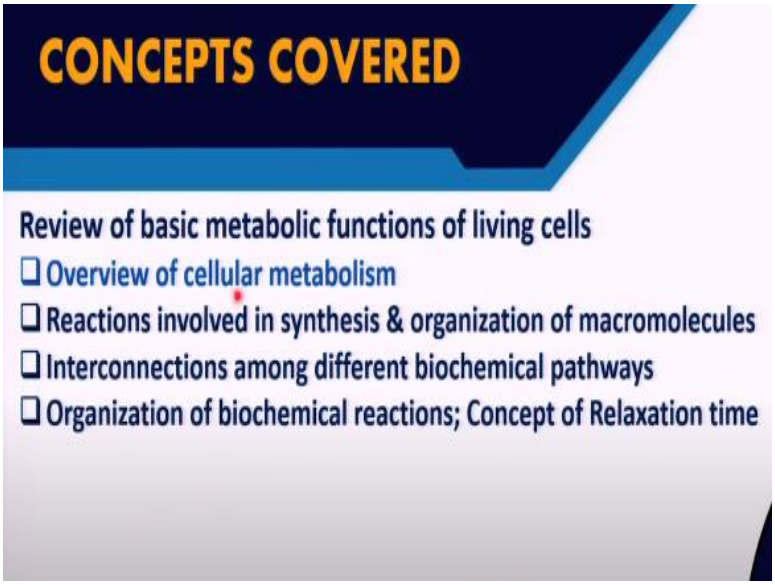
Metabolic Engineering
Prof. Pinaki Sar
Department of Biotechnology
Indian Institute of Technology-Kharagpur

Lecture - 07
Review of Cellular Metabolism - Part B

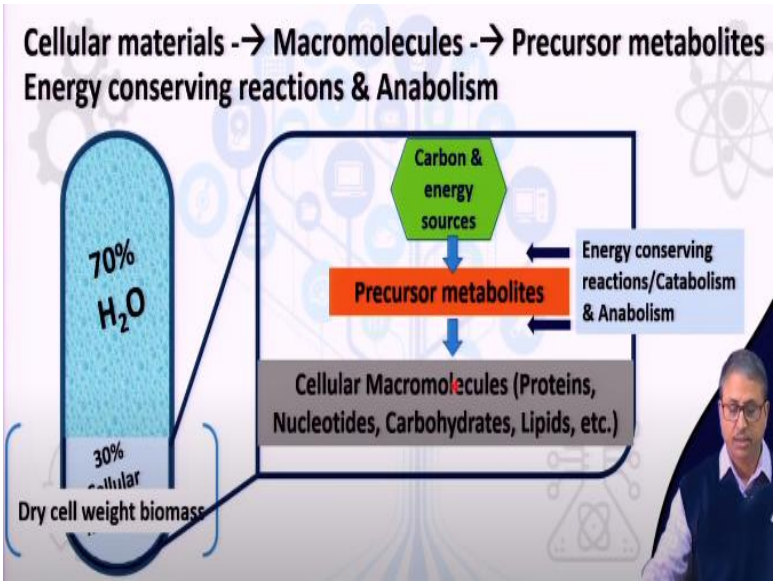
In today's metabolic engineering class, we are going to discuss about the metabolic frameworks operating within the cellular system.

(Refer Slide Time: 00:43)



So briefly we are going to continue our discussion on cellular metabolism, but today we will be emphasizing more towards the framework of the interactions or framework of the biochemical reactions especially reactions involved in synthesis and organization of macromolecules, interconnections among different biochemical pathways and organization of biochemical reactions and then the concept of relaxation time will be discussed.

(Refer Slide Time: 01:17)



As we discussed in our earlier class that within any cellular systems only about 30% of the cellular materials are present and the remaining or rest of the cell components are basically water corresponding to approximately 70%. Now this 30% cellular materials which are referred also as dry cell biomass is constituted or representing the cellular macromolecules which are different types of proteins, nucleotides, carbohydrates, lipids, etc.

