Introduction to Biomicrofluidics
Prof. Tapas Kumar Maiti
Department of Biotechnology
Indian Institute of Technology, Kharagpur

Lecture - 03
Engineers' Guide to the Cell

Start.

So in this lecture module I shall give the overview of membrane cell to the non-biologist. And at the same time I shall give that utility of micro fluidics platform to unravel that cellular behaviour like in vivo situation and at the same time cell at the single cell level how it behaves and to unravel their physiological, and other chemical and biochemical cues.

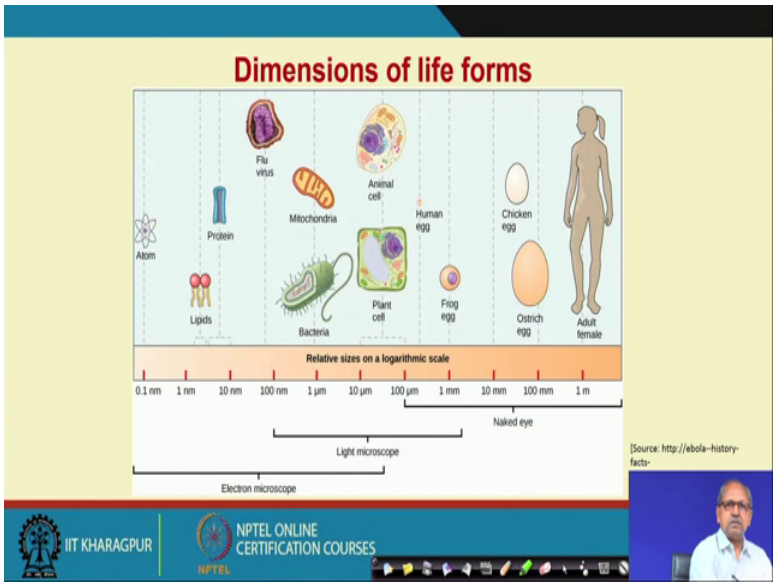
Now, if we look for that cells these are the unit of all leaving organisms is comprises of that different types of molecules.

(Refer Slide Time: 01:06)

And, so this is the cell this the unit of leaving organisms and it obeys that all the physical and bio physicochemical laws of nature which is obeys by that nonliving methods also.

So, these cells are organized to tissues, then tissues are organized, organ then organ to organ system then whole organisms. This hierarchy of organisms obeyed in all types of higher organisms. And if you look for the cells is a composite of molecules and atoms.

(Refer Slide Time: 01:59)

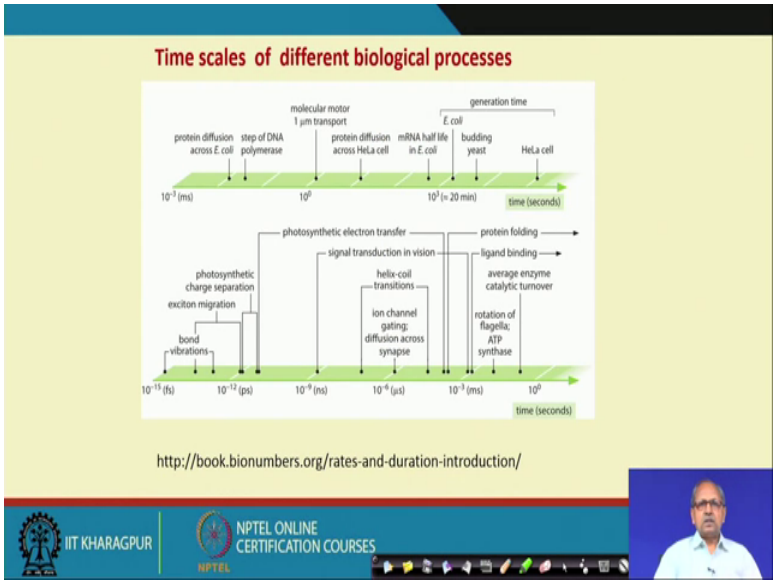


If we consider the dimension of the cells like say animal cell, plant cell is in the range of around say 10 micrometer to 100 micrometer. Whereas, in the bacterial cells is the range of 1 to 2 micrometer and some cell organelles is in the range of 1 to 2 micrometer. These cells are organized to whole animal which is the dimension around that 1 meter and that molecule is in the range of say 0.1 nanometer to say 4 nanometer.

Now, why these cells are have a diameter of around 10 to 100 micrometer whereas, that mammals the whole organisms the dimension is a very big one. Due to that unicellular system they can take that material by diffusion through high surface area, whereas in a animal system or whole organisms their surface to; surface area is to volume ratio decreases that is aspect ratio decreases and they cannot get that materials by diffusion only.

So, for this is higher organism develops that sophisticated transport system to transport that material actively, which is compensated by that diffusion limitation for passive transport.

