Cell Biology: Cellular Organization, Division and Processes
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Lecture - 02
Introduction to Cell Biology, Cell Components, Organization and Processes, Part II

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For this introductory lecture we will discuss cell compartments, plasma membrane, bio energetics, and mitochondria.

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So we have discussed that the cell is composed of various small and large molecules in an aqueous environment undergoing various reactions. Cells are highly organized and there are various complex interconnected processes going on within them.

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So let us try to understand the organization of a eukaryotic cell. A eukaryotic cell has got a nucleus which has the genetic material or DNA. Inside the nucleus various DNA related processes happen such as the transcription of genetic information into RNA. The nucleus also has this dense body known as the nucleolus, which is a site of ribosomal RNA synthesis and ribosome assembly.

The nucleolus usually has a peripheral localization within the nucleus and it is not itself bounded by a membrane. Cells also have endoplasmic reticulum and the Golgi apparatus, which are organelles where the sorting and transport of proteins destined for secretion occurs. The endoplasmic reticulum is an extensive network and it is important for processing and transport of proteins and also for synthesis of lipids. From the endoplasmic reticulum the proteins are transported within small vesicles to the Golgi apparatus.

And further processing and sorting for transport occurs there. The Golgi apparatus also has lipid synthesis and synthesis of certain cell wall polysaccharides going on. The cell also has numerous types of vesicles such as the secretory vesicles already mentioned. There are also ribosomes in the cell many of which are associated with the endoplasmic reticulum. And in such a configuration the endoplasmic reticulum is referred to as the rough endoplasmic reticulum.

There are mitochondria which are important organelles for generation of ATP from the breakdown of organic molecules and we will discuss much more about them in this lecture. There are also lysosomes, which are organelles for digestion of macromolecules and peroxisomes, which are compartments in cells for various oxidative reactions. Animal cells also have a vacuole, which is rather small.

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Structural integrity of cells is supported by a cytoskeleton. The cytoskeleton is composed of microtubules, intermediate filaments, and actin filaments. And there is also a centrosome, which has a got two centrioles surrounded by matrix but no membrane and this organelle ,which actually is not surrounded by any kind of membrane, it nucleates microtubules.

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Plant cells have a cell wall and they also have a prominent vacuole. And they have chloroplasts and they have got various kinds of plastids for example the amyloplast shown here that stores starch and various other similar organelles.

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So, coming to the plasma membrane, the plasma membrane of course it encloses all the contents of the cell and it is the outer boundary. Hence, it is a rather important part of the cell.

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The plasma membrane components in animal cells will be discussed here. So, the plasma membrane consists of lipids as well as proteins. Membrane lipids are mainly phospholipids, glycolipids and cholesterol. Phospholipids consist of 2 hydrophobic fatty acid chains, which are linked to 2 carbon atoms in a glycerol moiety. And the 3rd carbon atom of the glycerol is bound to a phosphate group, which is negatively charged and in turn attached to a small polar molecule such as choline, serine, ethanolamine or inositol to form phospholipids phosphatidyl choline phosphatidyl serine, phosphatidyl ethanolamine, phosphatidyl inositol and sphingomyelin. Sphingomyelin has got serine instead of glycerol. Glycolipids on the other hand have the 2 hydrocarbon chains linked to a polar group that contains carbohydrates. And cholesterol is structurally different. It has got 4 hydrocarbon rings that are strongly hydrophobic so it can insert within the membrane. However, 1 end of it has got a hydroxyl group that is weakly hydrophilic. So, these membrane lipids they are anphipathic molecules and they can form self-sealing lipid bilayers with a polar head group which is exposed to the aqueous and the hydrophobic tails are facing away from the water and they are facing each other in the interior of the bilayer.

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And this slide shows some more additional properties of membrane lipids such as mobility of these phospholipids in a bilayer, they can undergo lateral diffusion in the plane of the membrane, they can also undergo rotation or the side chains can undergo some level of flexion. However, the movement of the phospholipids from 1 layer of the membrane to the other, that is a flip flop movement, is rather rare because it is energetically unfavorable, of course.