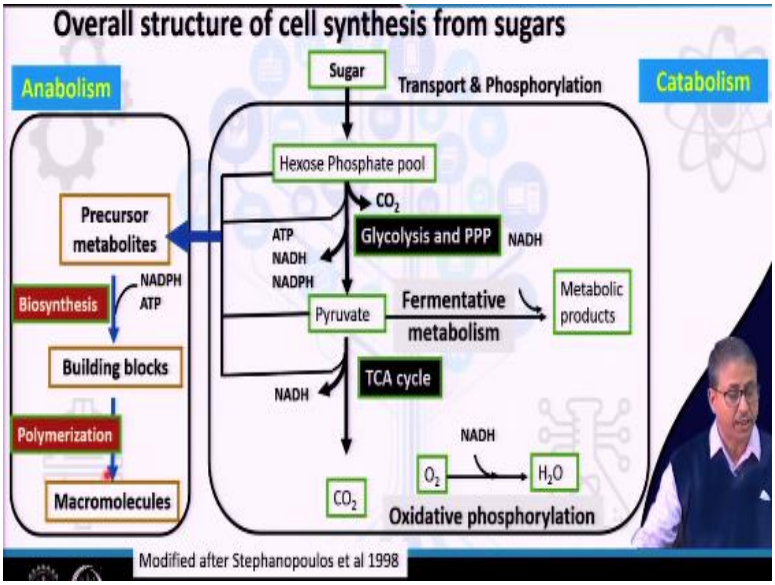
This macromolecules which are essential components of the cellular structure and cellular function are continuously produced through a number of independent biosynthetic reactions by the cells from a selective set of precursor metabolites which are small carbon molecules. Now this small pool of precursor metabolites which in our last lecture, we have learned that there are 12 such precursor metabolites and according to a recent report, there could be up to 13 precursor metabolites.

Now these precursor metabolites are used for diverse set of biosynthetic reactions to produce the cellular macromolecules. But at the same time, this precursor metabolites are continuously being produced from the carbon and energy sources, which the cells they acquire as their source of nutrients.

Now there are two set of reactions which we have also discussed that one set of reaction which is called energy conserving reaction or sometimes catabolism or many a times we call them catabolism. These set of reactions facilitate the conversion of the carbon and energy sources to the precursor metabolites.

Whereas, the set of reactions which are involved in converting the precursor metabolites into cellular macromolecules are conventionally called as the anabolism or anabolic reactions.

(Refer Slide Time: 03:47)



Now if we look at the overall cell structure or cell synthesis structure from sugar as a source of carbon and energy, during this energy harvesting reactions or the catabolic reactions, we could identify a set of very well defined reactions representing the biosynthetic the set of reactions, biochemical reactions. The sugar is transported inside the cell and it is phosphorylated to a number of hexose phosphate.

So we will talk about this hexose phosphate in some of the other lectures and these hexose phosphate pools represent the glucose phosphate glucose, 6-phosphate glucose, 1-phosphate, fructose 6-phosphate, erythrose 4-phosphate and other type of sugar phosphates. These hexose phosphate pool, it is called pool because there are number of different types of hexose phosphates are there or maybe other sugar phosphates are also there.

They provide the basic resource for rest of the reactions which are conventionally called the glycolic reactions and also the pentose phosphate pathway associated with that. These glycolysis and pentose phosphate pathway decarboxylate and produce the ATP as the major energy compound and reducing power which are basically NADH and NADPH.

The sugar molecules are eventually oxidized to smaller carbon compounds like pyruvic acid, and these pyruvic acid is eventually subjected to either the tricarboxylic acid cycle or TCA cycle or through the fermentative metabolism it is converted to a number of metabolic products. Now during the TCA cycle, a large number of reducing power is further produced like NADH or FADH2 and ATP molecules or ATP equivalent molecules are also produced.