(Refer Slide Time: 03:39)

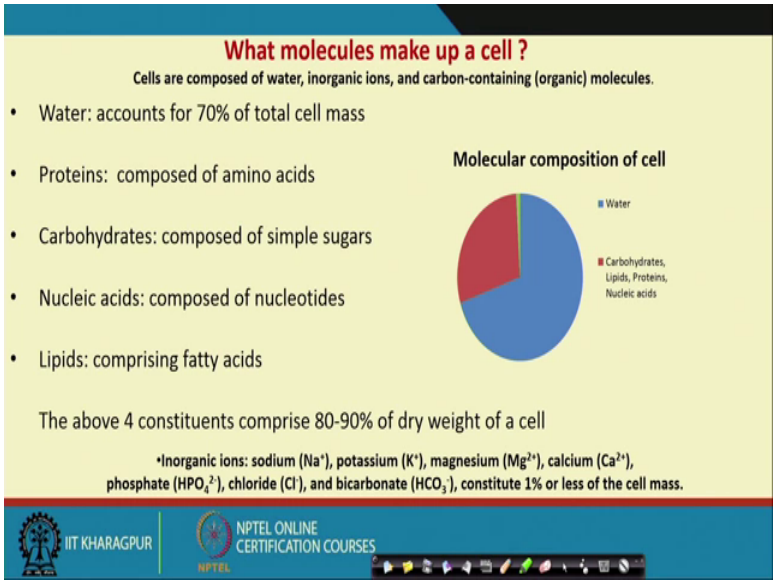


Now, if you look for that biochemical reactions which are manifested to that cellular behaviour what is the time scale? See if we look for that your enzymatic reactions like say it occurs at that millisecond level where in the signal transduction occurs at that nanosecond level.

What is the signal transduction? Signal transduction means it may be a chemical entity or may be biophysical entity, which transmit the signal from surface cell surface or cell membrane to the interior of the cytoplasm then goes to the nucleus. Then it is this message is processed then manifested in the terms of proteins. So, this signal transduction takes around say nanosecond level whereas, that protein expression and that ultimately enzyme activate the millisecond levels.

And all these events goes in to manifestation of say some cell division it in the bacterial system it took around 20 minutes whereas, in the equrates likes say hela cells or we say nevolian cells it takes around 20 minutes. So, you can see that how much scaling law occurs in the time scale, at the same time the dimensional scale where the aspect ratio is mattering the things.

(Refer Slide Time: 05:07)

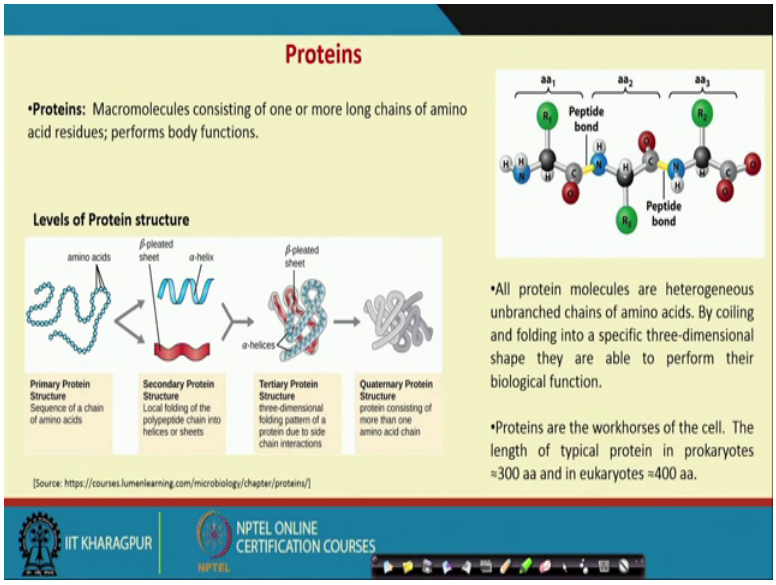


Now, what are the molecules constitute the cell say 70 percent of the cell must basically it is water. Then the remaining 28 percent or like that it is comprised of the 4 macromolecules I said protein nucleic acid hats and carbohydrate.

And the remaining 1 to 2 percent all the minerals basically, if we look for that dry weight of cell say around 50 percent of the dry weight is protein. Whereas, DNA comprises only 3 percent RNA is around 20 percents your lipids are around 10 percent. And the remaining percentage are comprised of your carbohydrate minerals etcetera.

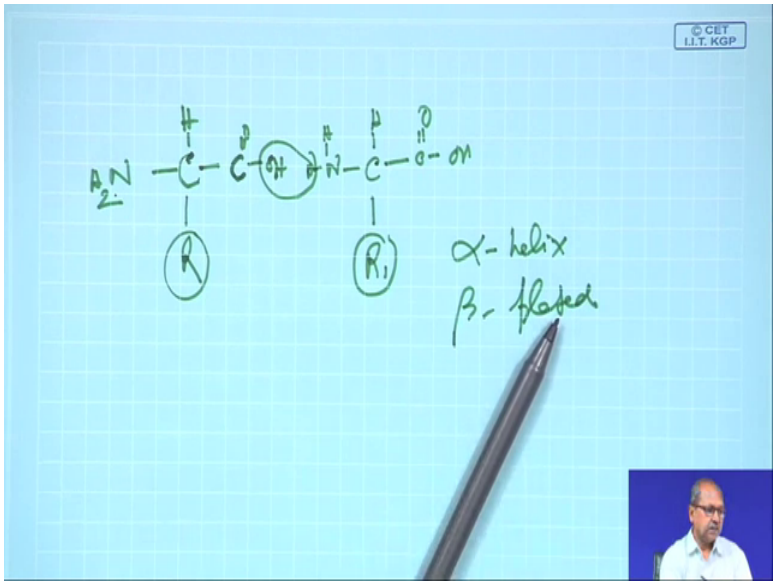
So, if you look for that protein and DNA these are the one at the higher end, and other in the lower end they are the main contributing molecules in the leaving cell they controls whole the organisms activity.

