Cell Biology: Cellular Organization, Division. And Processes

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Lecture 21

Nuclear organization

Hello everyone, I am Shikha Laloraya, Professor of Biochemistry at IISc. Welcome to this lecture on nuclear organization in the ongoing course on the introduction to cell biology.

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The nucleus as you know is the main feature that distinguishes eukaryotic cells from prokaryotic cells. The nucleus is a compartment that has a double membrane enclosing the DNA, which is the genetic material. Within the nucleus, important DNA transactions occur such as replication, transcription of genetic information to RNA, and RNA processing. The nucleus also has a nucleolus, which is this dense body over here, which is the site of ribosomal RNA synthesis and ribosome assembly.

The presence of the nuclear compartment also provides an opportunity for restricting the entry of some transcription regulators until the right time thereby controlling their access to DNA. So, this provides another strategy for the regulation of gene expression that is not present in prokaryotes. The outer nuclear membrane is continuous with the endoplasmic reticulum and the nuclear inter membrane space or the periplasmic space is continuous with the lumen of the endoplasmic reticulum.

The outer nuclear membrane and the endoplasmic reticulum has got ribosomes bound to them so that they are easily available to associate with the messenger RNA as it exits the nucleus from the nuclear pore to facilitate translation. The nuclear membrane effectively separates the nuclear content which includes important macromolecules required for all the DNA related processes, from the cytoplasm.

Now just to recap, that, various other organelles are also present in the eukaryotic cells, as is shown here.

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So, the nucleus actually is separated from the cytoplasm by this nuclear envelope shown here in blue. And this has got two concentric membranes the outer membrane and the inner membrane. The nuclear envelope is perforated by nuclear pore complexes through which transport of various molecules occurs. The outer and the inner membrane as you can see here, they are connected, or continuous with each other at the nuclear pore.

And here you can again see the nucleolus this dense body over here. And you can also see chromatin, these thread like structures. And you can see the variation, it is loosely arranged or loosely folded in some regions whereas in other regions, such as here, it is densely folded and more compact.

The inner membrane is also supported by a structure known as the nuclear lamina shown here in purple. So, this is a network of intermediate filaments of lamin proteins. And its function is to provide the mechanical support to the nuclear membrane.

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Now shown here is an image of a cell from the tubule of the kidney cortex showing the nucleus; this is how it looks in reality. And the nucleolus within the nucleus again can be seen very well as this darkly staining heterochromatin region. And then in addition, you can also see several other heterochromatin regions as well as these lightly staining euchromatin regions. In this slide the mitochondria are shown in red and they are quite numerous as you can see, and the basal membrane and the intercellular space can also be seen which is present in between the two cells.

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Now shown here is a colour enhanced electron micrograph of part of a pancreas cell, showing an enlarged view of the nucleus. So, the nucleus is here, this grayish or bluish segment of a sphere. And here you can see the double layered nature of the nuclear membrane quite clearly, as well as interestingly, there is a nuclear pore visible in the nuclear membrane towards this right hand side; it is over here.

And you can see again the darkly staining heterochromatin and the light staining euchromatin regions within the nucleus. Here the mitochondria are in orange and there is also a lysosome shown in reddish brown. And you can also see the numerous tubules of the endoplasmic reticulum, which I said are present near the nuclear membrane and in some locations they might be continuous with it.

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Now as mentioned, the outer and the inner membrane are continuous at the nuclear pore; you can see this quite clearly in this diagram that I made. So, you can see the lipid bilayer, the outer membrane curving over and also continuing as the inner membrane over here. Inspite of this they

have several different proteins. So, they have an overall different protein composition between the outer nuclear membrane and the inner nuclear membrane.

The inner nuclear membrane has got proteins that bind chromatin as well as the underlying nuclear lamina; the nuclear lamina shown here in pink this network like structure, mesh-like structure over here. And the outer membrane has got ribosomes bound outside. And in fact the proteins made on these ribosomes, they are actually translated into this perinuclear space present between the inner membrane and the outer membrane, which is also continuous with the endoplasmic reticulum lumen or the space which is within the endoplasmic reticulum.

In addition, the outer membrane has membrane proteins that can interact with the cytoskeleton, on one side they are interacting with the cytoskeleton and in this complex, on the other side with the lamina. And of course, you can also see the chromatin present over here and in some places you can see it is associated not only with the lamina but also with these inner membrane proteins. And this association is important for some aspects of gene expression that we will discuss later.

