Cell Biology: Cellular Organization, Division. And Processes

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Lecture 23

SMC Proteins and Chromosome Organization-Introduction

Hi, I am Shikha Laloraya, Professor of Biochemistry, at the Indian Institute of Science. Welcome to this lecture about the role of SMC proteins in chromosome organization, which is a part of the course on Cell Biology: Cellular organization, division and processes.

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How is it that a nearly 2 meters long DNA molecule can fit within a tiny nucleus having a diameter of only 5 or 10 microns? This is the conundrum that we will discuss today. Packaging of DNA with histones to form nucleosome arrays that further fold into a 30 nanometer fiber condenses DNA to a certain extent. But there are additional levels of folding of chromatin as well. So, today we will discuss a class of non-histone proteins, which are involved in higher order chromosome organization.

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Additional levels of higher order chromosome organization are seen in these chromosomes, which are entering mitosis. Shown here are the changes in the chromosome organization that occur as the cell enters mitosis and it reaches metaphase, at which point the chromosomes are maximally condensed. So, how does this happen? And also all these structural changes in the chromosomes are very important for their accurate segregation.

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This is a time lapse of mitosis by Jeremy Pickett-Heaps, contributed by Drew Berry. In this we can see that chromosomes are condensing and forming these rod-like compact metaphase chromosomes that are aligned at the equatorial plane. And after all the sister chromatids are aligned, they undergo the metaphase to anaphase transition and the chromosomes have separated and moved poleward.

The chromosomes are now decondensed and this is followed by cytokinesis resulting in two daughter cells. So, these structural transitions are crucial for accurate chromosome segregation.

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The question is how does this happen? The transition from the 30 nanometer chromatin to the metaphase chromosome probably involves formation of loops and then even their further folding. A conserved family of proteins, the SMC proteins, are important for this transition.

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Shown here is a metaphase spread of human chromosome showing the maximally condensed state of mitotic chromosomes. Note the cohesion between the sister chromatids, that is, along their length they appear to be paired and this cohesion is more intimate or close at the primary constriction region, which is where the centromere is located. Also note the high level of condensation that has occurred in these chromosomes to convert the sister chromatids into this X-shaped rod-like configuration. Both of these structural attributes, condensation as well as sister chromatid cohesion are dependent on SMC protein complexes and both of them are very important for accurate segregation of chromosomes during mitosis.

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Shown here is a schematic of a typical SMC protein complex. Now SMC stands for Structural maintenance of chromosomes. And these proteins are conserved in eukaryotes as well as prokaryotes. They are very important in terms of function. For example, in yeast they are essential for viability of the cells. They have a ATP binding head domain, a flexible coiled coil region and a hinge region. And these proteins, they dimerize with another SMC partner, to usually form a heterodimer and they dimerize at the hinge region. The head domains of these two SMC proteins are bridged by a non-SMC kleisin subunit, which is called Mcd1 or Scc1 or even Rad21, in budding yeast. Non-SMC subunits here are of course Scc1, Mcd1 or Rad21- this kleisin subunit and also the Scc3 or SA proteins.

There are other regulatory factors associated with cohesin such as Wapl1 and Pds5 and this complex and also the other SMC complexes are also regulated by post-translational modifications of various subunits, such as acetylation in case of cohesin by Eco1, sumoylation and phosphorylation. The SMC protein complexes bind DNA, they associate with DNA and they are involved in many important DNA transactions due to their inherent DNA binding and tethering abilities.

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Each SMC protein has got a hinge region and two coiled coil regions, which connect the hinge to the Walker A and the Walker B motifs. This molecule then folds up upon itself at the hinge and the two Walker A and Walker B motifs, they come together, and they form the head domain that can bind ATP and has ATPase activity. The SMC proteins can associate by the hinge region and their head domains as I already mentioned are bridged by this kleisin subunit. And this therefore closes the ring formed by this complex.

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Shown here are three of the conserved SMC complexes in eukaryotes, the cohesin complex, the condensin complex and the as yet unnamed SMC5/6 complex. A cartoon of the SMC monomer is shown at the top that folds and it associates with another SMC at the hinge, in the complex. So, each of the SMC complexes has got two different SMC proteins forming a heterodimer and additional non-SMC subunits. Cohesin has got two main non-SMC subunits, condensin has three whereas the SMC5/6 complex has got six non-SMC subunits.

