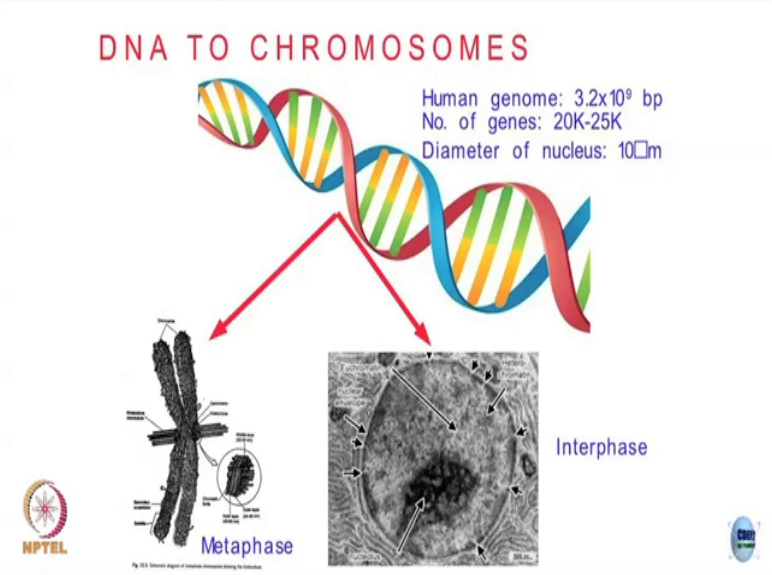
Physics of Biological Systems
Prof. Mithun Mitra
Department of Physics
Indian Institute of Technology, Bombay

Lecture – 32
The Hierarchical Chromatin Packing Model

(Refer Slide Time: 00:16)



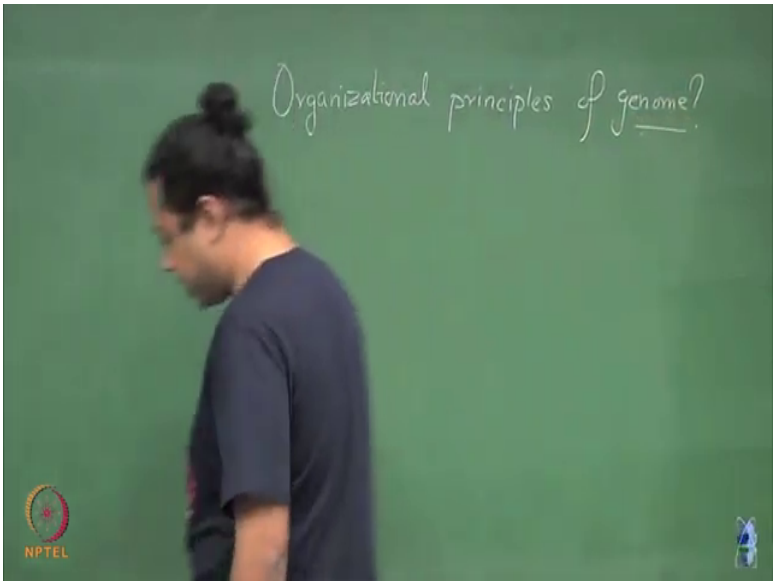
So, let us start; the thing is that if we think about the human genome, for example, as we have discussed that contains around some billion base pairs. So, around 3 into 10 to the power of 9 base pairs right. And the number of genes in there is around 20000 around 25000. So, this genome this is a polymer right, it is this double helix polymer the DNA and you need to package it inside this nucleus which is of the order of 10 microns.

So, you need to take these 10 to the power of 9 base pairs and you need to put it inside this 10 micron sized nucleus. And, eventually you get a structure which in metaphase you might be

familiar with. So, this is the famous sort of an x structure of chromosomes, but in general if you look at the interphase. So, the interphase is when the cell is not dividing the metaphase broadly is when the cell is dividing.