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The inner membrane is supported by the nuclear lamina, now you can see it clearly when all the other structures have been removed from this figure. So, this lamina you can see lies below the inner nuclear envelope; it is the mesh work of intermediate filaments, which are made up of lamin. So, here is a lamin dimer and a lamin polymer or a lamin filament; it is made up in this way where the lamin dimer they associate in a head to tail fashion. So, they are made up of lamin A and lamin B subunits and these filaments, they also then form the network, which is by associating with each other. And hence they form this mesh, which is the network present below the inner membrane. As I already mentioned the lamina can associate with the inner membrane proteins as well as a chromatin present within the nucleus that was shown in the earlier slide.

And I just want to remind you that we have previously also discussed that a mutation in lamin proteins can result in Progeria syndrome, which is a type of a premature aging syndrome in humans. So, it indicates how important this structure is for the cell and also of course for development in aging. So, it probably affects gene expression in some way. And we have also discussed how the morphology of the nucleus was altered in the patient's samples as well.

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So, this is a just a colour enhanced scanning electron micrograph of a cell nucleus. So, here this large spherical, semi-spherical structure, that's the nucleus, elliptical structure. And it is surrounded by the cytoplasm. And this is a freeze fractured specimen that is the tissue was frozen and then fractured causing it to split along the natural boundaries of the tissue. And reveal therefore the surface of the nuclear membrane, the outer part of the nucleus.

So, if you look at the enlarged view, you will see these dots or little round dark spots, and these spots they represent the nuclear pore. So, these pores they actually provide a channel for the diffusion of

small molecules through them however larger proteins in RNA are also transported by this nuclear pore complex, but this is an active process, which requires energy and certain proteins also helping in the transport.

So, you can see the nuclear pores, they are quite numerous here. And this number can vary; the higher the ongoing transcription, more numerous other nuclear pore complexes in the nuclear envelope. For example, in a typical mammalian cell, it may have about 3000 or so, pore complexes. Now if DNA replication is ongoing then the cell needs to import a lot of histones. And this is of course required to package the newly synthesized DNA into chromatin.

Again, in rapidly growing cells each of these nuclear pore complexes also has to export a certain number of ribosomal subunits, which are quite large but they can actually pass through the pore. And so, you can see that it is a very important structure and when cells are actively dividing and growing, there will be more nuclear pores because the transport requirements are greater. And also in addition to the molecules I mentioned many other molecules are also transported through this nuclear pore complex.

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Now this diagram shows a cross-sectional representation of the nuclear pore complex, which you have been seeing but I have not mentioned it so, far. So, this is just half of the nuclear pore complex it has been cut lengthwise. And you can see it is a very large structure. And that is even here you cannot really appreciate that.

So, the nuclear pore complex it is got four main structural building blocks. There are these column subunits, which form the bulk of the pore wall, okay, there is a pore in the middle and surrounded by these subunits. Then there are annular subunits which extend spokes, which are not shown here towards the center of the pore; there are eight spokes around a central channel. So, the channel would be somewhere here and the spokes also connected to the rings on the cytoplasmic side as well as the nuclear side.

Then there are also present subunits referred to as lumenal subunits, which connect the transmembrane proteins; the transmembrane proteins that anchor the complex to the nuclear membrane. So you can see on each side there are these proteins which are basically sort of very closely opposed to the nuclear membrane. So, they help in holding it in place in the membrane.

And then there are the ring subunits of course, which form the cytoplasmic which from the nuclear ring and the cytoplasmic ring of the complex. So, in addition you can see these fibrils, which are extending from both the cytosolic side as well as a nuclear side of the rings present in the nuclear pore complex. On the nuclear side you can see that these filaments or fibrils, they converge to form a basket like structure. Now here again this is just half of the basket and you will appreciate it in the upcoming slide.

So, a lot of localization studies have been done using immunoelectron microscopy and they show that the core, the subunits making up the core of the nuclear pore, are symmetrically distributed across the nuclear envelope. So, that the nuclear as well as the cytosolic sites they more or less are

identical. But this contrasts with the proteins which make up these fibrils, which are different on each side, on the cytosolic or nuclear side. As I already mentioned on the nuclear side they are connected and they form a basket, whereas they are sort of free floating on the cytoplasmic side they are not connected really with each other. And I already mentioned this in the previous slide but you can see that not only is a nuclear pore complex associated with the nuclear membrane, but also that the outer membrane and the inner membrane are continuous with each other at the nuclear pore.

