Cell Biology: Cellular Organization, Division and Processes

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Lecture – 06
Regulation of the Cell Cycle – Part II

Hello, everyone, welcome to the second lecture on cell cycle regulation.

(Video Starts: 00:26)

In the last lecture, we discussed the mitotic cell cycle. We discussed that the mitotic cell cycle consists of G_1, S, G_2 and M phases, and a cytoplasmic regulator, MPF, controls the entry into mitosis. We also discussed that mutants arrested in particular stages of the cell division cycle, the *cdc* mutants, were isolated in budding and fission yeasts.

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Another experiment done in Tim Hunt's lab also provided important insights into potential cell cycle regulators. In an experiment with sea urchin eggs, specific proteins whose levels fluctuated or cycled as the cell progresses through the cell cycle in successive divisions where discovered. In this case, the sea urchin eggs, they were fertilized and radioactive methionine was added after fertilization and then samples were taken at intervals of 10 minutes or so, and the proteins present in these samples were analyzed by gel electrophoresis to examine the proteins present in them. And a protein whose level which gradually built up, but was rapidly degraded when cells divided, was observed. The levels of the proteins were quantified and they were compared with the timing of cell division. And it was observed that each time it's level falls just before cleavage, or just before the cells divide, which corresponds with the end of mitosis.

So this protein appeared to be destroyed each time the cells divided. And similar cycling proteins were also observed in other species of sea urchins and also in clams, and hence they termed these proteins cyclins. Another interesting point is that the peak of cyclin also correlates with higher MPF activity.

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Recall that MPF is a factor that induces immature oocytes to become an egg. It's biochemical purification revealed that it had 2 subunits. It consists of a cyclin dependent kinase, CDK1, which is equivalent to p34cdc2 in fission yeast and Cdc28 in budding yeast, and cyclin B. CDK was always present while the cyclin levels fluctuate. And therefore, the MPF level or activity is transient. Cyclin is a regulatory subunit, it activates CDK or cyclin dependent kinase, and CDK induces mitosis by phosphorylating specific target proteins on Serine or Threonine residues.

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Shown here is the structure of human CDK2 determined by X ray crystallography. This protein has got 2 lobes, the N terminal smaller lobe in red and the C terminal larger lobe, which is shown in blue. And there is the active side in between, in this cleft over here with the ATP bound. The small N terminal lobe has got beta sheets and a helix referred to as a PSTAIRE helix. And the larger C terminal lobe has got several alpha helixes.

ATP can be seen in the active site cleft between the 2 lobes and its phosphates appear to be oriented outward towards the opening of the cleft. The T- loop, which is shown here in green color, blocks the access to the active site cleft.

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The cyclin dependent kinase is an important regulator of the cell cycle, and its activity is highly regulated at multiple levels. So, firstly it is regulated by association with cyclin. Association with cyclin activates the cyclin dependent kinase. Secondly, it's also regulated by phosphorylation. And there are 2 types of phosphorylations on the CDK. One is the inhibitory phosphorylation which occurs on tyrosine 15 and threonine 14 nearby. And there's also an activating phosphorylation present on threonine 161.

Of course, these numbers may vary among the different homologues of these proteins found in different species. And finally, there are certain proteins termed as cyclin dependent kinase inhibitors, which inhibit the activity of CDK cyclin complex. So, the binding of CKIs to the CDK cyclin complex inhibits the phosphorylation mediated by this complex.

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Shown here is the example of fission yeast. So, here the Cdc2, which we discussed as the Cdc2 mutant earlier, it stands for cyclin dependent kinase, and it is equivalent to Cdc28 of budding yeast. Cdc13 corresponds to the cyclin; Wee1 is an inhibitory kinase that brings about the phosphorylation of Y15 of Cdc2, and Cdc25- also coming out from the CDC screen, is a phosphatase, which removes the inhibitory phosphorylation on tyrosine 15 and 14, and it activates this Cdc2-Cdc13 complex.

The CDK activating enzyme is known as a CAK or the CDK Activating Kinase and it phosphorylates the CDK at threonine. And this is not regulated in the same way as the inhibitory phosphorylation. Now, the CDK cyclin is activated by the removal of the inhibitory phosphorylation by Cdc25. And after the removal of this only the activating phosphorylation is present, and this complex is active and now it can go in phosphorylate its various specific targets. At the end of mitosis, the M cyclin is degraded by ubiquitylation by the proteasome and thus the M-Cdk gets inactivated.

