Cell Biology: Cellular Organization, Division and Processes

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Lecture 14

Mitosis-Part I

Hello everyone! I am Shikha Laloraya, Professor of Biochemistry at IISc. Welcome to this new series of lectures on mitosis in the ongoing course, Introduction to cell biology.

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Today we will discuss M-phase. Shown here are some of the key regulators of the cell cycle control system. At the start of S phase the S-Cdk cyclin complex triggers the DNA replication machinery. The S cyclin is degraded at the end of S-phase. Following the completion of S-phase and transition through G2 the cell can undergo M-phase. M-phase it involves many dramatic structural changes and complex events to achieve the equal segregation of the genetic material into two daughter cells.

At the start of M-phase, the M-Cdk is activated and it triggers various mitotic events. Another important event within mitosis is the metaphase to anaphase transition, which results in the separation of sister chromatids, and this is brought about when APC, the Anaphase Promoting Complex, that is in E3 ligase triggers a destruction of securin. And this activates the separase enzyme that can cleave the cohesin complex that holds the sister chromatids together.

M-cyclin is also degraded at the end of M-phase by APC mediated ubiquitylation and so, now the M-Cdk is no longer active. The cells can exit mitosis and undergo cytokinesis to produce two daughter cells, which can enter the next G1 phase and can go through another round of the mitotic cell cycle if the conditions are suitable.

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So, here is a micrograph of dividing HeLa cells. HeLa cells are an immortal human epithelial cell line that was derived from a cervical cancer and adenocarcinoma. And it is a very old cell line and this was taken from a tumour biopsy from a lady Henrietta Lacks and it was the first human cell line that could survive and grow in the lab and it is used very widely in many experiments in cell biology. So, you will be hearing about this cell line a lot. Chromosomes are shown in purple and microtubules

are shown in green; microtubules as you know are cytoskeletal elements made up of the protein tubulin. You can see in this field of cells, there are two cells in the middle, which are in mitosis -they have sort of rounded off, they are less flat compared to the other cells. And you can see that the chromosomes are condensed and their shape is a bit different from the other cells where it is disorganized. And these chromosomes they have attached to the spindle microtubules. During anaphase, the spindle microtubules pull the condensed chromosomes, which have separated to the opposite poles of the cells, and then this is followed by the division of the cell into two daughter cells.

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So, when DNA replication is complete and the cell has also grown to an appropriate size in the G2 phase, then the entry into M-phase can occur. Entry into mitosis is driven by the activation of M-Cdk. You might recall MPF from my earlier lecture, which was a factor known as maturation promoting factor or mitosis promoting factor which in fact is a Cdk-cyclin complex, which is conserved in different organisms and is important for entry into mitosis.

So, there is an increase in M-Cdk activity at the start of M phase. This activation of M-Cdk, it depends on the accumulation of M cyclin due to the increase in the transcription of its gene in G2 and M. And also association with the cyclin is required to activate Cdk and it is very important but it is not enough. In addition, the Cdk is regulated by phosphorylation. There are both activating and inhibitory phosphorylations that occur on the cyclin-dependent kinase.

And in fact, its activation for M-phase is brought about by the removal of the inhibitory phosphorylation by Cdc25. So how Cdc25 is activated at this exact moment is not very clearly understood although it might possibly occur by S-Cdk, which is active in G2 and also early M. In addition, the inhibitory activity of the Wee1 kinase is also suppressed. Another interesting point is that M-Cdk can also activate Cdc25 partially and inhibit Wee1. So, this is a kind of a positive feedback loop to rapidly promote its own activation and therefore it favours a complete transition into M phase. M-Cdk along with some other mitotic kinases phosphorylates a variety of proteins leading to the assembly of the mitotic spindle and the attachment of sister chromatid pairs to the spindle microtubules. These phosphorylation events also trigger chromosome condensation and aspects of chromosome reorganization, the nuclear envelope breakdown, and also cytoskeletal rearrangements, all of which are important events in mitosis.

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The other kinases that are important for mitosis are the Polo like kinase, this is important for the assembly of the bipolar mitotic spindle. It phosphorylates proteins which are required for separation of the spindle poles early in mitosis. Aurora kinases are also important. Aurora A is important for the assembly and stability of the spindle whereas Aurora B is important for attachment of sister chromatids to the spindle and also some other events.