(Refer Slide Time: 06:18)



Now, you look for the protein which is the major part of the dry weight constituents. So, protein is made up of amino acids, so what is amino acid it is a deuter ionic compounds.

(Refer Slide Time: 06:43)



It is a N H 2 group and it is a carboxylic group and this is the (Refer Time: 06:57) R groups. So, depending upon the nature of the R groups these amino acids are grouped and we have 20 proteinogenic amino acids these R groups are hydrophobic, polar, nonpolar, anionic and cationic nature.

These amino acids are linked by that peptide bond like say another amino acid that peptide bound is found. And due to the restriction of that peptide bound that is carbons

are these peptide (Refer Time: 07:51) group have a that confirmation in alpha helix and beta plated sheet structure.

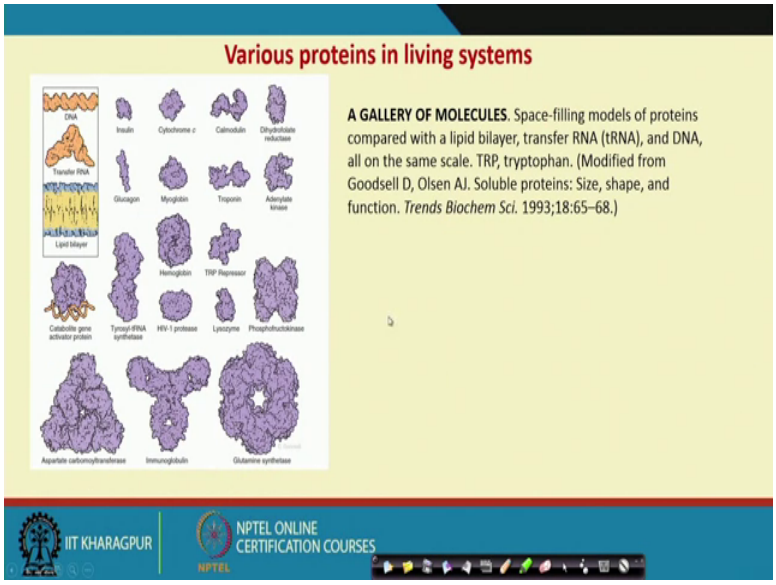
So, this primary sequence of the amino acids how these amino acid links by the peptide bond, and then this is go to there secondary structures. Now secondary structure elements like alpha helix and beta plated sheet they are arranged three dimensionally to make the tertiary structures.

And main contributing force for the tertiary structures are your hydrophilic, ionic, hydrophobic and with banderol forces and hydrogen bonds. And this tertiary structure is the real active form of the structure which imparts that activities of the proteins, the tertiary structure of proteins again goes to quaternary structure or you can tell the supra molecular structure by that aggregation.

This quaternary structure if we can look for that say example is myoglobin is carries that oxygen molecule it is a subunit one subunit structures, within four subunit structure like say alpha two and beta two the quaternary structures which carries the oxygen that is present the (Refer Time: 09:09). And these proteins are main workhorse of the cell say like say it has these molecules has a structural activity means like say phylementas proteins are mainly structures.

Where the globular proteins they have a lot of functions like say starting from that receptor function, transport function, enzymatic function. Then these proteins acts as a molecular machines basically and their efficiency of the machines from 50 percent to 100 percent they convert that mechanical energy to chemical energy and chemical energy to mechanical energy.

(Refer Slide Time: 09:54)

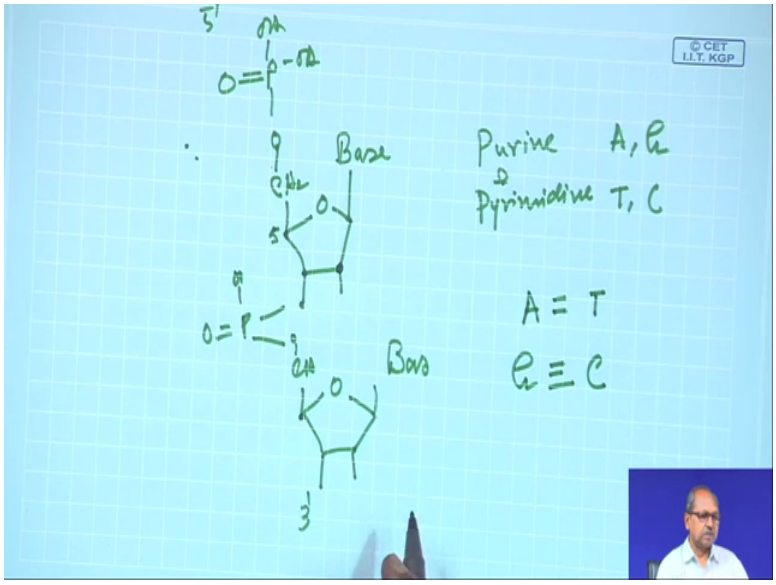


So, here we can see that different proteins of different sizes this is say insulin glucagon these are acts as a hormone this is cytochrome c acts as a electron transport carrier chalmodulin acts as a calcium binding proteins.