So, in the interphase the chromosomes sort of looks not like this, nice x structure that we see often in books, but like this mess over here. So, this whole thing over here is the chromosome; this dark spot in the center, this dark spot in the center is the nucleolus that has no genomic material, but everywhere outside. So, all over here you have this DNA which is this polymer the DNA floating around inside the nucleus. So, while there is; so, the question is that; the basic question that we want to understand is that is there any principle to this organization of the chromosome? The organizational principles of a genome, right.

(Refer Slide Time: 02:09)



So, when I have taken this polymer and I have put it stuffed it inside this nucleus do I need to follow some rules as to what I am allowed to do and what I am not allowed to do or do I just go about doing it randomly? The answer is that of course, you there are some rules that you need must follow and simply because you the DNA is a storehouse of information right.

So, whenever you have some gene sequence in the DNA and let see you want to produce the associated protein. So, the transcription translation machinery will need to come and find this gene and you will need to transcribe it and then produce the corresponding protein out of it right

So, ideally the cells should sort of you know roughly; where this gene is to be found. It cannot be completely random; it cannot be completely random that every cell has this in a different location. So, there needs to be certain organizational principles that one must follow the question is what are those and the answer is that we do not really know it is an unsolved problem and that many people work on.

But, at least I will tell you; what are the features that whatever model that you come up with what are the features that it should be and I can, I will probably discuss couple of model ok. So, for example, so I will mostly be talking about this interphase chromosome because this there is the state of the cell for the most of its lifecycle.

This metaphase when it is dividing is a special case its happens for a small window of time, most of the time of its life cycle the cell is in the interphase state and there this chromosomes look like a soup ok. It looks like a messy soup, but is it as messy as it looks. So, that is what we will try to understand.

So, one thing that I can show from this picture itself is that if you look at this picture, you will see that there are light spots and there are dark spots right. What these correlate to this intensity of this light and dark spots, what this correlates to is how tightly is the DNA pack. So, for example: if this DNA is packed like this very tightly versus something like this right.

This thing we will show up as a dark spot in this picture, this thing will show up as a dark spot, this thing will show up as a light spot. So, in this picture; whenever I see a dark spot over here for example, a region like this over here I know that the DNA over there is much more tightly packed as opposed to something over here which is much with more whiter in comparison. And, this has the functional role as well.

So, for example, this dark spots over here is what is called heterochromatin this is what is called heterochromatin; whereas, this light spots which are loosely packed or what are known as or what are called as euchromatic. And the difference functionally is that with human genomes codes for around these 20000, 25000 whatever genes, but not all of these genes are required for the functioning of every cell type right. Some cells might require only a subset of these genes, some other cell might required different subset of these genes.

So, the genes for a given a particular cell type; the genes that are necessary for its functioning, for the functioning of that cell type. We will generally be found in these euchromatin regions which are more loosely packed. So, these are euchromatin regions are gene rich, these are gene poor. And it sort of make sense from a naive point of view in that if I need to.

So, as I will show you; there are levels of organization, but ultimately if I need to produce a protein I need to read the genomic sequence which means that I need to sample the DNA at a base pair resolution right. ATCG whatever the sequence is and if this DNA is very tightly coiled around I cannot really access this information.

So, it make sense that; if I need to access some part of the information more often to for the functioning of that particular cell type I would like to package it more loosely as compared to something that I do not need. So, this heterochromatin therefore, contains transcriptionally silents genes for a particular cell type the euchromatin contains the genes that are required for that particular cell type broadly speaking, all of these are broad statements.