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Mitosis is usually a relatively short part of the cell cycle but the events that go on in mitosis are quite dramatic and they are dramatic structural changes and reorganizations of the chromosomes as you can see here. And other cellular structures such as the cytoskeleton shown in green- here we are looking at the microtubules. The various phases of mitosis are mainly prophase, metaphase, anaphase and telophase.

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Some of the complex events that occur during mitosis are listed here. These include formation of the mitotic spindle, which is a complex molecular machine important for chromosome segregation. Formation of the spindle involves spindle pole body duplication and separation. Nucleation of nuclear and cytoplasmic microtubules and the formation of microtubule and kinetochore associations. Nuclear envelope disassembly also occurs which helps in the binding of the chromosomes with the microtubules. There are also changes in chromosome organization- for example sister chromatid cohesion at the centromeres and arms facilitates bipolar orientation of the pairs of sister chromatids on the spindle.

Chromosome condensation occurs, which results in compaction of the chromosomes and their shortening along the long axis of the chromosome. Maturation of centromere and kinetochore complexes and their binding to microtubules and finally the congression of microtubule associated sister chromatid pairs to the equatorial region in metaphase.

The metaphase to anaphase transition is tightly regulated. And during this transition the sister chromatid dissociation and segregation occurs. Finally, there is exit from mitosis, which is regulated by MEN, the mitotic exit network. And towards the end of mitosis there is also nuclear envelope reassembly and chromosome decondensation also happens. The substantial reorganization of the actin cytoskeleton as well and this is all followed by cytokinesis or cell division.

And for this there will be contraction of the actin myosin ring and in some other cell types septum assembly is also important and for example in fungal cells in plant cells cell wall synthesis and septum formation also becomes important.

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An example of how Cdk activity affects mitotic events is shown here. So, upon activation we know that M-Cdk phosphorylates several target proteins. Phosphorylation changes the properties of these targets and it could inactivate or activate their functions or it could affect their interactions with other molecules. These changes may bring about events, which are required for cell cycle progression.

So, one of the examples of the role of M-Cdk mediated phosphorylation in affecting an important mitotic event is shown here. So, this event is the nuclear envelope disassembly in animal cells; this is facilitated by phosphorylation of lamins. Lamins are proteins that form a network under the nuclear

envelope in the nuclear lamina. It forms filaments and its phosphorylation dissociates the lamin filament into the monomers. So, it disassembles the nuclear lamina by breaking down the lamin filaments. And some other examples of Cdk targets are inner membrane proteins of the nucleus ; their phosphorylation causes detachment of the inner membrane from lamins and from chromatin. And also nuclear pore complex, which results in the disassembly of nuclear pores.

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The main stages of M phase or mitosis are depicted here. So, prophase is the phase where the chromosomes start condensing. So, you can start seeing the condensed chromosomes. In metaphase all these chromosomes are at the equator, they have arranged at this plane and they are also attached to the spindle microtubules, which are shown in green. In anaphase the sister chromatids separate from each other and move away and in telophase the sister chromatids have reached the poles. And now you can see the nuclear envelope being reformed and this is all followed by cytokinesis where actual cell division happens followed by entry into G1 and another interphase.

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Human cells showing different stages of cell division starting with interphase are shown here. So, the red is a DNA and green are microtubules again and we are progressing anticlockwise in this image. So, the different phases are early prophase where you can start seeing the chromosomes appearing in this filamentous form but here the centrosome is not yet separated. But in late prophase you can see that the centrosome has separated and you can see more of the DNA condensation has occurred and also the nuclear envelope breakdown has occurred here.

So, you can see the chromosomes they are more spread out. And this is followed by pro-metaphase where the chromosomes are attaching to the spindle microtubules, but this process is as yet incomplete. In metaphase, the chromosomes are all attached and aligned at the equatorial plane and this is followed by the metaphase to anaphase transition where the two sets of chromosomes or sister chromatids, they separate away from each other and they move away and they move to the pole of the spindle.

And the spindle poles also move away from each other. So now the cells in telophase they begin to flatten again and this formation of the mid body and you can see early cytokinesis where now the chromosomes are decondensing. And the nuclear envelope has been reformed also. Now in cytokinesis the cells divide and then they also move apart.