In that way traphonin acts in the acting myosin that is your stretch activity, in that way that is haemoglobin is a oxygen caviar protein this is escorted carbon (Refer Time: 10:28) and glutamines (Refer Time: 10:30) that the enzymes and this is haemoglobin and that is. It acts that antigen binding which protects our body from that external threats like say bacterial or microbial threats.

So, what is nucleic acid which comprises around 20 percent RNA and 30 percent DNA is nucleic acid basic building block is nucleotide; what is nucleotide?

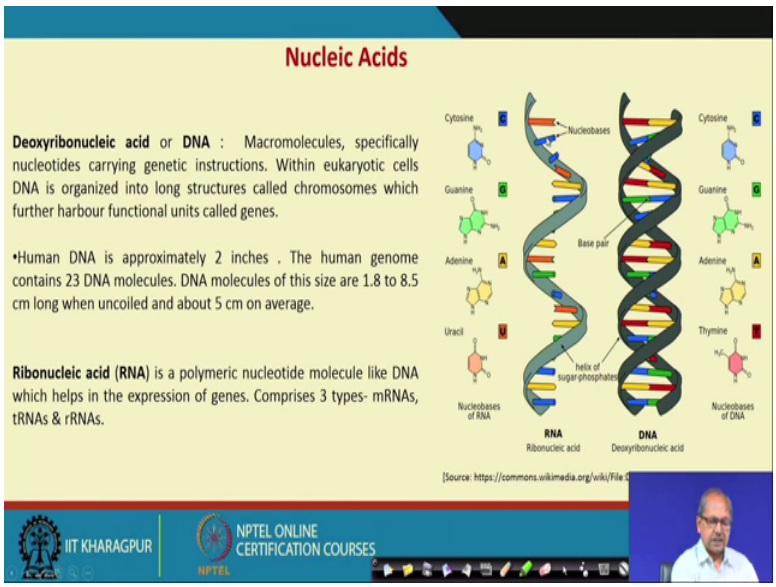
(Refer Slide Time: 11:01)



This is comprises of base these bases are purine and pyrimidine these purines are adenine and guanine and pyrimidine's are thymine and cytosine. So, this base is linked to pentose sugar that is ribose and one phosphate is link into the fifth position of that ribose sugar.

So, this is called the nucleotide. Now these nucleotides are linked by the phosphatised turban like say. So, in that way all the bases are linked by the phosphatised turban this is 5 fragment and this is your 3 fragment. In DNA these stands are anti parallel and this phosphatises are backbone is the backbone of DNA with the backbone of DNA that phosphatised turban.

(Refer Slide Time: 12:32)

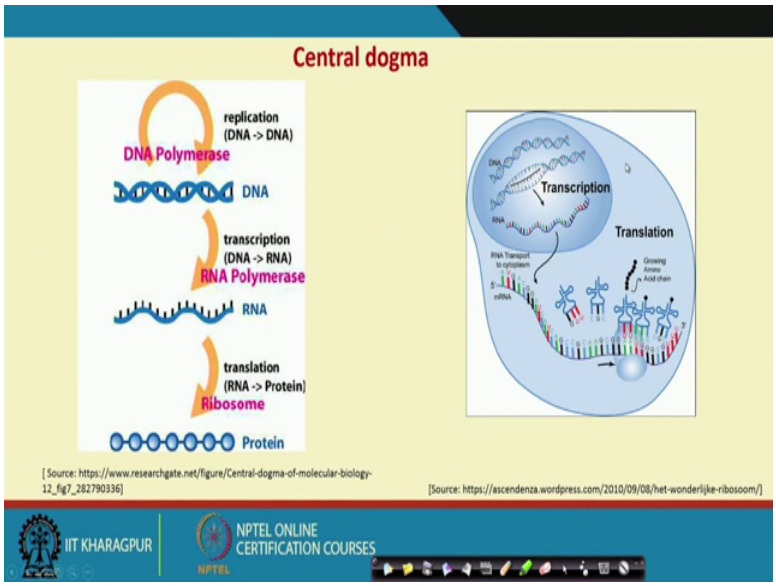


And that ATGC they are linked by that A is complimentary to T by 2 hydrogen bonds G is the complementary into C, they linked by the three hydrogen bonds.

So, these ATGC pairs is these are the means nucleotides and these three nucleotide means sequence mix the genetic codon. So, these genetic codons are the carrier means it is the message is stored in the form of genetic codes. And this for the difference between DNA and RNA, RNA is that they have a hydroxyl group two positions and these hydroxyl groups makes that RNA is unstable. Whereas DNA has no hydroxyl groups in the position this is the deoxidized ribose sugar so there is this stable one.

And this DNA is a double helix the anti parallel double helix and this carries that genetic information in the form of that genetic code. And in the human system around say 98 percent of the genetic codes are non coding for the proteins.

