Cell Biology: Cellular Organization, Division and Processes

Kshitza Mohan Dhyani

Department of Biochemistry

Indian Institute of Science – Bangalore

Lecture 20

Meiosis Part II

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Hi again! Let us begin by summarising what we learnt in the previous lecture on meiosis. So, meiosis is reductional division as we now know and it is essential in order to maintain genetic variation across the progeny and it is also essential to maintain the ploidy in offspring. There are different stages in the meiotic process starting with the G1 phase during which the cells prep to enter meiosis. During the S phase the DNA replicates, which is followed by the G2 phase. G2 phase is followed by meiosis 1, which is an extremely important and it is where the reduction of the number of chromosomes actually happens.

During prophase 1 in meiosis 1, which is subdivided into five different stages, in the pachytene stage the homologous chromosomes to form the synaptonemal complex. And during the pachytene stage crossing over or homologous recombination occurs. During diplotene stage the synaptonemal complex disassembles, which is followed by anaphase 1 where the homologous chromosome segregation occurs and the number of chromosomes is reduced to half.

After cytokinesis 1, meiosis 2 follows, which is again subdivided into prophase, metaphase, anaphase and telophase. What is noteworthy here is, during the anaphase two now sister chromatid segregation occurs while the number of chromosomes stays the same. Another very important point that is noted in the previous lecture was that in case there is premature segregation of the homologous chromosome, which means that either both the homologous chromosomes move towards the same pole and they do not move towards the different opposite poles as they should have been, and in cases where the sister chromatid segregation is premature or aberrant, the daughter cells that are formed will either be aneuploid or polyploid; in both the conditions it is deleterious to the cells and can lead to fertility issues and can also lead to you know genetic disorders in progeny.

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We left at the question where we asked that even though the siblings have the same parents why are they different from each other? And how does the exchange of genetic material occur between the two homologous chromosomes. And the answer to these questions lies in a process which is called homologous combination and we will get into the details of how homologous recombination occurs. And why is it very important during meiosis.

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Now the homologous recombination as we know happens during the pachytene stage during prophase 1 in meiosis 1. However, other than that when cells are mitotically dividing homologous recombination is a process which is essential to allow DNA repair. However, in contrast to during meiosis, the preferred template is the sister chromatid in case of mitotically dividing cells.

When homologous recombination occurs in context of meiosis, it is essential for the pairing of the homologs first. And the second thing is essential for the exchange of genetic material between homologs. Now in contrast to mitotic dividing cells during meiosis the preferred template for homologous recombination is the homologous non sister chromatids. Even though even in the case of meiosis the sister chromatid is constitutively present, some proteins actively make sure that the bias of using the preferred template is towards non sister chromatid rather than the sister chromatid. Now mechanistically homologous recombination can be divided into four different phases. Let us that start with the first phase which is called the initiation phase. During the initiation phase DNA double-strand breaks are induced in one of the homolog in the pair by a specialised protein which we will discuss soon.

Now, you would wonder that why are we calling it as programmed double-strand breaks. We are calling them programmed because this is one of the very few cases where the cell is inducing the DNA damage in form of double-strand breaks. And how you would wonder that in case there are a certain number of DNA double-strand breaks in the cell maybe it will be deleterious to the cells and you are right, which is why the number of DNA double-strand breaks during meiosis is highly regulated, as we will see soon.

The initiation process is followed by the second step which is called the DNA double-strand break processing wherein single stranded DNA tails are generated by a specialised set of proteins and these single stranded DNA tails are recombinogenic in nature. The second step is followed by the third step where the DNA is repaired using homolog as we just saw, there is a bias towards the homolog. And this process is followed by homologous recombination intermediate resolution and all of these four processes involve a set of proteins which are specialised to generate the homologous recombination intermediates, which are then resolved to give products, which are called crossovers or non-crossovers.

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On the left side we are showing a pair of homologous chromosomes one in green and one in blue. On the right side we are showing the stage wherein each of these mechanisms occur. Now let us follow the schematic. The first protein, which comes into picture during the initiation phase is called Spo11. Now Spol11 is a topoisomerase like protein, which is evolutionarily conserved across organisms. And what it does is, it induces the double-strand breaks in one of the homolog in the pair, as you can see here the double-strand break is induced on the green homolog in the pair. Following the DNA double-strand break induced by Spol11, there is another set of proteins which is called Sae2 and Com1. Now Sae2 and Com1 actively inhibit another form of DNA repair mechanism, which is called nonhomologous end joining such that now the repair of this damaged DNA or the DNA wherein the double strand breaks has been induced, occurs only using the homologous recombination mechanism rather than using the nonhomologous end joining mechanism.