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In prophase the sister chromatids, which is a pair of newly replicated DNA molecules or chromosomes that are produced in the last S-phase, they condense. And the mitotic spindle starts assembling between the two separated centrosomes. In pro-metaphase, which is a later phase in

between prophase and metaphase there is a breakdown of the nuclear envelope and the chromosomes are now accessible too and they can start attaching to the spindle microtubules.

In metaphase the chromosomes are aligned at the equatorial plane of the spindle. The spindle microtubules from the opposite spindle poles, they attach to the kinetochores of each sister chromatid; these are referred to as kinetochore microtubules.

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In anaphase the sister chromatids separate simultaneously to form the two daughter chromosomes. Each of the sister chromatid is pulled to the spindle pole it was oriented towards by its attachment to the kinetochore microtubules. And these kinetochore microtubules, they get shorter and therefore they help in moving the chromosomes poleward. In a later stage of anaphase, which is referred to as anaphase B, the spindle poles also move away from each other. So, there is an increase in the distance now between the two spindle poles and this is also fairly complex process.

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In telophase the two sets of daughter chromosomes have reached the spindle poles and the chromosomes decondense and the nuclear envelope assembly occurs reforming the nuclei and again enclosing these chromosomes. And the division of the cytoplasm also starts with the contraction of the contractile ring.

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In cytokinesis which is a part where cell division actually occurs, the cytoplasm is divided into two parts. And there is a contractile ring made of actin and myosin filaments, which contracts and partitions the cell into two parts to create two daughter cells, each of which has got a nucleus.

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All these events can be seen in quick succession in this time lapse of mitosis by Jeremy Pickett Heaps, which is provided by Drew Berry. You can see the chromosomes condensed, now they have reached the equatorial plane. And there you see the metaphase to anaphase transition where they moved away from each other, they have reached the poles. So, this is telophase you can see them decondensed you can see cell division has just occurred forming these two daughter cells.

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Two extremes of chromosome organization in human cells are shown here, that is interphase versus metaphase. The chromosomes or DNA are stained in red. So, you can note the thread like chromosomes which are seen here in this metaphase spread; they cannot be seen in interphase. In metaphase they are folded to form these rods or thread like structures. You can see the various chromosomes of different sizes and you can also note the primary constriction in each of the chromosomes. So there is a part of the chromosome, which is narrower than the other that is referred to as a primary constriction and that is where the centromere is present. Now in this there is a yellow signal and that is a part of the Y chromosome, which is being detected using a fluorescent probe using a technique termed as FISH.

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So, this shows just only the changes in the chromosome organization at different stages of the cell cycle again starting with interphase at the top and counter clockwise towards a more condensed organization. And you can see now the condensation is complete and the metaphase to anaphase transition has occurred here. And it is also clear how decondensation indeed happens in telophase.

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This is a metaphase spread of human chromosomes to show the structure of a metaphase chromosome. Once again you can see there are different sizes of the chromosomes you know in humans there are 23 pairs of chromosomes. So, here is one chromosome, which is a metacentric chromosome. So, it is this means that the centromere is in the center that is a primary constriction is in the center. And the arms on either side they are of equal size such a chromosome is referred to as a metacentric chromosome and you can see different kinds of chromosomes, there is submetacentric, telocentric and so, on depending on the relative position of this primary constriction.

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Now condensation of chromosomes is brought about by SMC protein complexes. SMC stands for Structural Maintenance of Chromosomes and these are essential and conserved protein complexes that are important for chromosome organization. One of these complexes is a condensin complex, which is shown here; it is got five different subunits SMC2, SMC4 and CAP-H, G and D2, which are the non-SMC subunits. These complexes are thought to form a ring-like structure; they have ATPase activity and they can bind DNA. So, these complexes are very important for chromosome organization and it is thought that for example condensin it may form a ring-like structure and bring about intramolecular interactions of different parts of the same chromosome forming a loop to help in condensation. The condensin head domain binds and it hydrolyzes ATP and in vitro it can change the coiling state of DNA and it can also bring about loop extrusion.