Although the membrane shown here is symmetrical, in fact, there is some asymmetry in the plasma membrane, there is certainly asymmetrical distribution of phospholipids. For example, on the outer layer of this membrane, glycolipids, phosphatidylcholine , sphingomyelin and cholesterol are more abundant whereas on the inner side which is facing the cytosol, phosphatidylserine and phosphatidylethnolamine are more abundant, phosphatidylinositol is also present and smaller amounts of cholesterol, phosphatidylcholine and sphingomyelin may be present. This asymmetry is functionally important because the cytosolic inside of the membrane has certain enzymes which

come to this part of the membrane and they are involved in important signalling events within the cell.

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Membrane proteins can be integral membrane proteins or they can be peripheral membrane proteins. Integral membrane proteins are transmembrane proteins; they may have 1 or more membrane spanning alpha helices with 20 to 25 non polar amino acids, or they may have a beta barrel that is beta sheets, which are folded into a barrel like structure. These integral membrane proteins have exposed hydrophilic portions on either side of the membrane.

Peripheral membrane proteins on the other hand are not directly inserted into the membrane but they associate via interactions with other proteins or with phospholipids. Some proteins can also be inserted into the membrane by covalent attachment to lipids, for example to the extracellular face of the plasma membrane via glycolipids. For example, the Thi1 antigen in lymphocytes via the C terminal GPI anchor, shown here.

Another example, which was not shown here could be the attachment of certain proteins to the intracellular face of the plasma membrane via covalent attachment to fatty acids. An example of this is this Src oncoprotein, which is attached via an N-terminal myristoyl group, or prenyl groups in case of the Ras protein, where the prenyl and palmitoyl groups are attached to 2 C- terminal cysteine residues in this protein.

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The fluid mosaic model of biological membrane structure was originally proposed in 1972 by Singer and Nicolson and this is still a valuable model for understanding the membrane organization. According to this model, the overall membrane structure is determined by a combination of hydrophobic and hydrophilic interactions. Globular integral membrane proteins are inserted into the bilayer; these membranes spanning proteins are amphipathic with non-polar regions which are inserted in the bilayer and polar regions exposed to the aqueous environment on either side. The membrane proteins according to this model can move laterally throughout the lipid bilayer, that is, they are free to move.

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Now, it was observed later on that many membrane proteins were restricted in contrast with the free movement in the bilayer as proposed by the fluid mosaic model and certain other observations indicating that membranes are composed of distinct domains were also made. And hence this model has been modified. And this is an interesting article by Nicolson in BBA that is worth reading.

So, according to this revised model, membranes are composed of distinct domains that have important structural and functional roles. Some of the plasma membrane proteins are associated with the underlying cytoskeleton elements and these cytoskeleton elements in the cortex are often referred to as a cortical fences. This association actually restricts their mobility and therefore they cannot move around freely and they also act as barriers to restrict the mobility of other proteins. In addition, some of the membrane lipids such as cholesterol, sphingomyelin, and glycolipids, tend to cluster, and they form semi solid lipid rafts that compartmentalize the plasma membrane into

distinct domains. These are enriched in GPI-anchored proteins, and also certain transmembrane proteins, and they are involved in specific functions. Caveolae are a subset of lipid rafts. There is a membrane protein caveolin, which interacts with a cytoplasmic protein, cavin, and cholesterol, and they form invaginations, which are important for endocytosis.

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Cell membranes are selectively permeable. So, gases, then hydrophobic molecules and small uncharged polar molecules such as water can diffuse freely across the membranes. However, large uncharged polar molecules for example glucose or charged molecules, and ions, cannot diffuse through, but some of them can still cross the cell membranes via transmembrane transporter proteins.

If this transport requires energy derived from ATP hydrolysis it is termed as active transport. Shown here are examples of certain ion transporters and channels present in neurons. The ion transporters actively move ions against the concentration gradient and they can therefore create ion concentration gradients. Ion channels on the other hand allow the ions to diffuse down the concentration gradient and they can cause selective permeability to certain ions.

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Now cells obtain energy from food. Enzymatic breakdown of food molecules occurs outside the cells in the intestine and multicellular organisms or inside the cell in the lysosomes. Digestive enzymes are present in the lysosomal compartment and they break down proteins into amino acids, polysaccharides into sugars, and fats into fatty acids and glycerol. The small organic molecules produced after digestion enter the cytosol of the cell where their oxidation starts.