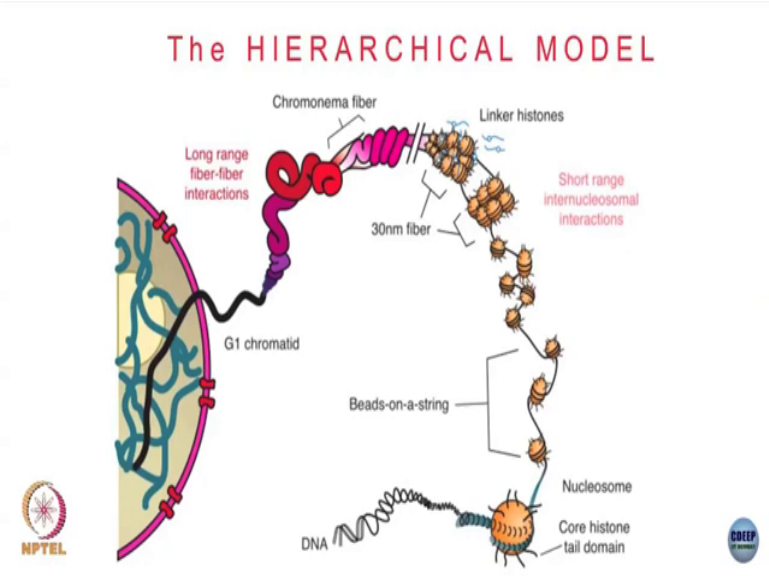
So, therefore, the packaging will also differ from cell type to cell type. If I were to image a different cell type the same region of the genome could come which was in the euchromatin part in one, could come in the heterochromatin part in the another.

Yes depends the for example, in the for a human genome I mean it is 46 polymers. Because, we have 23 chromosomes, 23 pairs of chromosomes, 23 into 246; each of them is a long polymer ok. So, it depends on the number of chromosomes that you have.

Student: E-coli

E-coli for example, is a single ring polymer ok. So, how what is known about this packaging; how do I go from this double helix which is whose structure we sort of more or less understand into this messy soup looking object.

(Refer Slide Time: 07:58)



As I said the complete answer is not known, but at least we know little bit. So, what we know is this initial part of the packaging; which says that well I have this double helical DNA at the very basic level or at the very base fair level what these DNA is do, is that they wrap around a protein. It is not a single protein is actually a multiple component protein, but I will discuss that a protein complex let us say which is called this histone protein complex.

So, it wraps around this histone protein complex, many many histone protein complex is like this to form something like beads on a string ok. So, here is my double helical DNA, here is my histone protein it wraps 2 and a it wraps 2 and a half times around this protein complex and then wraps around the next one and then wraps around the next one and so on. So, this object, this histone protein, this histone protein together with the DNA that is wrapped around

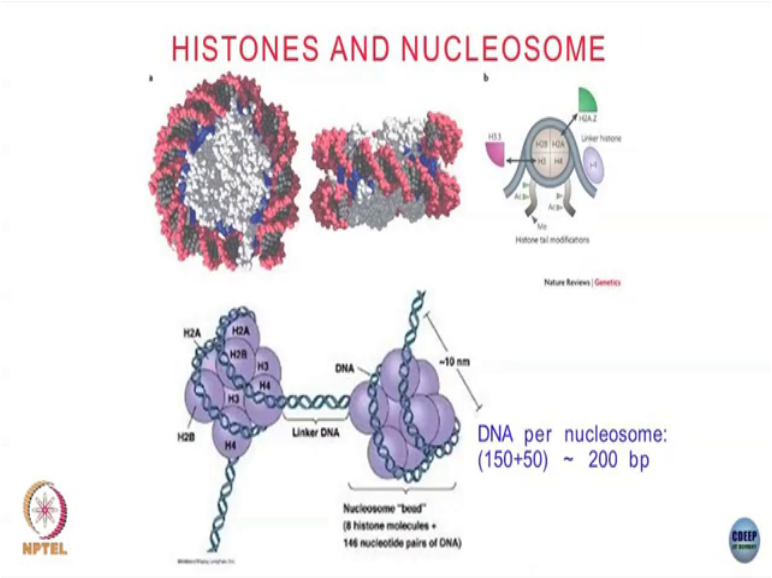
it; together with the DNA that is wrapped around it is what is called a nucleosome this is what is called nucleosome ok.