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How does cyclic degradation occur or how do cyclin levels cycle? This is dependent on a process known as ubiquitylation. Ubiquitin is a short 76 amino acid polypeptide, which is conjugated to substrate lysine residues by the E1, E2, E3 ligase enzymes. The polyubiquitinated ubiquitylated proteins are degraded in the cell by the 26S proteasome. There are 2 E3 ligase complexes, which are involved in cell cycle regulation. Although there are many different E3 ligases in cells, these are the 2 particular ones that impact cell cycle progression. One of them is referred to as SCF. This is a modular E3; It consists of Skp1, Cdc53 or Cullin and F-box containing protein subunits. And this is

associated with an E2 enzyme, Cdc34. Some of its substrates include the G1 cyclins, Sic1, Swe1 or Wee1, E2F and IκB etcetera.

APC is another important multisubunit, E3 ligase and this is an E3 with 12 subunits and it brings about destruction of particular mitotic substrates and this requires the presence of a 9 amino acid motif called the destruction box. So, examples of APC substrates are a protein known as Pds1 important for the metaphase to anaphase transition, and M-cyclin or cyclin B.

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So let's try to understand the mechanisms of CDK activation. In this slide are shown structures of CDK2 and cyclin-A, and the CDK2/cyclin-A complex. So, the activation of this CDK2 by cyclin-A at the structural level is represented here. Upon cyclin binding, as you can see in this complex, the cyclin dependent kinase undergoes some structural changes involving its activation segment, shown here in the cyan blue color here, and in the C-helix, which is shown in gold color. So, without the cyclin, this loop or the activation loop or the T-loop blocks the cleft as we mentioned earlier. And the position of several key amino acid residues is not optimal for ATP binding. But when cyclin binds, then there's rearrangement of 2 alpha helixes that allow ATP binding. And one of them, it's coming just before the T-loop, it becomes a beta strand and it helps to rearrange the T-loop. So, you can see the change in the configuration of the T-loop over here so that now it no longer blocks the cleft where the active site is present. And the other alpha helix, the PSTAIRE helix, also rearranges and it helps in changing the position of key amino acid residues in the active site.

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The activity of cyclin dependent kinase itself is also affected by its phosphorylation status. So in this paper by Welburn et al, they've attempted to understand the mechanism of inhibition of CDK activity by the inhibitory tyrosine 15 phosphorylation and the methods that they used were kinetic as well as crystallographic analysis of the Cdc2 cyclin-A complexes. They found that the phosphate at tyrosine 15 can cause steric blockade of the peptide substrate binding. It can create an environment that favors non-productive conformation of the terminal group of ATP such that it cannot be transferred. And this phosphorylation, also noted by them by their kinetic studies on the kinase and ATPase activities, they found inhibits the activity; but still there is some trace amount of activity remaining in the phosphorylated form. So, they speculated that additional phosphorylation at Threonine 14 may further block the peptide binding site and also may prevent the binding or appropriate configuration of ATP. And another interesting point discussed by the author's and I quote here is that “whereas phosphorylation of Threonine 160 leads to conformational change that embeds the phosphate group in a network of stable interactions required for structural integrity, the tyrosine 15 phosphate remains exposed and it's readily accessible to Wee1 as well as the Cdc25 phosphatases. And thus, the increased tyrosine phosphorylation can provide the cell with a rapid and potentially readily reversible mechanism for regulating the CDK activity in response to checkpoint activation. So, you know, the activating phosphorylation, it's embedded and it may be harder to remove, whereas what they're saying is that the tyrosine 15 phosphorylation is exposed and it's more accessible to the phosphatase and more available for modification as well as removal of the phosphate.

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This slide shows the potential mechanism of inhibition of the CDK cyclin complexes by CKIs. Recall that CKI are cyclin dependent kinase inhibitors, they are a class of proteins which bind this cyclin dependent kinase and cyclin complex, and prevent it from phosphorylating substrates. So, 2 types of CKIs are shown here. One is the INK4 inhibitor shown here and this binds to cyclin dependent kinase and it can cause a key active site loop shown here in the light blue color to rearrange, okay. So, you can see the shift in the configuration from this location to another side. And this inactivates the protein and it also impacts its interaction with cyclin. On the other hand, another inhibitor p27Kip1 is shown on the right side: this one binds to the CDK cyclin complex and by binding, you can see, it invades into the active site. Therefore, it blocks ATP binding over here.

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So far, we've discussed the discovery of key cell cycle regulators, the CDK cyclin complex, in yeast, budding and fission yeast, in frogs and in sea urchins. Does this have any relevance in understanding our own biology?