After the DSB's, that is the DNA double-strand breaks, are induced, another set of proteins which is called the Mre11 protein which has a 5 prime to 3 prime endonuclease activity cleaves the DNA from the 5 prime end and this is called resection, which means that the DNA is cleaved from the 5 prime end. Similarly on this strand, which leaves an overhang in the 3 prime end and these single-stranded tales are generated on the 3 prime ends.

And these single-stranded DNA tails are recombinogenic in nature and they are coated by another protein called Rad51 which leads to the formation of a nucleoprotein filament here. What is important to note here is that in most of the organisms the generation of the DNA double-strand break is a prerequisite for homolog paring to occur and as you have seen in the previous lecture the homolog start to co-align during the Leptotene stage. So the DNA double-strand breaks are generated during the Leptotene stage.

The single stranded DNA tails on the three prime ends that have been generated here are coated by Rad51 which forms nucleoprotein filament here. This is absolutely essential because this is what is required for homology search. And after the nucleoprotein filament is formed, it leads to homology search wherein it invades the homologous template on the nearby homolog in the pair.

And this kind of structure that is found here is called a D-loop structure. This intermediate that is formed during the process can now undergo one of the two pathways wherein one of the pathways is called the crossover pathway which will generate crossover products and the other pathway is called the non-crossover pathway which is which will generate the non-crossover products.

This entire process is facilitated by different proteins namely Rad51, Dmc1, both of them coat the single stranded DNA that is formed here. Sae3, Rec8, Hop2 and Mnd1 are accessory proteins, which allowed the formation of the D-loop intermediate here.

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During the zygotene stage as we know earlier the proximity between the two homologs strengthened furthermore by co-alignment. Let us see mechanistically how that happens? We ended up at a D-loop intermediate from where there are two pathways that the intermediate can

follow. The first pathway is non-crossover pathway. In this case the single stranded DNA end which extends itself or invades the homologous template. DNA synthesis is primed on this template and a certain length of DNA synthesized. After this, this extended end is extruded and this sort of intermediate is formed.

On the contrary, in the crossover pathway, there are several proteins namely Zip1, Zip2, Zip3, Mer3 and Msh5. These proteins facilitate single end invasion. So, what happens is, as we see here the single stranded DNA invades the homologous template, DNA synthesis is similarly primed. However now in this case, another sort of intermediate, which is called as double Holliday junction will be formed, which is not formed in the non-crossover pathway. During the pachytene stage we know that the synaptonemal complex formation is complete, which means that the two homologs are in such close proximity to each other that the distance between them is almost equal to 100 nanometres, which means that the formation of these intermediates is very intimate.

In case of the non-crossover pathway, the extruded end anneals to the primary homolog and the DNA synthesis continues. Now as the DNA is synthesized as you see in the white dotted line here, this entire homolog is repaired and these products are formed which are called non-crossover products.

However, in the crossover pathway when the single end innovation occurs in DNA synthesis occurs another homolog forms the second end annealing, the DNA synthesis continues and ligation occurs as you can see here in white colour dotted line. And an intermediate which is characteristic to the crossover pathway is formed, which is called a double Holliday junction. Now there are very important proteins, which are responsible in resolving this kind of structure because DNA cannot be left in this kind of a mesh. Therefore, specialised proteins called Holliday junction resolvases act on the cross structures that are formed here and resolve the Holliday junction into the products that are called the crossover products. This process is completed during the diplotene stage of meiosis 1.

Note that per chromosome the meiotic machinery makes sure that there is one crossover. And this one crossover per chromosome is called obligate crossover. The obligate crossover is absolutely essential in order for the homologous chromosomes to correctly segregate. Also note that the number of DNA double-strand breaks that are generated by Spol11 during the initiation phase is much, much larger than the number of crossover products that are generated at the end, which means that not all DNA double-strand breaks end in the formation of crossover products.

How the selection process occurs is as yet a matter of investigation and researchers are actively looking into it.

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Now let us discuss the significance of homologous recombination during meiotic prophase. The exchange of genetic material between homologs is the primary reason for genetic variation. And genetic variation in almost all of the organisms prove to be the basis for better survival of offspring. The next very important aspect is the proper segregation of chromosomes. So, in many organisms, if the homologous recombination is impaired or the homologous recombination does not occur, it adversely affects the segregation of chromosomes in a way that nondisjunction occurs.

Now nondisjunction can lead to aneuploidy or polyploidy or genetic disorders such as Turner syndrome, Down syndrome and Klinefelter syndrome. The third very important aspect is homologous recombination in the canonical meiotic program leads to homolog pairing. In *Saccharomyces cerevisiae*, *S. pombe*, mammals, plants and filamentous fungi, homologous recombination is absolutely essential to facilitate the homolog pairing. However, this is a general rule, but there are alternative programs. For example, in fission yeast and *Asperigillus nidulans,* what happens is both recombination independent and recombination mediated pairing occurs, but the synaptonemal complex is absent. In female *Drosophila* and *C. elegans*, the pairing between the homologous is independent of recombination. In male *Drosophila*, recombination is absent and the pairing between the homologs is recombination independent. We shall see how the recombination independent paring occurs in a short while.