So, at this level I have these beads on a string structure then these beads on a string can further fold among themselves to form what is called as the 30 nanometer fiber. And then, somehow it folds amongst it folds more and more until ultimately you get this soupy looking structure that is that you see in these pictures. So, the thing is that up till here we sort of know very well that it forms these nucleosome complexes this beads on a spring structure. This thing there are conflicting evidences, some studies have seen a 30 nanometer fiber where you have these multiple sort of beads folding on top of each other.

So, each of these are some histones to form a thick sort of thick polymer which is called this 30 nano because the width is roughly 30 nanometers this is called the 30 nanometer fiber. So, there have been some studies which have seen sort of this 30 nanometer fiber, but increasingly evidence seems to suggest that this is not true; this is more of a something that is seen in a test tube, but not inside an actual cell, but anyway.

So, this is a gray area whether this 30 nanometer fiber exists or not. Beyond this as you can make out from this picture nothing really is known because it is just some hand waving picture where I have put coils and coils. So, at this level at this higher order folding nothing really is known all that is known is this part of the picture with some degree of confidence ok.

(Refer Slide Time: 10:36)



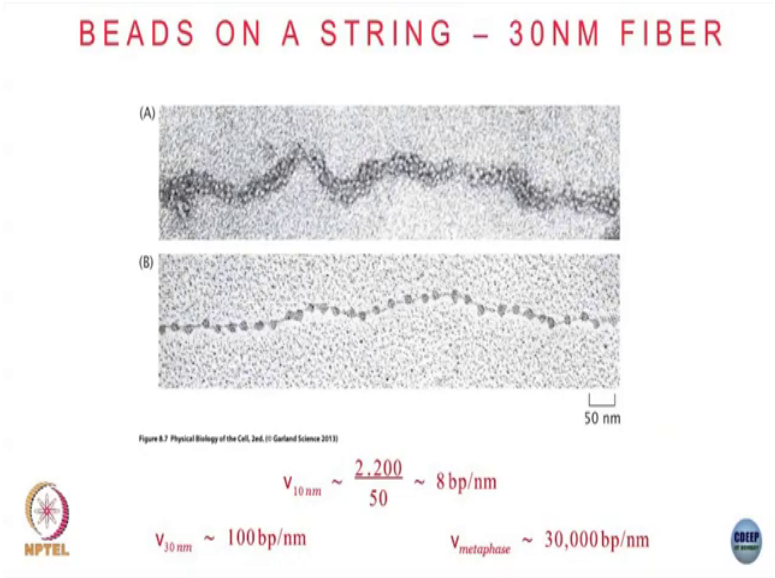
How do these histones look like? So, I said this is a multi protein complex. So, here is what the histones look like it has 8 proteins 4 of soul see H 2 A, H 2 B H3 and H 4, these are the 4 proteins of this histone complex each protein has two copies. So, it is an octamer this protein complex is an octamer.

So, you will see 2H2A is hopefully somewhere one ism. So, if you see 2H2B is one here one here 2H4 one here one here 2H3 and 2H2s. So, each of these four proteins are present in two copies. So, and together this octamer forms what is called as this histone complex and the DNA comes and it reacts two and a half times around this histone complex; then there is some region of DNA which is not bound to the bound to the histone and then there is DNA bound to another histone and so on and so forth.

So, this region this interconnecting DNA region which is not bound to the histone is what is called as linker DNA. And typically if you see the numbers; so, the amount of DNA that is coiled around each is around these histone protein complex is around 150 base pairs it is 146 roughly to be more precise and this linker DNA length can actually vary on an average its roughly like 50 it can go anywhere from 20 to 100 or 150 then, but roughly of this order fifty seventy something like this

So, per nucleosome the amount of DNA that is there per nucleosome is something around two hundred base pairs and then this structure continues and then there is other the structure which we do not know.

(Refer Slide Time: 12:28)



So, if you take pictures of this let me show it over here. So, this bottom this bottom figure is this DNA wrapped around these histones. So, these beads are these individual d a individual

histone proteins and you can. So, and this is sort of this 30 nanometer fiber where you have multiple beads that have folded into forming this thick fiber right.

