Cell Biology: Cellular Organization, Division and Processes

Kshitiza Mohan Dhyani

Department of Biochemistry

Indian Institute of Science – Bangalore

Lecture 26

Meiosis Part III

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Hi everyone! We have now read that the meiotic process is a tightly controlled process and it should be, because in case the chromosome segregation is compromised in one way or the other, it can be deleterious to the cells in form of aneuploids or in form of polyploids. We also understand that during the homologous recombination process, DNA double strand breaks are generated and these are induced by a protein, which is topoisomerase like protein called Spo11.

Now Spo11 is also evolutionarily conserved. Now understand that the proteins that are evolutionarily conserved are good in a way such that the cell has to define a certain means of regulation and that means of regulating the protein activity is conserved across the organisms. Let us understand how the meiotic process is regulated at different levels. Starting with the generation of DNA double-strand breaks.

When we were learning about the generation of DNA double-strand breaks we also discussed that in case the number of DNA double-strand that are generated or induced by the cell are randomly induced or in case they are induced in a number that is higher than what the cell can accommodate, it can be deleterious to the cell. Therefore, one would intuitively understand that the DNA double-strand breaks that are generated during the meiotic homologous recombination is a non-random process.

Studies in yeast and other modular organisms have shown that the generation of DNA double-strand breaks preferentially occurs in intergenic promoter containing intervals and is depleted from other regions. It is also important to note that the DNA double stand break formation is highly repressed in telomere and centromere proximal regions. One other phenomenon, double-strand break interference, means that if double-strand break is induced at certain point, it will make sure that

another double-strand break is not induced close by. Such a phenomenon is called a double strand break interference.

Yet another phenomenon is called double stand break compensation. This is a homeostatic process and it is able to redistribute double-strand breaks across chromosomes. Now, what happens is, there are certain hotspots with the DNA double-strand breaks are induced. However, if for some reason the double-strand breaks are not induced over those hotspots, other dormant double strand break hotspots takeover and this time the double-strand breaks are induced over the dormant double strand break hotspot. What happens is the entire landscape of the DNA double-strand break hotspots can be changed.

Another important factor that influences the generation of DNA double-strand breaks is the histone modifications. Notably the H3K9 acetylation falls very close the DNA double-strand break hotspots. This is followed by histone H3K4 methylation and H3K4 methylation is a fundamental mark of meiotic double strand information. So much so, that in budding yeast, the enzymes that are responsible for H3K4 methylation when deleted lead to a significantly reduced frequency of DNA double-strand breaks.

Other than all these factors one very important factor are chromatin remodelling factors. Importantly, the chromatin remodelling factors are responsible to allow the rapid access of Spo11 enzyme to DNA to induce the DNA double-strand breaks. So these are all the processes or all the levels where the generation of DNA double-strand breaks is regulated by the cell during meiotic process.

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After the DNA double-strand breaks are induced, another point at which meiosis is regulated is while it is deciding the spatial patterning of crossovers. We have already understood that there is something called an obligate crossover, which meant that every set of chromosomes, every homolog pair should have at least one crossover, which is called an obligate crossover and in certain cases if the obligate crossover is not present it can lead to mis-segregation of chromosomes.

Another phenomenon which is similar to the double standard interference phenomenon is called the crossover interference. Crossover interference phenomenon means that if a crossover has occurred at a certain region across the chromosomes, it will reduce the tendency of another crossover to form nearby. Therefore, the spatial patterning of crossovers make sure that the chromosomes are not mis-segregated and there is an interference such that the number of crossovers are regulated.

From the first lecture, if you remember, the meiotic G_1 phase is followed by S phase where the DNA is duplicated, and S phase is followed by the G_2 phase after which the cells enter meiosis 1 and go through meiosis 2, after which meiosis is completed.

Let us look at the first step of how the meiotic G_1 to S phase transition is regulated. Now you would wonder what makes the cell enter meiosis at the first place. In organisms like budding yeast and fission yeast, nutrient deprivation conditions are the conditions which force the cells to enter meiosis. And if you understand it in a way that meiosis is responsible for maintaining genetic

variation and genetic variation is what drives survival. So, under nutrient deprivation conditions what happens is, the daughter cells that are formed due to genetic variation, there is chance that they are having a better survival.

In multicellular organisms, extrinsic cues from surrounding cells are the deciding factors, which made the cell enter meiosis. Let us start with budding yeast. In budding yeast nutrient deprivation conditions allow expression of regulators namely Ime1 and Ime2. Ime1 is a transcription factor and the target of the transcription factor is a gene called *IME2*. *IME2* encodes the meiotic specific kinase, which has homology to CDKs.