(Refer Slide Time: 14:05)



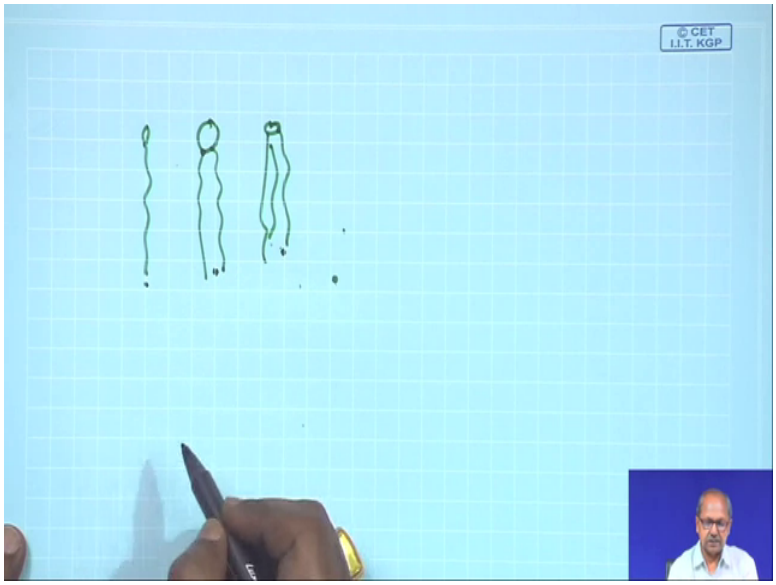
This is the central dogma of on leaving system. So, how that that linear code of DNA is transcribed to messenger RNA then this is translated to proteins. So, now if you look for the locations this translation is going on in that is your nucleus messenger RNA and it is transported to cytoplasm with the help of ribosome TRN and amino acids translated to protein, which is a main workhorse of that cell.

Now, if we look for that next molecule that is carbohydrate which is the main energy source of the cell say these are the varieties of carbohydrates are there monosaccharide,

disaccharide and oligosaccharides and polysaccharides. And in disaccharides, trisaccharides they are linked by that glycosidic bonds and they are in the oligosaccharides, there may be from 3 to 10 glycosidic bonds and the when their members are going larger we are telling these are the complex polysaccharides.

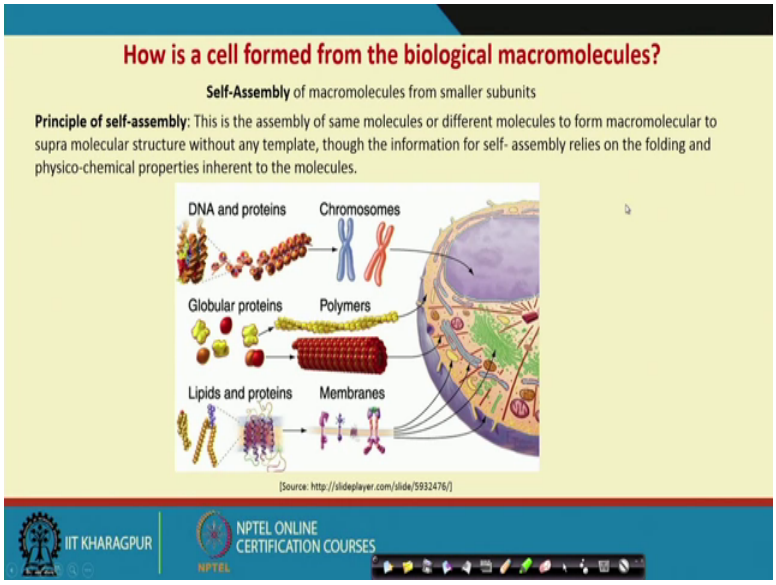
And these carbohydrates are highly branched in animal system they are stored as a glycogen, whereas in the plant system they store as a starts cellular hemi cellular etcetera. Next molecular lipid which comprises 10 percent of the dry weight

(Refer Slide Time: 15:45)



So, these are the antibiotic molecules which have a hydrophilic head groups and hydrophobic 10 this is one and this is triglycerides this is phosphoglycerides this is the fatty acids. And this triglycerides acts as a oil what we are taking in our daily life and phosphoglycerides are the main components of membrane. And what is membrane it is that boundary or the cells from the external environment with internal environment.

(Refer Slide Time: 16:30)



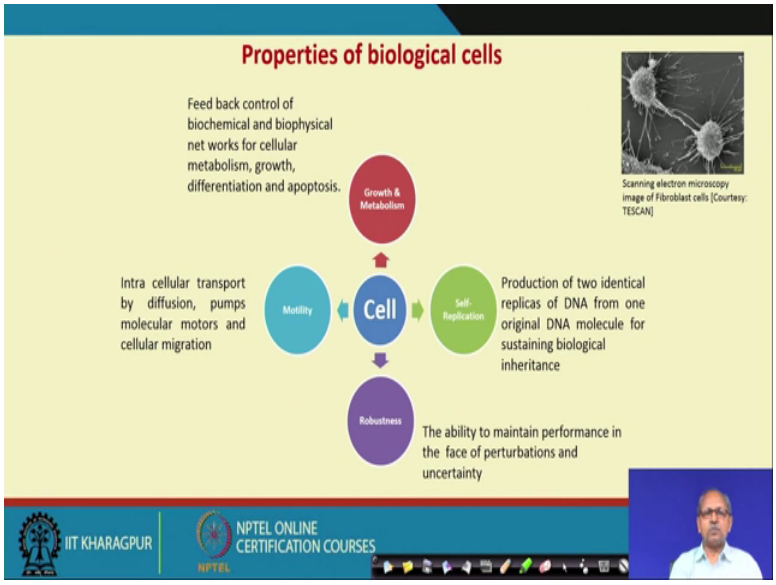
So, these are the macromolecules now how these macromolecules are arranged to make that organelles or the leaving system, so these are addicted by self-assembly. So, what is self assembly say it is an non template driven process it is a non template driven process, but the information present in a molecule itself.