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Now this is a computer graphic of a nuclear pore in a eukaryotic cell; this is the complete model, not half, unlike what I was showing you earlier. So, you may note the octagonal symmetry. The nuclear pores as I already mentioned, they are very large protein complexes. And each nuclear pore complex has an estimated molecular mass of 125 million or so. And it is thought to be composed of more than 50 different proteins called nucleoporins.

In yeast, the native nuclear power complex has a mass of 52 Mega Daltons, or it may be 87 Mega Daltons when you consider the membrane, the cargo, and the nuclear transport factors associated with it. It has eightfold symmetric cylindrical assembly, consisting of about 500 copies of 30 different proteins, which are referred to as nucleoporins or Nups in yeast. And each nuclear pore complex has got an eight-fold symmetry. And it is a cylindrical assembly; you can see this, it is sort of tilted over here. And here you can see the complete basket, the nuclear basket, which is present towards the nuclear side of the pore complex. So, you can see it in its entirety over here.

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Small molecules can diffuse freely, up to 5000 Daltons or even 17000 Daltons, takes a couple of minutes to equilibrate across the nuclear envelope. Whereas proteins which are larger than that, say greater than 68000 Daltons, they cannot cross the nuclear envelope by mere diffusion. Active import processes are required to transport such large molecules into the nucleus. So, nuclear proteins, that is proteins which are destined to go into the nucleus, they have got targeting signals.

For example, a nuclear localization signal or an NLS. In many nuclear proteins, the NLS consists of one or two short sequences which are rich in positively charged amino acids such as lysines and arginines. For example, in case of SV40 T antigen, which is one of the first NLSs to be discovered, the sequence is quite rich in lysine and arginine, it is the short sequence PKKKRKV. And in some other nuclear proteins there are different signals, which are not following this consensus formula. And hence these are probably recognized by a different receptor. Now the NLS can be located anywhere within the sequence, its precise location per se is not important. And it can even target a cytosolic protein to the nucleus if this sequence is artificially fused to these proteins. The NLS is recognized by nuclear import receptors, which are encoded by family of related genes. Each family member encodes a receptor, which is specialized to transport a group of nuclear proteins that share a similar nuclear localization signals that share similar features, that is.

And we can talk about the classical NLSs; these are the ones which are rich in the lysines and arginines and these are recognized by transport receptors known as importins. The nuclear import receptors bind to the NLS on the protein to be transported as well as to the nucleoporins. Some of the nucleoporins, they form filaments that extend into the cytosol from the cytoplasmic side of the nuclear pore complex as I mentioned earlier. So, these filaments as well as some of the other nucleoporins that make up the nuclear pore complex, have a very large number of short amino acid repeats of phenylalanine and glycine, referred to as FG repeats. These repeats serve as binding sites for the import receptors. So, these repeats, the FG repeats that is, they line the path taken by the import receptors and their bound cargo proteins as they are moving via the NPC's.

The import receptor complexes repeatedly bind, dissociate, and then rebind to adjacent repeat sequences. And thus they move along the particular path within the channel of the nuclear pore complex. Inside the nucleus, the cargo is released from the receptor. And then the receptors can also return to the cytosol.

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Now the importins, they work with a protein known as Ran. Ran is a monomeric GTP binding and hydrolyzing protein, it is a GTPase. Import of nuclear proteins through the nuclear pore complex concentrates specific proteins in the nucleus increasing overall order in the cell and as we discussed in one of the introductory lectures, this consumes energy. And this energy is thought to be provided by the hydrolysis of GTP by this GTPase known as Ran. Now Ran is present in the cytosol as well as the nucleus and it is required for both nuclear import, as well as export. Ran can be thought of as a molecular switch that exists in two conformational states depending upon whether GTP or GTP is bound to it. And the conversion between the two states is triggered by two regulatory proteins; there is a cytosolic GTPase activating protein or a GAP, Ran-GAP, that triggers GTP hydrolysis. And thus converts Ran-GTP to Ran-GDP. And there is a nuclear guanine exchange factor or GEF that promotes the exchange of GDP for GTP and thus it converts Ran-GDP to Ran-GTP.