Glucose is converted into pyruvate; that is 2 molecules of pyruvate arise from one molecule of glucose via glycolysis. ATP and NADH are also produced. Pyruvate then passes from the cytosol into mitochondria. In the mitochondria each pyruvate molecule is converted into carbon dioxide + 2 carbon acetyl group, which becomes attached to coenzyme A forming acetyl coenzyme A, which is an activated carrier molecule, by oxidative decarboxylation.

So, CO_2 and NADH are also produced in this process. The acetyl group is linked to coenzyme A via a high energy linkage and therefore it can be easily transferred to other molecules. In the matrix the acetyl group of acetyl Coenzyme A is transferred to the 4-carbon molecule oxaloacetate, to form citrate, which then enters a series of reactions known as the citric acid cycle. Two carbons of citrate are oxidized to carbon dioxide and oxaloacetate can be regenerated. And this would complete a full cycle which is of course a multi-step process. And in this process one molecule of ATP, one of FADH2 and 3 of NADH form for each turn of the cycle.

Now electrons from NADH and FADH2 are transferred by the electron transport chain which is located in the inner mitochondrial membrane, to molecular oxygen coupled to the formation of ATP molecules by a process known as oxidative phosphorylation.

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Shown here is glycolysis and respiration from a biochemist's perspective. Note that the breakdown of glucose results in two molecules of pyruvate by a multienzyme pathway. This pyruvate then enters into the mitochondria. In the mitochondria, pyruvate is converted to acetyl coenzyme A by oxidative decarboxylation. This acetyl group, as I already mentioned, is linked to coenzyme A via a high energy linkage and therefore it can be easily transferred to other molecules such as oxaloacetate in the beginning of the citric acid cycle, and it forms citrate. Now this is a multistep process again, and in the end the 2 carbon atoms from citrate are oxidized to carbon dioxide and oxaloacetate is regenerated by going through the cycle. In this process one molecule of ATP, one of $FADH_2$, and 3 molecules of NADH, are formed with each turn of the cycle.

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To understand ATP synthesis, the chemiosmotic theory was proposed by Peter Mitchell. He proposed that ATP synthesis is driven by an electrochemical gradient across a membrane rather than a high energy intermediate. Because such a high energy intermediate could not be found by biochemists at that time. And it was also observed that intact membranes are required for phosphorylation. So he proposed that the intermediate that couples electron transport to ATP synthesis, is a proton electrochemical gradient across the membrane. And this proton gradient is produced by electron transport. The flow of protons back across the membrane in the energetically favorable direction, is coupled to ATP synthesis.

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ATP synthesis occurs in the inner membrane via the electron transport chain and the enzyme ATP synthase. The electrons from NADH and $FADH_2$ are transferred ultimately to molecular oxygen. And this process is coupled to the formation of ATP molecules by oxidative phosphorylation. This process occurs via the passage of electrons through a series of electron carrier molecules shown here which are inserted in the inner membrane and these molecules, they comprise the electron transport chain. The transfer of electrons through these complexes is associated with a decrease in free energy, which is in fact used to pump protons or hydrogen ions from the matrix into the intermembrane space. And this results in a proton gradient across the inner membrane; there are more protons in the intermembrane space in the end. So, these protons can then flow back into the matrix via the ATP synthase complex. And the energy which is released in this process is coupled to the synthesis of ATP from ADP and Pi.

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Shown here is the structure of mitochondria to understand ATP synthesis from a cell biology perspective. Mitochondria have an outer membrane and an inner membrane and an inter membrane space between the two. The inner membrane encloses a mitochondrial matrix. Pyruvate and fatty acids enter into the mitochondria from the cytosol. Each pyruvate molecule is converted into CO_2 + a two carbon acetyl group, which becomes attached to coenzyme A forming acetyl coenzyme A, an activated carrier molecule, by oxidative decarboxylation.

Fatty acids are also converted to acetyl coenzyme A in the mitochondria. Their acetyl group is linked to coenzyme a via high energy linkage and it can be easily transferred to other molecules such as a 4 carbon molecule oxaloacetate to form citrate, and it then enters a series of reactions called the citric

acid cycle. 2 carbons of citrate are oxidized to carbon dioxide and oxaloacetate is regenerated in 1 turn of the citric acid cycle. 1 molecule of ATP, one of $FADH_2$ and 3 of NADH are formed for each turn of the citric acid cycle.