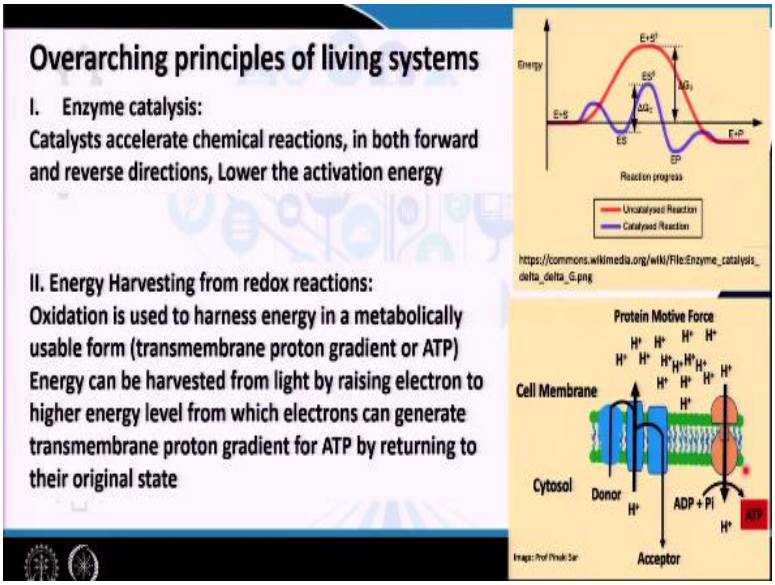
And the substrate like pyruvate in this case is completely oxidized to carbon dioxide. In case the pyruvate is entering towards the fermentative metabolism, it is reduced by accepting the electrons from NADH which is produced during the preceding reactions of glycolysis and converted to reduced substrate like lactic acid and other compounds.

Now these set of metabolites or intermediate metabolites of this connected biochemical reactions like hexose phosphate, pyruvic acid and many other intermediates are representing the precursor pool which when connected to the set of anabolic reactions, facilitate the biosynthesis of macromolecules. Now these large number of small metabolites represent the precursor metabolites.

They are taken by the appropriate reactions, which are called biosynthetic reactions to produce the building blocks. So from the precursor metabolites the building blocks are produced and these building blocks are assembled, polymerized and modified to different macromolecules. Now these biosynthetic reactions or the polymerization and assembly reactions are most of the time they are reducing reaction.

So they require supply of reducing power or electrons and they are energy consuming reactions. So they require a large number of ATP. So the ATP or the reducing power like NADH or NADPH produced during these glycolysis reaction or the catabolic reactions rather are providing are used to provide the energy source and the reducing power for the biosynthesis and polymerization and other required reactions to synthesize the macromolecules.

(Refer Slide Time: 07:54)



Now there are four overarching principles operating with respect to the metabolism of living systems. The first one is the enzyme catalysis. As we know the enzymes are the bio catalyst of cellular system and they accelerate the chemical reactions in both forward and reverse direction lowering the activation energy of the desired reaction. The second one is the energy harvesting from redox reactions.

So a number of oxidation and reduction reactions are occurring in a cellular metabolism. For example, oxidation is used to harness the energy in a metabolically usable form in the sense either the transmembrane proton gradient or the production of ATP and once this metabolically usable form of energy is produced, this energy is available to all reactions, which are requiring energy sources or the required supply of energy.

Now energy can be harvested not only by oxidizing the organic carbons but also by harvesting it from light sources as it is done by the phototrophic organisms. So when energy is harvested from light, the electrons are raised to higher energy level from which the electrons can generate the transmembrane proton gradient for ATP production by returning to their original state.

So this simple diagram represents the oxidative mechanism through which the electron donor which is getting oxidized it may be the NADH H+ is donating the electron to the enzyme complex sitting over the membrane, bilayer structure. And

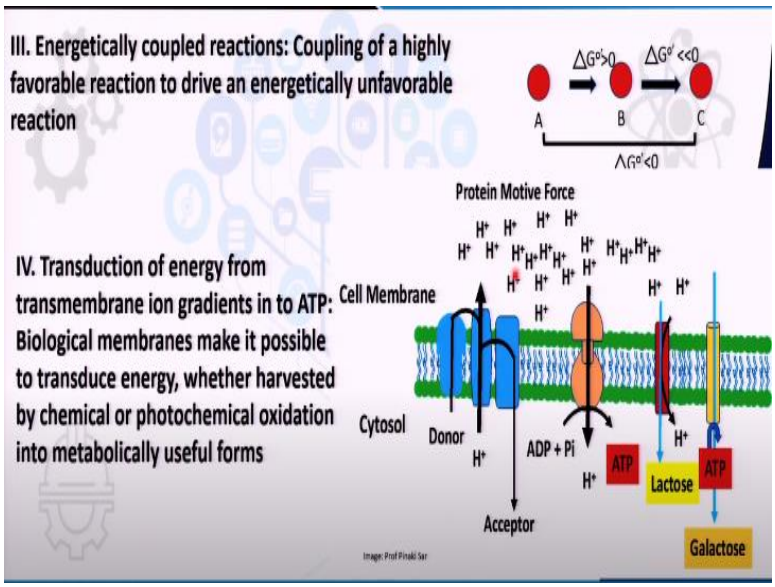
these electrons are eventually transferred to electron acceptor which is also called terminal electron acceptor for example, oxygen and converting oxygen to water.

