached to the sides of the microtubules holding them. The kinetochore is also a mechanical tension sensing system that can feel whether everything's attached and positioned properly; it is also a stop signal broadcasting system that sends signal to the rest of the cell about whether it is ready for the chromosome separation to occur or not.

Now you can note some of the subunits turning green and this kinetochore has sensed that the tension is right and the chromosome is correctly attached. The last little bit of red which you can see is a stop signal broadcasting system, it is being carried away now by the dynein motor which is walking away from the kinetochore along the microtubule. In yellow or orange proteins walking leftward are the kinesins and these are the plus end directed motors shown carrying proteins on their back and this large motor walking here with the red complex is the dynein which is carrying the stop signal broadcasting system.

So, when all the kinetochores are ready the cell can transition into anaphase, the sister chromatids can separate and they are pulled apart to the opposite poles of the spindle as you can see here, all the chromosomes have segregated, reached the spindle poles. And this is followed by decondensation and cytokinesis to produce the two daughter cells.

(Video Ends: 1:03:29)

So, I hope you have enjoyed this lecture on mitosis and also begun to appreciate the complexity of the dynamic processes, which are crucial for chromosome segregation and inheritance. Thank you.