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Cohesin was first identified based on its requirement for the process of sister chromatid cohesion in budding yeast mitosis. Budding yeast mutants, which were defective in Mcd1, the kleisin subunit of cohesin, showed cohesion as well as condensation defects by fluorescent in situ hybridization analysis of budding yeast chromosomes. Now we know that one of its main roles is to hold the two sister chromatids together until the metaphase to the anaphase transition.

At the metaphase to anahase transition, Mcd1, this purple subunit shown here, is cleaved by an enzyme named Separase. And this cleavage opens up the ring and the two sister chromatids can move apart, they can come apart. This results in the dissolution of sister chromatid cohesion. And in anaphase now the two sister chromatids, they can move apart; they were already attached to microtubules coming from opposite poles. So, they move towards the poles as well and thus chromosome segregation happens.

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The cohesin subunits are Smc1, Sms3 and then Mcd1 or Scc1 and Scc3. All the subunits are essential and conserved. This complex is of course very important for sister chromatid cohesion. But it also has roles in mitotic chromosome condensation, in DNA double strand break repair, in gene expression, and also in replication. The complex subunits are sumoylated by Mms21 or Nse2, which is a subunit of the SMC5/6 complex.

Defects in the cohesin subunits or their loading factors can result in severe developmental defects in humans, highlighting the importance of its function even in multicellular organisms in development.

So, when cohesin was first discovered, we asked the question where does cohesin bind to chromosomes, because this was not known at all?!!

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For this we employed a technique termed chromatin immunoprecipitation or ChIP in which one of the components of cohesin, Mcd1, was immunoprecipitated from sheared formaldehyde crosslinked chromatin from yeast cells. So, in this method, that is in chromatin immunoprecipitation, chromatin is crosslinked first using formaldehyde and then it is fragmented and then a DNA binding protein specific antibody, which will be an antibody to your protein of interest, is used to pull down that protein and its associated DNA sequences.

Then the crosslinks are reversed and the DNA is purified and then this DNA which co-IPed with your chromosomal protein can be analyzed by a number of methods such as qPCR, microarray hybridization, or even next generation sequencing. And the DNA sequences, which are found to be enriched in the immunoprecipitates represent the binding sites of the DNA binding protein of interest *in* vivo.

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So, to identify sequences to which cohesin binds in yeast cells, one of the components of cohesin, Mcd1, was immunoprecipitated from sheared formaldehyde cross-linked chromatin. By the way, this experiment was done by myself several years ago during my post-doc in the Koshland lab; cohesin in budding yeast was also identified by Doug Koshland's lab, when he was at the Carnegie Institution of Washington.

We observed the selective enrichment of certain sequences but not of others, in the Mcd1 chromatin immunoprecipitates. Thus, we identified specific sequences to which cohesin binds in yeast cells and we named these sequences as Cohesin-Associated Regions, abbreviated as *CARs*, in analogy with *SARs* and *MARs* that are other previously identified putative DNA elements that were thought to be determinants of higher order chromosome structure. *SARs* are Scaffold Associated Regions and *MARs* stands for Matrix Associated Regions, that are DNA sequences that were found to be associated with the mitotic chromosomal scaffold and the nuclear matrix respectively.

So *CARs* on yeast chromosomes show a conserved periodicity, that is they recur every eight to nine kilobases, at least for the CARs that we found in this study. We could also find cohesin binding sites within the tandemly repeated ribosomal DNA locus. There was one site in each ribosomal DNA repeat, and we also observed that cohesin seemed to be bound to most of the repeats that is most of the rDNA repeats were occupied with cohesin binding to the rDNA *CAR*. We also found that there was higher binding near the centromere in pericentric regions and that there was lower binding to sites near the ends of chromosomes or near the telomeres.