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The Ime2 regulator promotes entry of cells from G_1 to S phase by 2 ways. The first way is it promotes the degradation of S phase CDK inhibitors and the second way is it inhibits the ubiquitin dependent proteolysis of cyclin B which will lead to the stabilization of cyclin B. Now cyclin B is what is responsible for promoting S phase and later chromosome segregation.

Similar to budding yeast, nutrient deprivation conditions are what drive the cells of fission yeast towards meiosis. Let us look at how? There is RNA binding protein called Mei2 protein. And Mei2 protein is necessary and sufficient for initiating meiosis. However, a protein kinase called Pat1 phosphorylates RNA binding Mei2 protein leading to the inhibition of meiosis. Now under nutrient deprivation conditions a Pat1 inhibitor called Mei3 is expressed which leads to the inhibition of the Pat1 itself and the release of Mei2 repression. As soon as the Mei2 repression is released it is necessary and it is sufficient for meiosis to progress. And therefore, under nutrient deprivation condition meiosis is progressed.

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The regulation of pre-meiotic S phase, that is where the DNA replication occurs, is brought about by S phase CDKs . Now the S phase CDKs are composed of 2 B-type cyclins, Clb5 and Clb6, along with Cdc28. It is very interesting to note that DNA replication has been shown to be essential for establishing inter-homologue interactions and also in the formation of a synaptonemal complex. So, much so that if the; DNA replication is impaired by some way the synaptonemal complex is also compromised.

Another point at which meiosis is regulated and is a very important checkpoint is called the pachytene or recombination checkpoint. Recall that homologous recombination occurs during the pachytene stage therefore there has to be regulated mechanism wherein we can check whether the double-strand breaks that are formed by Spo11 during the homologous recombination are repaired by HR.

Now after the double-strand breaks are induced, homologous of recombination (HR) occurs and in case the double-strand breaks are left unrepaired the cells need to be arrested in the G_2 phase. How this happens is, in the budding yeast process has been characterized extensively. So, in budding

yeast the meiotic CDK activation is inhibited by activation of a protein, which is called Wee1. Now Wee1 protein phosphorylates and inhibits Cdc28. And this entire process blocks meiotic progression and the cells are arrested in the G_2 phase.

Another way by which this entire process happens is by inhibiting the transcription factor, which is called Ndt80. Now Ndt80 is a transcription factor, which is required for expression of meiotic cyclins. So, if Ndt80 is inhibited the transcription of meiotic cyclins namely Clb1, Clb3 and Clb4 is inhibited, and therefore this is one way by which meiotic progression is prevented and cells are arrested in the G_2 phase.

This is the scenario, which is the checkpoint scenario. However, there is another case wherein the cells are arrested in G_2 and you must have read this read about this during oogenesis. In female germline in most metazoans the cells are arrested in the G_2 phase namely in the diplotene stage and the maturation is triggered only during ovulation.

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We have emphasised over and over again about the chromosome segregation in the meiosis and its significance. Now homologous chromosomes are the ones that segregate during meiosis 1 and sister chromatids are the ones that segregate during meiosis 2. Let us look at meiosis 1. Three important processes are responsible for regulating the homologous chromosome segregation during meiosis 1, as you can see here, in purple and in green are the homologs in a pair and here is the chiasma where the crossing over has occurred between the 2 homologs.

Therefore, homologs must be linked by the chiasma during meiosis 1. Secondly, in order to avoid the premature separation of the sister chromatids during meiosis 1, it is essential that the linkage between the sister chromatids is maintained during meiosis 1. However, we need to separate the sister chromatids during mitosis 2. So the linkage between sister chromatids should be broken during meiosis 2.

Third and very important process means that the sister chromatids must bind the spindle that is arising from the same pole during meiosis 1 as you can see here and at the different pole during meiosis 2. This will ensure that the sister chromatids are segregated towards the same pole during meiosis 1; however, they are segregated towards different pole during meiosis 2.

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Proteins called cohesins and their subunits are responsible for keeping the sister chromatids together as well as responsible for keeping the homologous chromosomes together. During mitosis, the mitotic cohesin subunit is called Scc1/Mcd1 and the meiotic specific variant of Scc1 is called Rec8. Now we know that during anaphase 1 the homologous chromosomes need to be segregated from each other.

But we need to make sure that the sister chromatids stay together. Therefore, a mechanism was designed by the cells such that the cohesins in the arms are lost during anaphase 1. However, during

anaphase 2 when the sister chromatids need to be separated from each other, the cohesin in the centromere is lost. Note in the figure, the rings around the sister chromatids are lost during anaphase 1. But the cohesin is retained at the centromere such that the sister chromatids are retained together and segregated towards the same pole and when the centromeric cohesion is lost sister chromatids are now segregated from each other.