So, you can calculate sort of this what is the base pair density in these different states. So, for example, if I look at this bottom figure this ten nanometer sort of a histone this nucleosomal beads on a string sort of structure the scale bar is around 50 nanometers right and how many beads do I have roughly in their fifty nanometers. I would say around two right roughly two beat. So, you can calculate what is the density of base pairs.

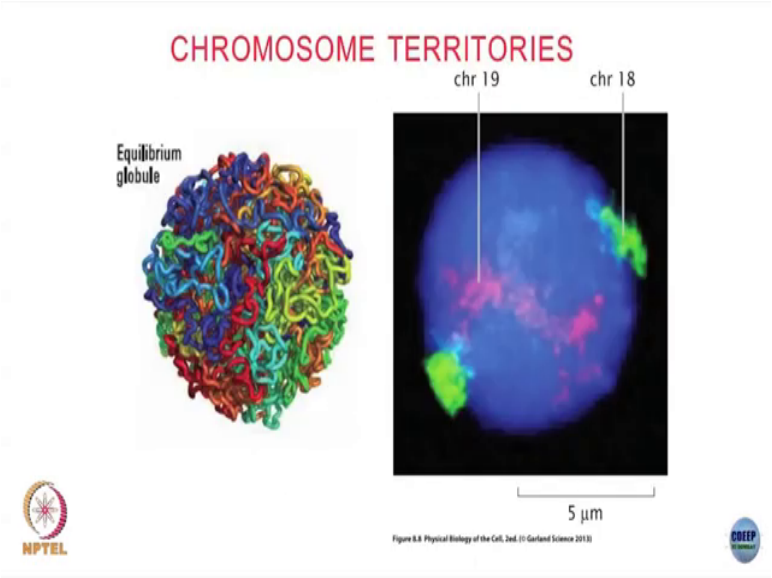
So, exactly they worked it out it is around 2.2; so, 2.2 by 50. So, that is around eight base pairs per nanometer. So, that is the density for this 10 nanometer fiber. If you look at this higher if you look at this 30 nanometer fiber of course, there is much more DNA in the same 50 in the same unit of length this 50 nanometer. So, if you look at this 30 nanometer fiber that has around 100 base pairs per nanometer and if you looked at this folded nucleosome this folded chromosome in the metaphase that x sort of a structure that is even more dense to around 30000 base pairs per nanometer.

So, somehow the cell or the body has sort of evolved mechanism to go from these densities of around eight base pairs per nanometers through some hierarchical folding principles into these extremely high densities of around 30 thousand base pairs per nanometer ok. So, this is fact number one, but these are actually extremely dense objects this chromosome assembles.

So, then how do I put them together? So, one thing I might imagine. So, I have this nucleus right and in this I must package my DNA as some are thread this is not a single polymer, but depending on the number of chromosomes that I have. So, let say I have human chromosomes I have 46 polymers floating around in here right and these are highly packed these are highly compacted because these exists at a very high density.

So, naively if I thought about it if I were just putting these polymers randomly inside the nucleus what I might expect, if I manage to color code each of these chromosomes separately is that they would all be mixed together right.

(Refer Slide Time: 15:15)