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Here is a depiction of a metacentric metaphase chromosome and its organization. So, as already explained in the middle is the centromere flanked by the arms, chromosome arms, on each side. So, here is where the centromeric DNA would be there and it forms a primary constriction that is the DNA in that region is very closely paired with each other; you cannot see the difference between the two molecules. However, it is important to understand that in this chromosome there are actually two DNA molecules termed sister chromatids; this is one sister chromatid and this is the other one and. So, these are the two DNA molecules that were produced from one chromosome in the last S phase and they are paired with each other with the help of a protein complex known as cohesin. So, some of the important things to know are that the centromere DNA is bound to specific proteins and those proteins in turn do bind microtubules; that is how it helps in attaching the centromeric region to the microtubules.

And the centromere has got specific histones. So, there are centromere specific histones and therefore particular specialized nucleosomes which are bound at the centromere. So, the nucleosomes here differ in composition from nucleosomes elsewhere in the chromatin and also there is a difference in organization. So, this pericentric and centromeric chromatin is more compact and it forms a heterochromatin, relative to other parts of the chromosomes.

Now as already mentioned there are centromere specific binding proteins, which form the kinetochore. So, the kinetochore itself, it is a large multi subunit protein complex on the centromere. It is composed of an inner plate and outer plate if you look at it by EM and the kinetochore outer plate proteins bind the spindle microtubules. So, this is how the chromosome associates or attaches to the microtubules.

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Here is a fluorescence microscope image of a metaphase in HeLa cells again. So, here the whitish blue is a DAPI stained DNA and microtubules in this case are shown in blue. And you can see in green the Aurora B kinase protein, which is involved in polymerization/depolymerization of the microtubules during mitosis. You can see it is present at the site of action and in red is CENP-F which is an outer kinetochore protein. So, it is showing a localization that you would expect from its known function as an outer kinetochore protein and so, this is also very important for mitosis.

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Cohesin is another SMC complex, which is important for sister chromatid cohesion or pairing. And it has got SMC1, SMC3 and other non-SMC subunits, Rad 21 and SCC3 also known as SA1 here. And also it associates with other regulatory proteins. It binds chromosomes periodically at intervals of a few kilobases at specific sites. Now this pattern of binding of budding yeast cohesin complex was deduced by chromatin immunoprecipitation. In fact, I was involved in this work and I termed these sites as *CARs* Cohesin-Associated Regions in analogy with MARs which are Matrix Associated Regions and SARs or Scaffold Associated Regions that are other structural determinants of chromosome

organization that had been found earlier. You can see in this cohesin distribution map that there is more binding of cohesin near the centromere. And that may explain the close pairing that is observed in this region.

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It is currently thought that the cohesin ring encircles two DNA molecules to bring about cohesion as is shown here. We will talk more about it in future lectures.

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This image shows the staining of chromosomes for cohesin in yellow. In fact, you can see it shows an axial staining pattern and in fact condensin also shows a similar staining pattern.

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The mechanism of dissolution of cohesion at the metaphase to anaphase transition is shown here. So, an enzyme known as separase is activated after all the chromosomes have aligned at the equatorial plane and they have attached to microtubules and there is no empty kinetochore left. Then this enzyme separase is activated and it cleaves a cohesin subunit shown here the kleisin subunit termed here as Rad21, which is also known as SCC1 or MCD 1 in budding yeast. So, when this subunit is cleaved the cohesin ring opens up and then the DNA molecule can be released. So in this way the two sister chromatids they can separate from each other; they are no longer bound or paired to each other with cohesin. So, now the chromosomes can separate and the metaphase to anaphase transition has occurred. And because they are able to separate they do come apart and then they are further pulled away from each other by the spindle microtubules.

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To summarize some of the key points about today's introduction to mitosis. It is the activation of M-Cdk, which brings about entry into mitosis. Phosphorylation of many proteins by M-Cdk and other kinases, the polokinase, Aurora A and Aurora B, are important for mitotic events. Spindle assembly, chromosome reorganization and bipolar attachment of sister chromatids to the spindle poles via the kinetochore microtubules, are important events in mitosis.

Condensin and cohesin are important for mitotic chromosome reorganization such as condensation and cohesion. The dissolution of cohesion at the metaphase to anaphase transition occurs by cleavage of a cohesin-subunit by the separase enzyme and this is followed by separation of chromosomes, their movement to the poles in telophase and then decondensation, which is followed by cytokinesis to produce two daughter cells. Thank you.