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The loss of the cohesin from the sister chromatid arms during the metaphase 1 to anaphase 1 transition is brought about by a protein which is called separase. The separase is a protease and it cleaves Rec8 during both meiosis 1 and meiosis 2. Now you would wonder what makes the centromeric Rec8 so strong or so resistant to separase that during the meiosis 1 the centromeric Rec8 is retained. However, the arm cohesin is lost. Also, it is interesting to note that during meiosis 2 again the centromeric cohesion needs to be degraded and the function is again brought about by the same protein which is called separase. Research has been conducted over this and there are several hypotheses that are proposed to explain how this mechanism occurs. So, in order to protect the Rec8 at the centromere during meiosis 1 there are three processes that are involved.

By research we know that the phosphorylation of Rec8 by kinase Cdc5 is essential for the cleavage of Rec8 by separase. Now the centromeric Rec8 is resistant to degradation by separase because the centromeric Rec8 is not phosphorylated by the kinase called Cdc5. This is one hypothesis. Another hypothesis is that at the centromere, the Rec8 that is present at the centromere interacts with other centromere specific proteins. And that interaction is what prevents the cleavage of Rec8 by separase and therefore protects centromeric Rec8 during meiosis 1. A specialised protein called Spol13 is present only during meiosis 1 and it has been shown that Spol13 is essential to protect centromeric Rec8 during meiosis 1 and loss of Spol13 leads to premature separation of the sister chromatids because in this case the centromeric Rec8 is also degraded by the separase.

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The kinetochore attachment during both meiosis 1 and meiosis 2 is an important point of regulation. Now let us look at these two sister chromatids with their respective kinetochores. These are capable of attaching to the spindle arising from the poles in three different ways. The first one is merotelic attachment; in the merotelic attachment the kinetochores are joined towards the spindle arising from the same pole as well as one of the kinetochores is attached to spindle arising from both the opposite poles. In case of amphitelic attachment the kinetochores of the sister chromatids are joined towards opposite poles. In case of syntelic attachment the kinetochores of the sister chromatid joined to the spindle, which are rising from the same pole.

Now observe that when meiosis 1 happens we need the homologous chromosomes to segregate from each other. In that case syntelic attachment is what will be preferred because in this case both the sister chromatid has kinetochores that are attached to the spindle from the same pole therefore both the sister chromatids will be segregated towards one pole. However, during mitosis and meiosis 2, the sister chromatids need to be segregated from each other therefore, what is preferable is an

amphitelic attachment wherein kinetochores from the sister chromatids are joined to spindle arising from opposite poles.

Now let us look at during mitosis and meiosis 2. A protein called Pcs1 inhibits the merotelic attachment, which is unfavourable. Similarly, syntelic attachment is prevented by a protein kinase which is called Ipl1. There is one possibility wherein the kinetochores are unattached, which is called unattached kinetochore condition. This case needs to be corrected to form an amphitelic attachment. However, if the kinetochores remained unattached it can lead to induction of the spindle checkpoint, which will inhibit anaphase onset. Therefore, anaphase onset will only occur when the correct amphitelic attachment is maintained between the sister chromatids during mitosis and meiosis 2.

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During meiosis 1 as we just discussed, the syntelic attachment is what will be the preferable form of attachment. A protein called monopolin inhibits the amphitelic attachment. Unattached kinetochores similarly activates the spindle assembly checkpoint and prevent anaphase onset. If the homologs are co-oriented instead of bi-oriented, a protein called Mad2 is responsible for correcting it. And when the syntelic attachment is formed, is the case when anaphase onset will be induced.

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Let us summarise what we understood about the regulation of meiosis and the various points at which meiosis is regulated. Starting from the induction of DNA double-strand breaks by Spo11, we understood that the generation of DNA double-strand breaks is not a random process and is highly regulated both in terms of frequency and in terms of positions. We also understood that there is a restriction to the number of crossovers. However, there is one obligate crossover that is required for correct chromosome segregation to occur. We understood that CDKs are required for meiotic G_1 to S phase transition and CDKs are what induce pre-meiotic S phase DNA replication. The pachytene and recombination checkpoint is essential to understand whether DNA double strand breaks that were generated by Spol11 have been repaired. If not, the cells are arrested in the G_2 phase

Homologous chromosome segregation is controlled by the enzyme called separase in meiosis 1. in meiosis 1, after meiosis 1, the centromeric Rec8 is lost by separase. But during meiosis 1 centromeric Rec8 is protected and retained. Kinetochore orientations maintain the chromosome segregation to homolog segregation during meiosis 1 and sister chromatid segregation during meiosis 2.