So, Ran-GAP is located in the cytosol, the Ran-GEF is located in the nucleus. And therefore, the cytosol has mostly Ran-GDP and the nucleus mostly has got Ran-GTP. This differential localization of Ran-GTP in the nucleus, and Ran-GDP in the cytosol, provides directionality to this nuclear import process. The hydrolysis of GTP to produce Ran-GDP is mediated by Ran-GAP and Ran binding protein on the cytosolic side of the nuclear pore complex. And on the other hand the factors that stimulate the exchange of GDP for GTP are associated with chromatin in the nucleus.

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So, here is an example, here is shown the import of TRPS1. This is a repressive transcriptional factor it is involved in the regulation of chondrocyte and perichondrium development; the details of its activity are not so important here. But the NLS of TRPS1, shown here in pink, is recognized by importin molecules, which they come in bind to this and then they move the protein through the nuclear pores. And once they are inside the nucleus, then TRPS1 protein is released and it can go ahead and complete its function. So, inside the nucleus the TRPS1 is released from the complex by

Ran-GTP that binds to importin-beta. And also, this process releases the importin alpha subunit. The Ran-GTP importin beta can now return to the cytosol again via the NPC the nuclear pore complex where its conversion to Ran-GDP by Ran-GAP occurs, the GTP is activating protein. And then this releases importin-beta which can now associate again with another cargo.

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Many of the imported proteins shuttle back to the cytoplasm. And some of these may be carriers during the transport of other molecules such as RNAs or may even regulate transcription factors. So, the nuclear export really occurs by a similar process or similar mechanism, but it is in reverse. So, proteins that are exported from the nucleus have nuclear export signals. And these signals are rich in hydrophobic amino acids such as leucine. In this case, the Ran GTP in the nucleus promotes cargo binding to the export receptor or exportins and the binding of the loaded receptor to the nuclear side of the NPC. In the cytosol, Ran-GAP and Ran binding protein bring about hydrolysis of the Ran-bound GTP. The export receptor then releases both its cargo and the Ran-GDP in the cytosol and it then dissociates also from the pore complex. Now these free export receptors are then returned to the nucleus. Many of these import and export receptors are members of a family of transport receptors known as karyopherins.

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RNAs are also transported via the nuclear pore complex. So, many RNAs are exported as ribonucleoprotein complexes. Export of tRNAs that is transfer RNAs, ribosomal RNAs, and miRNAs is mediated by specific karyopherin proteins. rRNAs associate with ribosomal proteins some of which have got export signals, in the nucleolus, and then the assembled 40S and 60S subunits are exported to the cytoplasm by an exportin termed Crm1.

mRNA export is independent of karyopherins and also Ran-GTP. It follows a different mechanism. There is a distinct mRNA exporter complex, which associates with pre-mRNAs. And then after processing, it transports the mRNAs through the nuclear pore complex. And there is an RNA helicase on the cytoplasmic side of the nuclear pore complex, which remodels the mRNA and thereby it releases the exporter complex and the mRNA on the cytosolic side.

In contrast with these RNAs, many non-coding RNAs for example snRNAs, that are required for pre-mRNA splicing, and snoRNAs, which are required for rRNA processing, function in the nucleus. So, the snoRNAs are formed and they stay in the nucleus, but the snRNAs although they are formed they are nevertheless transported to the cytoplasm by the exportin Crm1. And in the cytoplasm interestingly they bind proteins to form snRNPs. And then an import receptor referred to as snurportin, recognizes the sequences in the snRNP proteins and transports them back into the nucleus. So, there is this business of export-import going on for the snRNAs, in the nucleus.

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Now regulation of this process of import can have beneficial effects. The regulation of import of transcription factors can allow for another way to regulate gene expression, which is a possibility not present in prokaryotes. So, for example in mammalian cells, the transcription factor NF-kappaB is retained in the cytoplasm in an inactive complex with an inhibitory subunit, I-kappa B. I-kappa B actually masks the NLS of NF-kappa B preventing it from being transported into the nucleus. Upon stimulation of the cells the I-kappa B is phosphorylated. And then it is degraded by ubiquitin mediated proteolytic pathway. So, now NF-kappa B whose NLS is exposed can be imported by recognition of its unmasked NLS and it goes inside the nucleus and it activates the transcription of its target genes.