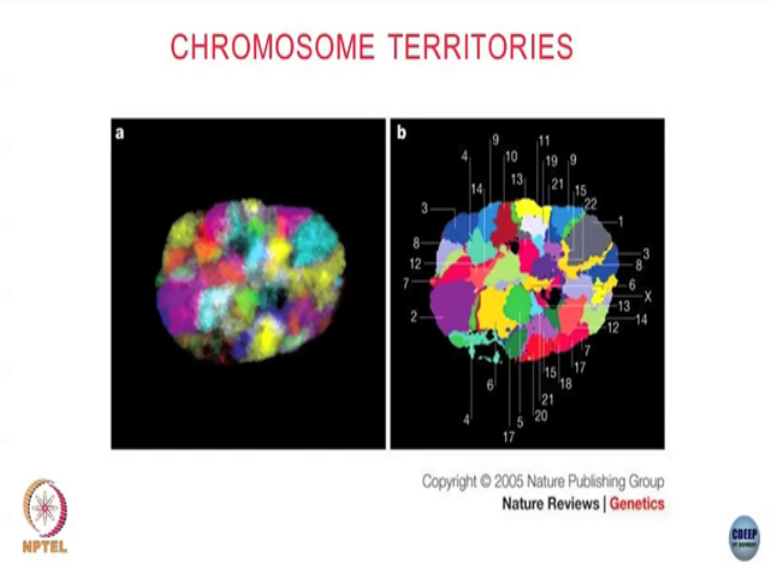
So, this is something what I might expect. So, the different colors denote different chromosomes for example. So, this is what I would expect in equilibrium entropy maximization would mean that everything is sort of mixed very well with everything else and all the chromosomes are sort of interconnected in the soup ok.

If you do experiments; you find that this is not actually the case. The chromosomes occupy extremely well separated regions known as territories. So, for example, here is an experiment where two chromosomes were labeled chromosomes 18 and 19 ok. And chromosome 19 occupied this interior region chromosome eighteen occupied this outs on region on the nuclear periphery and you can actually see two copies of this chromosome eighteen right.

And there is not at all. So, chromosome 18 is green, chromosome 19 is red and there is not much intermingling among these two at all. Also you see that there is difference in packaging

for example, this one is much more tightly package this one is much more loose packaged right. In fact, it is a genetic fact that these heterochromatin regions, the ones which are more tightly packaged will be found more often near the nuclear periphery and this euchromatin of the loosely packaged are found more towards the nuclear center.

(Refer Slide Time: 16:47)



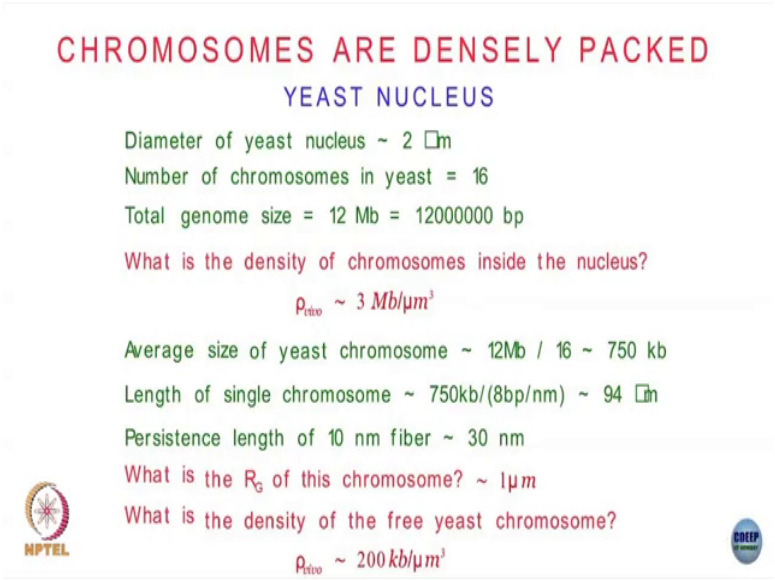
If you now do this for all the chromosomes in the human in the human nucleus; here is the picture that you get. So, this is an actual experimental picture this is slightly cleaned up where each chromosome has been colored according to different colors and you see there is not at all much intermingling between these colors right the chromosomes occupy extremely well separated regions there is not at all much crosstalk between the chromosomes.

So, this could never be in some sense an equilibrium configuration of polymers. If I took polymer configuration and I let it come to equilibrium I would never get something like this I

would see rather something like this. So, it is not really an equilibrium system although, we will use some level of equilibrium modeling, but or at least we will see what factors can give rise to sort of compact as give rise to this sort of territory formation. So, these are called chromosome territories in that in each chromosome occupies its own territory.

So, before I go on let me just estimate a little bit more about the density of chromosomes using some actual numbers.