When the molecules comes together by diffusion like an example of lock and key model they binds each other by hydrophobic interaction by losing the water molecules tightly, and in that way, that they makes that macro molecular structure to supra molecular structures. So, like in this example we can show that that nucleic acids binds with protein histone they makes that chromatin which is in the chromosome that is present in the nucleus.

That globular proteins like say acting's they make that acting filament which is the cytoskeleton protein. Then tubulins are formed from the manometers they make that large macromolecular structures for the, which are used for transport system intercellular system as well as it helps the stability of that membrane system.

And lipids and proteins is the two different class of molecules they are aggregated or organized as self assembled we can develop to make that membrane. So, this is self assembly it is one of the manifestation the leaving system.

(Refer Slide Time: 18:20)



So, what are the attributes or properties of the cells by we can tell that it is an leaving cell. So, first attribute is your production of two identical replicas of DNA from one original DNA molecule to sustain their biological inheritance means. From one cell to its progeny that whole the information is percolated by that your transcription of the DNA without much error.

This is self replication, then we are coming growth and metabolism this is encompasses by that huge amount of biochemical and biophysical networks, for the survival growth differentiation and ultimate apoptosis that is your program cell width. And these networks are controlled by that feedback mechanism, but all the means complex manifestation of these cells cannot be explained simple by your feedback mechanism.

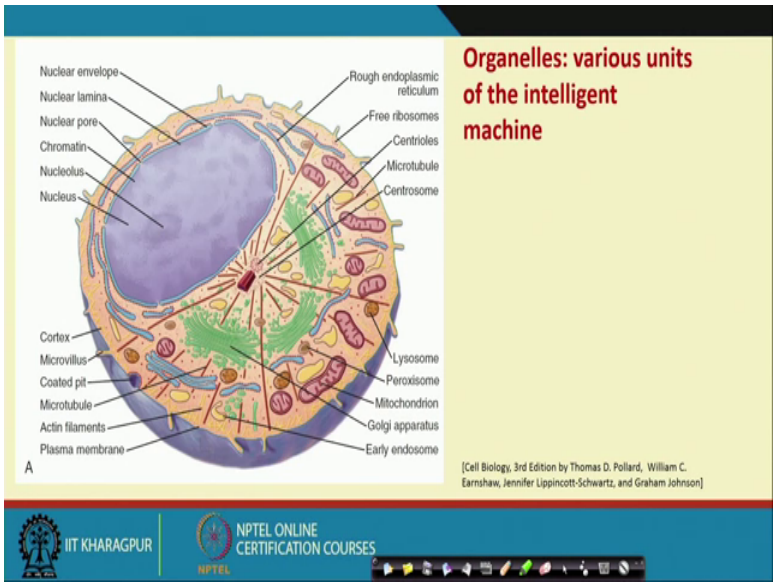
So, next that manifestation of that cell is the transport system both intercellular and that total cellular transport. In inter cellular space molecules are transported by that your passive diffusion process, but when the molecules are bigger than thirty kilo Dalton or also some vesicles, we cannot be transported by simple diffusion it will take lot of time.

So, that is why cells are the developed their machinery to transport these macromolecules and vesicles by active transport using that protein roads and that molecular machines like say actinamyosin cyanosis. Then we are coming that robustness of the cell what is robustness say it is a robustness nature is that if we part up that internally or externally both physically and physico chemical ways they same has a property to sustains that steady state.

So, we can take that or you can take that, perturbations or you can take that robustness like homeostasis. Homeostasis means cell has a behaviour to maintain that steady state if we change that temperature ph even if cell constants in within a tolerable limit. It can be a steady state and it can be a dynamic state dynamic state means like say with tine nib and way silicon synthesis or express some group of proteins, so this is called circadian rhythms. So, these circadian rhythms will not be affected by that external or internal physical and physicochemical perturbation.

So, this is that how this is maintained this is maintained by high level of control system. So, these are the four manifestation we can tell that all the leaving cells has.

(Refer Slide Time: 21:44)



Now, if you look for that what are the parts of a leaving system. So, this is that your unit of leaving system that is cell they are mainly described that mammalian cells.

This is the boundary between external and that internal of the cells with the boundary made up of your lipids mainly phospholipids its composition is mainly 40-60 protein and lipids. And it harvers that lot of proteins which acts as a receptor is a transporter and ion channels by which it can be exchanged the material from outside the inside and this membrane is the hydrophobic in nature.

So, that polar ionic molecule cannot transport easily. And this next is the cytoplasm that is that whole that the viscous like liquid like honey. And here that protein concentration

is around 180 to say 400 milligram per ml. And this high concentration protein this that crowding nature of that cytoplasm and viscosity like honey and molecules cannot transport from one place to another by simple diffusion. So, this is a diffusion restricted, so for that how that big molecules will transport as I told earlier also these are the cytoskeleton elements like say turbulence by which that big molecules are transported by using the molecular machines.