The sites that we identified were quite broad; the breadth ranging from 0.8 to 1.5 kilobases. We also found most of them were located in intergenic regions and that their distribution correlated more or less with AT-rich regions. So, this distribution of cohesin has been depicted in this diagram of a chromosome where you can see in the pericentric regions or near the centromere, there is more binding of cohesin. Cohesin, by the way, is shown as this blue rod like structure, which is just diagrammatic depiction of cohesin and we also observed that there is binding of cohesin every few kilo bases, with some sort of periodicity along the chromosomal arms. And of course, I already mentioned that the binding near the ends of the chromosomes appear to be lesser.

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This is one of the current models of how the cohesion ring might hold the two sister chromatids together. This might be by encircling these two DNA molecules, which can pass through the hole in this ring, as is shown here, to bring about cohesion. So, at the metaphase to anaphase transition if you recall, cleavage of this subunit opens up the ring and the DNA molecules can slip out. So, this is one of the current models which is *in vogue* trying to explain how cohesin brings about cohesion.

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This image shows the localization of cohesin. Cohesin is shown in yellow on the condensed chromosomes, which are depicted in red. Cohesin here in this figure, it seems to have an axial localization on the chromosome which is consistent with its known functions in cohesin and condensation.

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Although cohesin was first identified by its requirement for sister chromatid cohesion, in a screen for mutants that were defective in sister chromatid cohesion, these mutants were referred to as *pds* mutants, precocious dissociation of sisters, during mitosis. We now know that cohesin has several other functions as well. Cohesin is also important for mitotic chromosome condensation, it is also required for DNA double strand break repair by homologous recombination. It is also important for DNA replication, in fact cohesin was shown to be speeding up the progression of the replication fork. Cohesin is also important for gene expression and for silencing barrier activity. Silencing barriers are sequences that can limit the spread of silencing coming from a silencer or silencing initiator sequence. And these sequences indeed are present in eukaryotic cells and it has been found that their barrier activity or boundary activity is dependent on cohesin.

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The condensin complex, has SMC2, SMC4 and 3 non-SMC subunits CAP-H, CAP-D2 and CAP-G. All the subunits again, in yeast at least, are essential and also they are conserved. It is important for mitotic

chromosome condensation. In addition, the condensin complex also has roles in cohesion, in rDNA stability, and also silencing at the ribosomal DNA, in telomere silencing, and in tRNA gene clustering within the nucleus.

Vertebrates have got two different condensin complexes and defects in condensin subunits can result in a developmental defect in humans, for example, primary microcephaly.

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So, condensin has been shown to be required not only for condensation but also cohesion, rDNA stability, ribosomal DNA silencing, telomere silencing, tRNA gene clustering within the nucleus; I think we already mentioned this.

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Condensation and resolution of sister chromatids depend on the condensin complex. Like cohesin, condensin and may also form a ring-like structure and bring about intramolecular interactions in a DNA molecule forming a loop to help in condensation. So, here also there is a kleisin subunit, CAP-H, which bridges the two head domains of SMC2 and SMC4 and also the head domains can bind and hydrolyze ATP. Condensin can change the coiling state of DNA in vitro which is also stimulated by M-Cdk. And also it can bring about loop extrusion in vitro. These properties of condensin may help in chromosome reorganization by the condensin complex in mitosis.

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The SMC5/6 complex has Smc5 and Smc6 as its SMC subunits and it also has six non-SMC subunits, Nse1 through 6. All of these subunits again are essential and conserved. And one interesting feature of this complex is that it has got additional enzymatic activities; in fact, two of its subunits Nse2 or in budding yeast it is also known as Mms21, and Nse1 have E3 ligase activity. So, Nse2 or Mms21 is a SUMO E3-ligase and it sumoylates several chromosomal proteins.

In fact, several of the SMC proteins are sumoylated by Nse2 whereas Nse1 has been shown to have ubiquitin ligase activity in humans. The SMC5/6 complex also is being studied a lot and it has numerous functions. It is important for repair. It also has roles in DNA double strand break repair by sister chromatid recombination mediated homologous recombination. It is important for replication, for stability, and also for telomere clustering and the peripheral localization of telomeres.

And also, it is important for stability of repetitive DNA in cells. The SMC5/6 complex also is important for meiosis. Nevertheless, a lot more still needs to be learned about this intriguing complex, the SMC5/6 complex, and also a lot needs to be still learned about the SMC family of proteins and a lot of scientists including my own lab are working towards solving these problems. Thank you.