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Indeed, proteins which are similar to CDK and cyclin, are present in humans. Surprisingly, it was found by Lee et al, a long time ago, that a cDNA clone from a human cDNA library, which is a library which is copied from mRNA, could complement the fission yeast *cdc2* mutant phenotype. And they also found that the complementing clone had the coding sequence for human Cdc2 or CDK1 homologue. So, this establishes that the structure and function of CDK is conserved in humans as well, and highlights the significance of studies done using model organisms.

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Shown here are the CDKs and cyclins of vertebrates and budding yeast. So budding yeast has only 1 cyclin dependent kinase, but vertebrates have got many. And budding yeast has got multiple cyclins though, as do vertebrates. Note here, that in mammals there are 3 cyclin D versions, cyclin D 1, 2 and 3. So, these form different stage specific complexes as shown: the G1-CDK, the G1S-CDK, the S-CDK and the M- CDK, in various combinations. M-CDK is CDK1 cyclin B complex in vertebrates and the CDK1 or Cdc28 complexed with these cyclins shown here, in budding yeast.

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Shown here is a simplistic view of cell cycle control system. At the start of S phase, the S-CDK cyclin complex is active, and it triggers the DNA replication machinery. At the end of S phase, the S phase cyclin is degraded. Now, again at the start of M phase, the M-CDK cyclin complex is active and it phosphorylates many targets in order to trigger the mitosis machinery and bring about various complex events that occur during mitosis.

The M-CDK is degraded again at the end of M, by APC-mediated ubiquitylation. And now the M-CDK is no longer active. The cells then divide and then enter G1 and now they can go through another round of the cell cycle.

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So how does CDK activation drive the cell cycle forward? Upon activation, the cyclin dependent kinase phosphorylates several of its target proteins. Phosphorylation, being negatively charged modification changes the properties of these targets. It may inactivate or activate their functions or affect their interactions with other molecules. And these changes may bring about events that are required for cell cycle progression.

So, one example is shown here, which is of Lamins. Lamin is a target of CDK and lamin forms a part of the nuclear lamina, which is a region under the nuclear envelope; it forms a network under the nuclear envelope, and it does this by forming these long filamentous structures. Now, phosphorylation by the CDK1 cyclin B complex dissociates these filaments into the dimers of lamin and therefore, it breaks that network present in the nuclear lamina. So, the nuclear lamina disintegrates.

Furthermore, there are other targets also. So, there are some inner membrane proteins, their phosphorylation causes detachment of the inner membrane from lamins and chromatin and also in the nuclear pore complex there is phosphorylation, which results in disassembly of the nuclear pores and all of these events they cause disassembly of the nuclear envelope, which I already mentioned, is an important event in mitosis.

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Another form of cell cycle regulation was proposed by Weinert and Hartwell and this is the concept of cell cycle checkpoints. They define checkpoint controls as signaling pathways that act to delay cell cycle progression, when perturbations delay completion of certain cell cycle events. So, these type of checkpoint pathways involve the detection of errors or problems in cellular process, for example, is there DNA damage or has the attachment of chromosomes to spindle been completed or not?

And then transmission of the signal to an effector that then inhibits cell cycle progression. These checkpoints slow down or they arrest the cell cycle to allow or give time to cells to repair the damage before proceeding further. Checkpoint mechanisms are critical for the fidelity of ongoing cell division and mutations in some of these checkpoint genes have been associated with cancer predisposition and also with cancer progression. Also a cell which is unable to fix the damage may undergo cell death by apoptosis.

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To summarize, we have discussed today that MPF is a cyclin dependent kinase complexed with a cyclin, and CDK1 is equivalent to Cdc2 in *Schizosaccharomyces pombe* and Cdc28 in the budding yeast *Saccharomyces cerevisiae*. Cyclin levels fluctuate during cell cycle progression and association with cyclin activates CDK. CDK is regulated by phosphorylation, both activating and inhibitory phosphorylation can occur and removal of the inhibitory phosphorylation by Cdc25 activates the CDK cyclin complex. There are a class of proteins termed the CKIs, the cyclin dependent kinase inhibitors, that inhibit the activity of the CDK cyclin complex. The activated CDK cyclin complex phosphorylates important targets triggering the events which are required for cell cycle progression. And furthermore, checkpoint pathways monitor errors or problems in the cell, and they slow down or arrest the cell cycle progression until the damage is fixed.

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So, the cell cycle can be thought of as a highly complex, regulated, ordered, and dynamic process. It is a temporally organized sequence of events that are linked together in an orderly fashion. Late events in the cell cycle depend on successful completion of earlier ones. Thank you.