Now, we are coming to the nucleus which the atrophies of that cell, which contains that DNA. And that DNA length is around 2 meter it is encompass in the space of 4 micrometer and they are highly packed basically in the nucleus also there are lot of proteins are there.

So, like say DNA polymerase RNA polymerase and other transporters also by which that nucleus can exchange that information from inside to outside. This is the organelles of endoplasmic reticulum is a continuation of the outer membrane of nucleus. And here that proteins are translated with the help of ribosome most of the proteins are translated here and processed.

These proteins are finally, processed in that Golgi which is a packaging system of the cells and from that place that processed proteins and lipids and other complexes are transported various location of the cells very precisely. This is the mitochondria that powerhouse of cell where that metabolites are burn to carbon dioxide and that heat generated for that burning.

It is converted to energy called energy currents of the cells ATP and that ATP is generated by using that is your molecular machine f 0 f 1 ATPs. It is efficiency around 100 percent which cannot be achievable by your manmade machine.

This is the lysosome that is the recycle bin of a cell where that used proteins, which should be recycled here by that lot of lighting enzymes using that protein edges, light edges, your glycoside edges which are clipped and the recycle to make that other organisms other organelles.

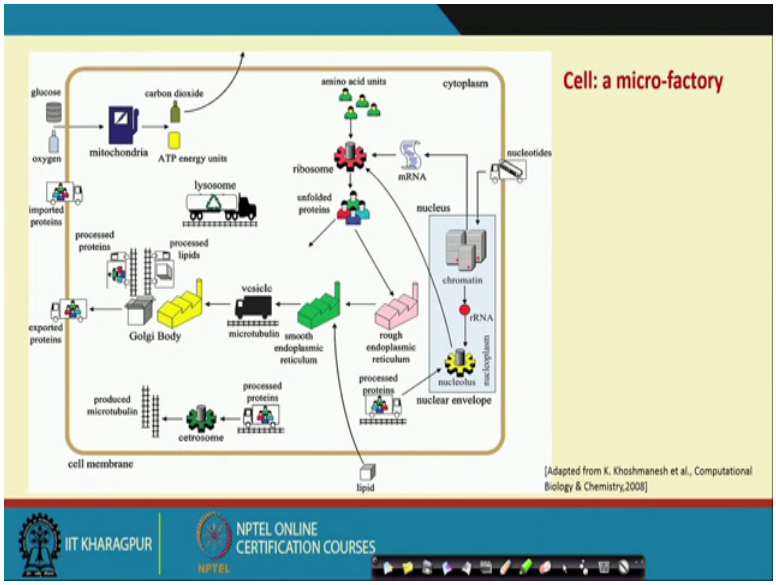
In that way all the organelles are arranged in the cytoplasm. So, we can tell that cytoplasm is the place where that that high concentration of proteins and that contains that cytoskeleton elements and their organelles. So, we can tell that this is a highly

crowded space we can take an example of that say vehicles are transported in a highly traffic zone. Where that pedestrians like bicycle they can go easily where as big molecules cannot transport so easily.

So, they needs that some transport system for direction on transport or active transport through that that is protein road turbulent by using your actin myosin kinesin that dynein type of your molecular machine to transport that cargoes from one place to other.

So, with this context we can tell that cell acts as a smart and robust machine, because all the attributes of machine characteristics present in the cell that is high level of automation high level of integration of that molecular networks.

(Refer Slide Time: 27:42)



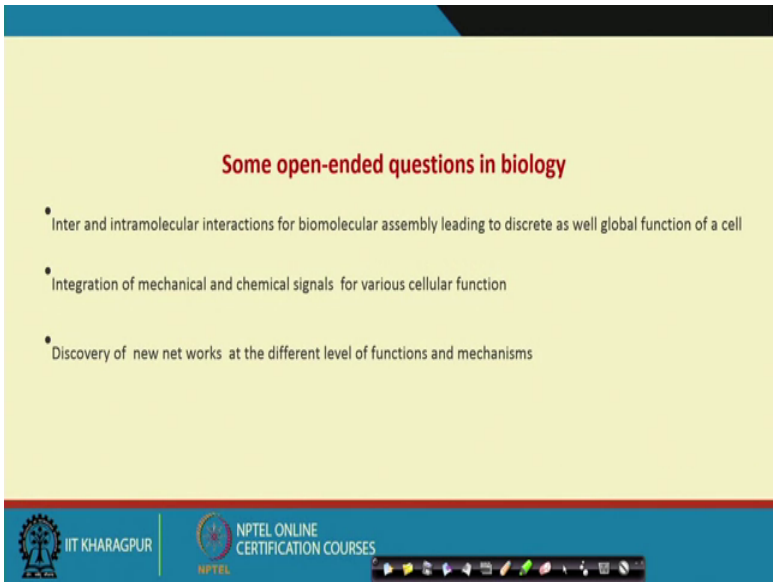
On the other hand you can compare the cell as a smart factory to give an understanding that how that cellular organelles are comparable with that organization of a highly automated modern their factory. So, this is that membrane which can be comparable to that your factory boundary they are the gates of that boundary walls means it is the transporters or ion channels.

