Cell Biology: Cellular Organization, Division, and Processes

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Lecture 24

SMC Proteins and Chromosome Organization: DNA loop extrusion by SMC complexes.

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Hello everyone, welcome to this short lecture on DNA loop extrusion activity by SMC complexes. So, in this short lecture I am going to explain you about how SMC complexes introduce loops in the genome. So, for this I utilized a single molecule visualization assay where I visualize the DNA molecule and then see how SMC complexes change the conformation of the DNA by their action. So, let us get started.

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So, here is the schematic structure of the SMC complex that I used in this study that is condensin, it is one of the SMC proteins. So, it has a typical structure of SMC dimers, in this case it is Smc2, Smc4 they come together by a hinge domain. And then each of these SMC subunits has an ATP binding domain, they can hydrolyze the ATP to do some work and then they have accessory proteins like Ycg1, Brn1, Ycs4.

So, Brn1 has a DNA binding motif, we know that from previous research, and then we purified this protein after over expressing in yeast cells and then we can basically see each of those subunits in SDS-PAGE gel; it is a denaturing gel, you can see the individual subunits there all the way from Smc4 to Brn1. So, this is a purified complex then we have it in hand, then let us see how we visualize the function of this protein at single molecule level.

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So, for that I developed this assay that lets us you look at the DNA molecule at single molecule level. So, we immobilize the DNA molecule on a quartz surface via biotin-streptavidin-biotin interactions. The way we basically immobilize biotin on the surface is through PEG molecules; PEG stands for

polyethylene glycol, which is a hydrophilic molecule that reflects off the proteins from the surface that does not let any proteins to stick on the surface, that is important for our study because if a protein binds on the DNA and then that sticks on the surface you cannot see any action.

So, that is why we need to pass away this surface, that is we do it by linking the PEG molecules on the surface. So, and then we immobilize the DNA molecule on the surface via biotin-streptavidin-biotin interactions the DNA carries the biotin at the ends. So, in the DNA molecule here has 48 kilobase pairs in length. So, we visualize this DNA under fluorescence microscopy after staining with an intercalating dye. What typically intercalating dye molecules do is that when they are in the solution, they do not give you as much fluorescence as they are on the DNA. So, the change in the fluorescence intensity is more than 500-fold compared to the in solution to the on the DNA when it binds on the DNA. So, that is why you can see the DNA molecule that is immobilized on one end here, another end here, and then you know we have flexible DNA in the middle like you know freely floating DNA sort of in the middle part of that DNA. Then that looks very nicely homogeneous intensity under fluorescence microscopy. So, now we will add condensig on top of this one and see how that changes the structure of the DNA.

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So, here again this as I showed you, DNA, immobilized DNA molecule; we add condensin plus ATP. ATP is the energy that condensin consumes to do its work and then we expect condensin will do some action on the DNA, some sort of structuring, some compaction would happen on the DNA. Here is the data basically that you have an immobilized DNA molecule and then there is a bright spot you can see that appears because of the condensin activity. That bright spot is only appearing because of condensin consuming ATP and then doing its action. So, if we remove ATP from the solution nothing would happen that is one control. Another control is that we added ATP-gamma-S, which is non-hydrolyzable ATP that can bind on the condensin but it does not hydrolyze. So, that means condensin cannot do its work then we do not see or we would not see any of this structuring this kind of a bright spot appearing there.

Also, we mutated the ATP binding domains of the condensin molecule and then in that case condensin can still bind to the ATP but it cannot hydrolyze then again we did not see any compaction happening on the DNA molecule. So, meaning that whatever bright spot here see we are seeing here is on top of the homogeneously distributed DNA is because of the action of the condensin molecule after consuming the ATP.

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To see the compacted structure or the bright spot on the DNA, what it is there, whether it is a loop or is it is a mesh like structure that is like sort of hooked together by different condensin molecules or so. So, we wanted to see what is there in this structure for that we started applying a buffer flow perpendicular to the immobilized DNA molecule. So, here we have one DNA molecule and two DNA molecules, second one, both of them has this compacted structure there.

And then we apply a buffer flow and you will see what is there what kind of structure it is now. So, now I start the buffer flow in this direction, I show with this arrow and you see that but bright spot gets extended really nicely it looks like a sort of like you know a loop emanating from the here and then ending there. So, the same here you have a loop there but you know if you visualize under a faster imaging then you can see sometimes it splits into a sort of a loop, like a ring-like structure there in the DNA.

So, now the question is how does the loop appearing, like how was the loop appearing there by condensin activity, was it by the condensin would bind on the two distant elements on the DNA and then brings together or was it condensin binds there and then sort of like increases the loop size by gradual loop extrusion. So those are the two questions that we asked, that is like whether it is by random grabbing of the DNA and then bringing the loop, or was it by active loop extrusion process?

So, to answer that question we started visualizing the DNA from the start or from the beginning when we add the condensin and the ATP and then we will see what we get.

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Here we are visualizing the DNA right from the beginning from the start when we add the condensin during the its action. Now I am playing the video. So, the DNA is stretched because there is a buffer flow and then condensin activity like basically increasing this loop size over the time by gradually extruding the loop. I play again, that you know initially there is a small bright spot because condensin started to do its action, then it increases its size over the time by bringing the DNA loop into the DNA.

So, now the question is how fast is this loop extrusion happening? So, the video I am showing here is 10 times faster. So, the entire video is around 200 seconds and then. So, I showed you in 20 seconds or so. What is the rate of loop extrusion or the kinetics of loop extrusion that is the question that we ask next.