In budding yeast also, the transcription factor Pho4 is retained in the cytoplasm because there is a phosphorylation near its NLS. There is regulated dephosphorylation of Pho4 and that allows Pho4 to be now transported into the nucleus and activate the transcription of its target genes.

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So, what is inside the nucleus? Of course, there is DNA which is organized in the form of chromatin but also there are various other nuclear bodies. These nuclear bodies include the nucleolus, we have mentioned this many times a large nuclear body, the dense, darkly staining body. This is a place where rRNA synthesis and ribosomal subunit assembly occurs. There also polycomb-bodies or P-bodies, which are sites of transcriptional repression. And Cajal bodies and speckles where the processing and storage of snRNPs occurs; snRNPs by the way if I did not mention it stands for small nuclear RNPs ribonucleoproteins.

Now of course chromatin as you know is a complex of DNA with histones and other non-histone proteins and it can be of different types we already mentioned euchromatin and heterochromatin the lightly staining and the darkly staining densely packed regions within the nucleus and these are sites of gene expression versus gene repression or silencing, which occurs in heterochromatin which is a repressive structure.

It has also been observed that there is actually non-random distribution of each chromosome within the nuclear space to form regions, which are referred to as chromosome territories. Also, key processes which occur in the nucleus such as the DNA transactions, which occur on DNA they are actually localized to clustered regions of the nucleus. For example actively transcribing genes may be found at transcription factories. And there are also regions known as replication factories where you can find lots of replication factors and ongoing replication, the forks tend to cluster over there.

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This slide shows various kinds of nuclear bodies in an actual nucleus, we mentioned some of them earlier. So, here the nucleolus is shown in red and we already mentioned this is a site of ribosomal RNA synthesis and ribosome subunit assembly. The Cajal bodies are shown in pink and speckles are labelled in green over here. And these are places where the processing and storage snRNP occurs. And of course the chromatin or the DNA as always is in blue.

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Other levels of organization within the nucleus are also there. For example, the subnuclear chromatin localization within the nucleus can influence the transcription activity of the genes, which are present on these parts of chromatin. So, we already mentioned that within the nucleus darkly staining regions of compact, dense, chromatin are termed heterochromatin. And the genes located here are transcriptionally repressed.

The light staining regions correspond to euchromatin that has a more open chromatin organization and it is transcriptionally active. Histone modifications are also important for establishing the heterochromatin versus euchromatin states and they affect transcription activities. Histones as you know are basic proteins that associate with the DNA in the form of an octamer of histones around which the DNA is wound and this is known as a nucleosome.

So, there are four different histones and they have projections termed histone tails. And there often the residues, many of the residues on these histone tail extensions may get post-translationally modified. Histone deacetylation by the proteins Sir2, which is a silencing protein, and also related histone deacetylases, promotes heterochromatin formation and gene silencing.

The transcriptional activity of chromosomal domains is also influenced by its subnuclear localization. Transcriptionally inactive chromatin often has a more peripheral localization in the nucleus. And it is associated with the nuclear lamina where it is referred to as LADs or Lamina Associated Domains of chromatin or it may be associated with the nucleolus in which case it is referred to as NADs or Nucleus Associated Domains. So, transcriptionally active chromatin on the other hand is localized towards the interior of the nucleus.

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In addition, another interesting aspect of nuclear organization, which I referred to briefly is the organization of chromosomes in interphase into chromosome territories. So, in the interphase nucleus you can see here, there is actually non-random distribution of chromatin. So, there are different chromosomes and they occupy specific regions; they are not unfolded all over the place, mingling with each other indiscriminately. So, you can see this: each chromosome, it occupies a distinct region of the nucleus. And this region is termed a chromosome territory. And this actually is done by a technique known as FISH (Fluorescent in situ Hybridization). And here in this case, they did a 24-colour 3D-FISH representation, as shown here. And this is in an interphase human G_0 fibroblast nucleus. So, each of these chromosomes they occupy a distinct region and also they have a particular position in relation to each other.

I hope this lecture has helped you understand the organization of the nuclear compartment in the eukaryotic cell in a better way, Thank you.