And this is that head office means which transmit the message in the form of messenger RNA or you can that this paper to that your cytosol means other places this is the endoplasmic reticulum. Where these message it translated to that proteins and which are transported the Golgi bodies processed for.

And they are going to different places using the vehicles this is the turbulence which are the roads basically by which vehicles are goes and going to the different destinations. And this is that mitochondria where that energy is generated by burning that the fuels that is going to carbon dioxide and it will be an energy.

So, this analogy will give that idea of the cell as a factory and earlier (Refer Time: 29:08) design machine property. In the omics area we know that proteomics, genomics, metabolomics transcriptomics and nowadays these coming the interactoms. With these omics and ohms we are able to get the knowledge of that how the cell works what are the networks are involved.

(Refer Slide Time: 29:40)



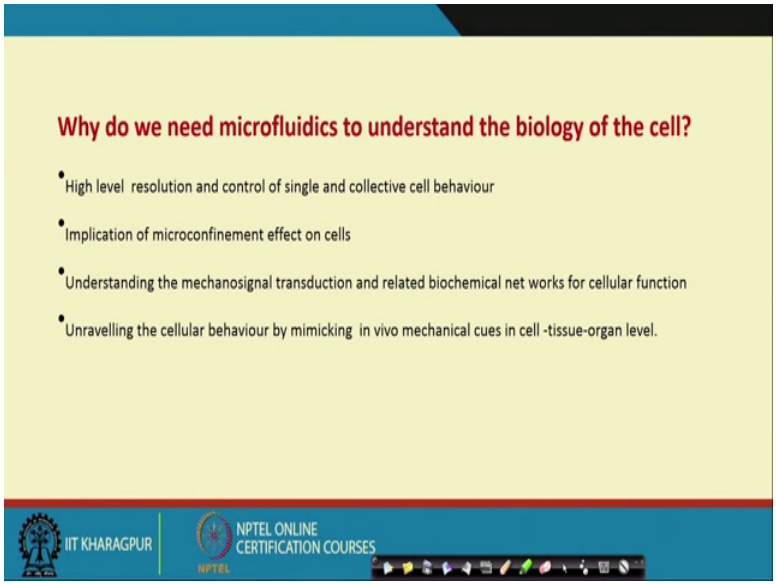
But there are lot of things to tell in that biology, like these are the some open ended questions in biology like say inter and intra molecular interactions or bio molecular assembly leading to discrete as well as global function of the cells.

Means say how that protein, protein interaction protein DNA interaction, lipid protein interaction, lipid carbohydrate interactions, leads to that global function of the cell likes a cell division motility differentiation and apoptosis. And how that integration of mechanical and chemical signals for the various cellular functions because we know that biochemical functions more than mechanical functions.

So, that mechanical signal transaction how these two processes are integrated for the cellular functions. And the discovery of new networks or you can tell that hidden networks and the different level of organization it means. What I want to mean that from a outsider we see on that machine is working and we do not note the what are the hidden network in the machine and this is known by that who have prepared the machine on the networks then also.

But in a perspective of cell we are the outsider we do not know because we have not created the cell what are the hidden networks present at the different level of organism. So, these are yet to discover to know that complexity of the leaving system. Now, how micro fluidic system will address these problems or related problems.

(Refer Slide Time: 31:23)



First is that high level resolution and control of single and collective cell behaviour. This can be addressed by that hydrodynamic wrapping as well as micro fluidic different types of bulbs to trap single cells or a group of cell together and study their behaviour precisely by using high resolution microscopy. Then implication of micro confinement effect on cells carrying on to define that if you put the cell in a different types of three dimensional biophysical cues, which will deform that membrane as well as nucleus and manifested there phenotypic characteristics. Like say, if you put the cell in a micro tube where that both nucleus and cellular membrane is deformed, how these things effect that cell viability cell division cell migration and apoptosis.

Third point is that understanding that mechanosignals transduction and related biochemical networks for cellular functions, means how that we can generate that mechanical signal by using different types of fluid flow. And how these fluid flows generated mechanical signal transmitted or transformed to biochemical signal and effecting cellular behaviour.

Like say in cancer situation say cell are disseminated from that tumour to distant place through metastasis. The or that that cellular means tumour stress involves to come out of the cells come out of the tumours by using this platform it can unravel.

And last of all that unravelling the cellular behaviour by mimicking in vivo mechanical cues in cell it is organic level. You take an example like say capillary bed network for endothelial system we can mimic this in vivo system in vitro.

And we can study that how that capillary bed networks that fluid flow like say bloods can affect that endothelial cells and it related immune cells like t cell v cells any other protein cells take another example in lymphatic node system, how that your lymphatic conduits where that antigen presenting cell and that t cells v cells interact to recognise the particular t cells. This is also can be mimicked in vitro system our system like bone canonically and immunological and neurological synapses. All these things which are occurring in the leaving system at that capillary level or micro scale level, we can mimic these systems in vitro to understand that biological phenomenon at the single cell level to your cell or multiple cell level by using this fluidic platform ok.

Next two classes we shall discuss all these phenomena sequentially to unravel that power of micro fluidics platform for that biological questions.