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Let us look at the rate of loop extrusion. Now here I am showing you DNA molecule under the absence of buffer flow, you can see the homogeneous fluorescence intensity along the DNA. I play the video now and you can see how the loop happens over the time, looping happens over the time. So, condensin bound there first and started to increase the loop size until increased to the maximum loop size. So, I play again. So, here a small bright spot appears because condensin lands and start to do the loop extrusion. And its size increased over the time until it reaches to the maximum. So, now we calculate/ divide this DNA into three regions, a region that is in behind the loop in the region the loop itself and the third region is the region in front of the loop. So, these three regions we calculate the size of these three regions over the time how it changes. So, here is the quantification- the red

curve shows the change in size of the loop that is region two, and that you can see that it increased from almost from 2.5 or zero kilo base pairs to 30 kilo base pairs in 20 seconds of time.

That means 30 kilo base pair by 20 seconds nothing but 1500 base pair per second, that is the loop extrusion speed by condensin molecule for this particular molecule. And then you have the green curve that is the region one that is in front of the loop that decreases its size from around 25 to 30 kilo base pair until to zero kilo base pairs within similar amount of time, that is like within 20 seconds. Again, if you calculate the rate of decrease in the region 1 is negative 1500 base pairs per second that is exactly inverse to what we see in the loop extrusion rate and then region 3 that is the DNA piece in front of the region 2 or the loop that does not change at all like in this molecule it is almost constant. So, these are the; three different loop kinetics we can get from the DNA molecule.

One the loop extrusion rate, the change in the loop size itself, another one is changing the size of the DNA behind the loop and third one is the in front of the loop. So, this is for one molecule but the similar behaviour we see for like many numbers of molecules. So, now I am going to show you. So, many, many molecules here this is the part for region 3 that kept constant that does not change over the time for many, each curve is for different molecule.

And then here is the loop size that itself for the region two in this case that increases over the time and stays constant and again each curve represents two different molecules that we imaged and then region one, that is the size of the DNA behind the DNA loop that is where the DNA loop being pulled by condensin that you see, that decreases over the time of each of the molecule that we image and the state stays constant at certain point.

So, this says that the DNA loop extrusion is actually happening by pulling the DNA from one side in this case from region one, but not from the region three. So, it is a one-sided DNA loop extrusion or in other words it is an asymmetric loop extrusion.

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Now we wanted to look at the number of condensin molecules that are acting during the loop extrusion process. So for that we basically stain the condensin molecule or label the condensin molecule with a fluorophore in that case it is in this case it is Atto647N, that is a molecule we label the condensin with and then we basically can visualize this condensin under fluorescence microscopy with appropriate excitation wavelength.

So, one thing I want to tell you that any fluorophore, in this case this Atoo647N, has a finite lifetime meaning that when you start to image the fluorophore it will photobleach at some point meaning that it does not give you fluorescence. It gives you fluorescence at a certain time and then falls back to like you know background because there will be a photo damage on the fluorophore.

So, we utilize that photo damage on the fluorophore to see the number of molecules in this complex or in the when the condensin is acting on the loop. So, what you are seeing on the left side is that you know here is a DNA molecule that say there is a buffer flow in this direction, the loop gets stretched here and the yellow arrow points to the loop base and that is exactly where you see a condensin molecule with a fluorescence a bright spot that you can see. So, that means condensin is

acting at the neck of the loop. Now if we basically overlay these two images you see there is a yellow spot appearing because DNA is shown here with the green and condensin is showing with red, red plus green you get the yellow. So, you can see that is at the neck of the loop. To calculate the number of molecules acting here we monitor the fluorescence intensity over time and we can see the number of photobleaching steps in this bright spot and then we can basically calculate the number of condensins there.

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So, here is the quantification for the number of condensin molecules that are acting during the loop extrusion. So, what you are seeing here is the background fluorescence intensity and the DNA molecule when there are no condensins there. As soon as condensin arrives onto the DNA you see increasing the fluorescence bright spot by the after Atto647 to certain level and then the condensation stays there and does its action that is the DNA loop extrusion.

And during which at some point the fluorophore will photobleach because of the photo damage and then that leads to the fluorescence intensity to the background level of the intensity. So, that means we are seeing a single step increase in the fluorescence intensity and then single step photo bleaching or single step decrease to the background level meaning that there is a single condensin molecule appeared and then single condensin molecule sort of photo bleached meaning that one molecule acting on that molecule on the DNA molecule.

So, here is the statistics, we almost all the time see predominantly single condensin molecule of which we imaged like 50 molecules of image, we always see single condensin molecule appearing. And sometimes we see no fluorescence intensity of the condensin because like you know this is a labelling chemistry we are doing here. So, we only had 75 to 80% of the condensin molecules labelled.

So, those 25% or 30 or 20% molecules will show no fluorescence intensity at all. So, this is predominantly single condensin molecule that is acting on the DNA loop extrusion.

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To conclude:

So, here is the summary of what I showed you so far. So, I showed you that first direct evidence for DNA loop extrusion by any SMC complex, in this case that is condensin, doing the loop extrusion. And I showed you that condensin binds on the DNA itself at one side that is we call anchor point and then does the loop extrusion by consuming ATP in an active loop extrusion manner. And I also showed you that it is a single condensin complex that acts at the neck of the DNA. Thank you very much for listening.