(Refer Slide Time: 17:53)



So, for so what we will choose is this yeast nucleus ok. So, the diameter of a yeast nucleus is around 2 microns. The number of chromosomes is 16 and the total genome size is around 12 mega bases ok. So, 12 into 10 to the power of 6 base pairs. So, we can ask that therefore, I know that I know the radius of the nucleus I know the number of base pairs what is a density of chromosomes inside this nucleus.

And that comes out to be around 3 mega bases per micron cube. So, that is the density of these chromosomes inside the nucleus. Now, I could go ahead and ask that, now that I sort of know I know that if I have a polymer of length N I know typically what radius it should occupy and that is so, that R size or R G square as we saw scales as N. At least if I if it was a random walk sort of a polymer. So, if I do that what would be the sort of typical radius that I would come up with.

So, if I think about a single yeast chromosome. So, there are 16 chromosomes and the total length is 12 mega bases. So, assuming all chromosomes are roughly equal each chromosome is around 750 kilo bases. And, if I think that the densities of this beads on a string density the 8 base pair per nanometer the length of a single chromosome is around 94 microns, the persistence length of DNA as we said is around 30 nanometers 30 to 50 nanometers whatever.

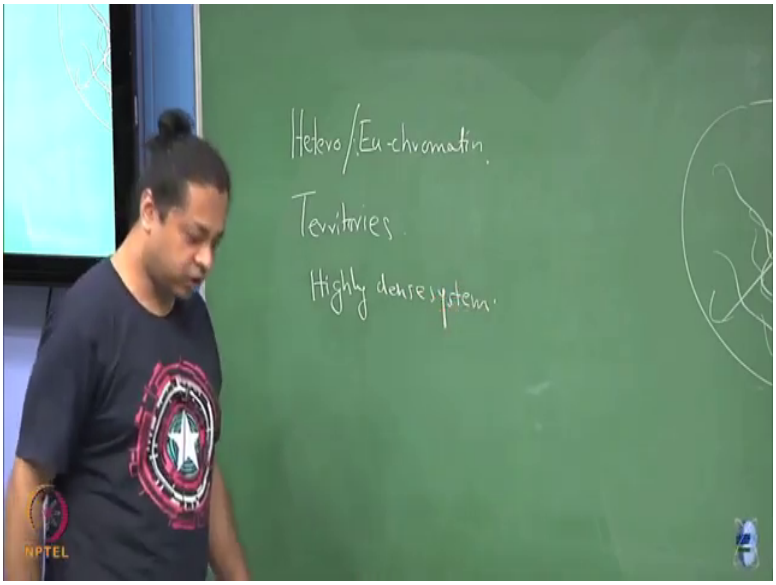
So, the persistence length I will use for the persistence length this same 30 nanometer number that I spoke about last class and then I can ask what is the typical size of a chromosome like this and it turns out that the typical R G of a chromosome like this is one micron and the density of this free yeast chromosome is around 200 kilo bases per micron cube. So, if I let yeast chromosome sort of freely float about in solution, it will form some sort of a polymer I can calculate the typical sizes and the typical densities and the density comes out to around 200 kilo base per micron cube whereas, inside the nucleus its around 3 mega bases per micron cube.

So, exactly it is an order of magnitude higher. So, it is much more densely packed then I would expect from a free random walk polymer sort of a model if I were estimating the density for free random walk model and, the density sort of set by this scale of the nucleus itself, because after all you must fit everything within this nucleus. So, whatever is the size of this nucleus that sets the scale of the density is in vitro.

This is the in vitro sort of thing if you just took out the yeast chromosome outside of the nuclear wall and just let it float in solution it would calculate what would be the typical

densities alright. So, that is one. So, let me just write down. So, what all have been learned. So, we have seen that there is heterochromatin versus euchromatin hetero versus euchromatin.

(Refer Slide Time: 21:23)



We have seen that chromosomes from territories in that each chromosome occupies its own space. We have seen that this is a highly dense system, this is a highly dense system much more than I would predict from typical random walks or Palestinian.