Electrons from NADH and $FADH_2$ are transferred to molecular oxygen coupled to the formation of ATP molecules by oxidative phosphorylation. This process occurs via the passage of electrons through a series of electron carrier molecules in the mitochondrial inner membrane comprising the electron transport chain. Electron transfer via the electron transport chain is associated with a decrease in free energy that is used to pump the protons from the mitochondrial matrix into the intermembrane space and this generates a proton gradient across the inner membrane. The proton gradient drives the production of ATP. Protons flow back into the mitochondrial matrix through the ATP synthase enzyme which is also embedded in the inner mitochondrial membrane. This flow of protons is coupled with release of energy, which is utilized for the synthesis of ATP from ADP and inorganic phosphate.

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The ATP synthase is a fascinating molecular machine that produces ATP by rotary catalysis. It is very interesting to look into its mechanism and its structure. ATP synthase is composed of a head portion referred to as the F_1 ATPase and it also has a transmembrane portion, the proton carrier region known as F_0; both F_1, and F_0 are formed from multiple subunits. So they are rather complex entities and they are connected in 2 ways, 1 by a rotating stalk which turns with the rotor formed by this ring of 10 to 14 subunits, which are inserted in the membrane.

And then there is also another connector referred to as the stator or the peripheral stalk, which actually has an arm which connects the subunits inserted in the membrane with the F_1 head. Okay, so the head region of the F_1, it consists of 3 alpha and 3 beta subunits and there is a path for protons which is created by the a subunit along with the c subunits of the ring, which rotates past it; there is a transfer of protons from this complex along a somewhat convoluted path, which makes this part rotate.

The free energy of the proton movement down this electro chemical gradient is harvested by the synthesis of ATP. So F_0 is embedded in the inner membrane and it forms a spinning channel through which the protons cross the membrane. As it spins, this rotor stalk, which is attached to it, also spins and it is in contact with the inside of the head region, which has these catalytic subunits. So the beta subunit has got a catalytic nucleotide binding site, which is present at the alpha/beta interface.

And these catalytic sites they have got different conformations depending on their interaction with the stalk. So as the stalk rotates it changes the conformation of these beta subunits sequentially. So, there could be one conformation which has high affinity for ADP and Pi, and the rotating rotor stalk can push it into a different conformation driving the formation of ATP, and then the release of ATP.

So in this way mechanical force exerted by the central rotor stalk is converted into the chemical energy of the ATP high-energy phosphate bond. This is a really efficient machine; it spins at 8000 revolutions per minute and it can generate 3 ATP molecules per turn coupled with the flow of 3 or 4 protons into the matrix and therefore it will result in the formation of 400 ATP molecules per second.

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Now, shown here is an animated 3-dimensional model of adenosine triphosphate or ATP, and also the mechanism of its synthesis within the mitochondria that has been made and provided by Dr. Drew Berry and Etsuko Uno. ATP provides energy for the biochemical activity in cells and it is also a building block of DNA and RNA within the cell. Mitochondria form elongated structures that form an intricate network shown here in yellow. They produce ATP using the energy from the breakdown of food. The inner membrane of the mitochondria has got rows of the rotary molecular machines consisting of the multiple subunit enzyme, the ATP synthase, that generates ATP from ADP and Pi. A ring of enzymatic subunits work in step producing 3 molecules of ATP with each rotation cycle, converting the mechanical energy into chemical energy. The rotating axle powers a sequence that is attached to a rotary molecular motor in the inner membrane moved by the force of protons, which are coming from the intermembrane side of the membrane where their concentration is higher, resulting from the pumping of protons into the intermembrane space during electron transport. So, a difference in the proton concentration propels the mechanism as the protons flow through this F_1-F_0, rotor ring, it rotates along with the central stalk of the axle that in turn brings about conformational changes in the subunits of the F_1 head region near it. A subunit bound to ADP and Pi can change conformation favoring conversion to ATP and its release and in this way with repeated cycles driven by the return of protons to the matrix, 1 ATP synthase can produce up to 400 molecules of ATP per second.

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So finally, in this lecture we have discussed the organization of cells and key examples of complex molecular processes that occur within the cells for their sustenance and maintenance. I hope this lecture has provided a clearer picture of the miniature world within the cell. Thank you.