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We just saw that DNA double-strand breaks lead to the formation of intermediates, which facilitate homolog pairing and this is the canonical meiotic program. However homologous pairing is also facilitated by interactions which are independent of DNA double-strand breaks. And you will see how the centromeres and the telomeres of the chromosomes are involved in this process.

In cases of yeast and in mouse spermatocytes as you see here, centromeric associations are formed long before synapses has occurred in the prophase stage. As you can see the homologous chromosomes in green and purple are shown here. Now, there are two events which are called centromeric coupling and centromeric pairing. Centromeric coupling usually occurs between homologous and can also occur between nonhomologous chromosomes. However, centromeric pairing occurs only between homologous chromosomes. In yeast it has been shown that is Zip1 protein is essential for both centromeric coupling and centromeric pairing and the localisation of Zip1 to the centromeres is facilitated by another protein, which is called Rec8. After centromeric coupling has occurred during the prophase synapsis occurs and the homologous chromosomes pair together, which is called centromeric paring. And then the cell cycle proceeds towards metaphase.

However, in case of mouse spermatocytes centromeric coupling has not been detected. Synapsis occurs and is followed by centromeric pairing which is followed by metaphase. So what we see here is, centromeres play an important role in homolog pairing even before double-strand breaks are generated. Therefore, this is one of the mechanisms wherein homolog pairing occurs independent of the DNA double-strand breaks.

Now you would wonder why this kind of process occurs? Now inside a cell where one homolog has to find the other homolog in order to pair together, the centromeric coupling allows them to check transient interactions and in case they find the correct homolog this interaction is stabilized during synapsis and is matured into pairing. So therefore, these transient interactions that occur long before actual synapsis has occurred, make sure that the pairing process is made easier and the finding of the homologous is made, correct.

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Another way by which homolog paring occurs independently of the DNA double-strand break generation is by exploiting the telomeres of the chromosomes. Now in early Leptotene all the telomeres tether to the nuclear envelope, which is shown here in the blue boundary. During the late Leptotene, the telomeres start moving towards one of the ends of the nuclear envelop. This clustering of telomeers is completed during the zygotene stage as you see here. And during the pachytene stage the scattering of telomeres starts.

Now in plants and vertebrates, telomeric interaction leads to formation of a bouquet structure also called the REBL orientation. The telomeres tethered to the nuclear envelope. In fission yeast it has been shown that mutations that affect the telomeric clustering lead to reduced recombination. And such mutations are used to study the effect of the telomere clustering on homolog pairing and subsequently on homologous recombination.

Experiments in *Saccharomyces cerevisiae* have shown that homolog recognition requires telomeres and telomere associated protein called Ndj1. Now mutations in Ndj1 show impaired chromosome pairing and bouquet formation. Except *C. elegans* and *Drosophila* bouquet formation is found in all studied organisms. Other than the role of telomeres and centromeres in homolog pairing during double-strand break independent homolog pairing, there is another candidate, which is absolutely essential in order to allow homolog pairing and these are called the non-coding RNA's. Studies in fission yeast show that Mei2 encodes an RNA binding protein which functions at two stages. Primarily, Mei2-RNA complex sequesters a negative regulator of meiosis called Mmi1, which binds meiotic specific transcripts during mitotically dividing cells and prevents meiosis in mitotically dividing cells.

During meiosis however the Mei2 RNA accumulates at *SME2* locus and they promote homolog recognition and pairing of homologous chromosomes.

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Let us now look at the summary of the lecture of part 2. We started with homologous recombination. We now know that during meiosis inter homolog recombination is preferred over inter-sister recombination, which is preferable during mitosis when the cells are divide mitotically. We also studied how homologous recombination occurs. It starts with the introduction of DNA double-strand breaks by a topoisomerase like protein called Spol11.

Mre11 and other endonucleases generate single strand tails which are recombinogenic in nature, they are bound by proteins called Rad51, that nucleoprotein filament is now ready for strand invasion. After strand invasion intermediates are formed typically during crossover process. The double Holliday junction is formed; proteins called Holliday junction resolvases resolve such intermediates. And then there is the generation of crossover and non-crossover products.

We also studied that homologous recombination is responsible for homolog pairing in the canonical meiotic pathway. It is essential for proper chromosome segregation and it is absolutely essential for genetic variation. Next, we studied that homolog pairing is not just double strand break dependent it is also independent of the DNA double-strand breaks. It can be mediated either by centromeres. It can also be mediated by telomeres and can also be mediated by small RNAs.