And some of this electron carriers, which are membrane bound electron carriers or electron transport complex are acting as proton efflux pump. So as they operate like a proton efflux pump, the proton molecules are efflux out. So eventually on the outside of the cellular system in the periplasmic space for example, in a gram negative bacteria, a concentration of protons build up.

Now as this concentration of proton builds up, the proton motive force is generated and this proton motive force is a usable form of energy. These protons can be allowed to enter inside the cell or cytoplasm through a specific enzyme complex which is called ATPase. And whenever the protons are allowed to come inside the cell through the plasma membrane, ADP molecules are phosphorylated to produce ATP.

So the proton motive force generated by oxidizing a reduced substrate is eventually utilized to convert ADP molecule to ATP. And as we all know the ATP is one of the most useful form of the energy which is called energy currency within the cellular system.

(Refer Slide Time: 11:43)



The third principle is energetically coupled reactions. So wherein coupling of highly favorable reactions, favorable means thermodynamically favorable reactions to unfavorable reactions or energetically unfavorable reactions are achieved.

For example, if we consider the conversion of B to C, which is having a strongly negative or highly negative delta G zero prime value or the reaction is highly favorable thermodynamically when it is coupled to a del G positive reaction, which is otherwise thermodynamically not favorable, the overall conversion from A to C is having a negative del G value.

That is the A to C conversion could be favorable thermodynamically when these two reactions are interconnected. So one of the fundamental principles is that the energetical reactions are coupled. The last principle is transduction of energy from transmembrane ion gradients into ATP. Now biological membranes make it possible to transduce energy whether harvested by chemical or photochemical oxidation into metabolically useful form.

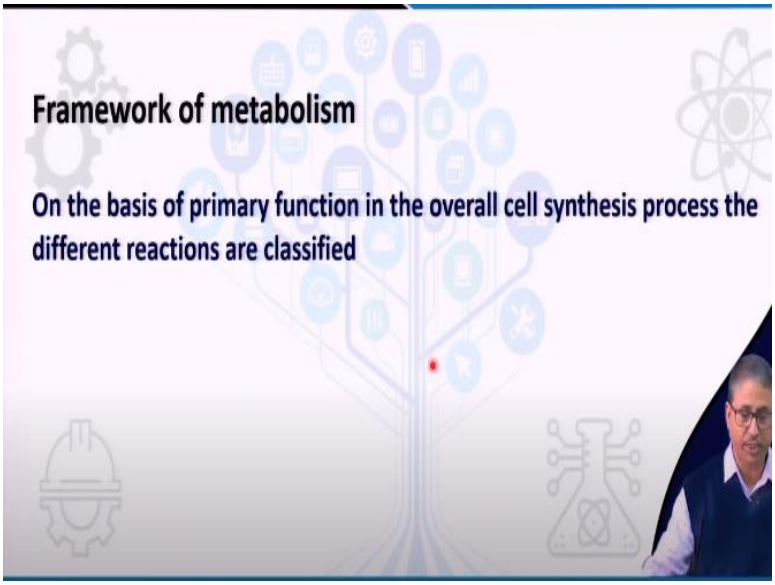
Earlier we have seen that how a reduced substrate which can donate electrons, for example the NADH+H+ or FADH2 can donate the electrons to the appropriate complex who can receive the electron from the NADH H+ or FADH2 and transfer the electrons to the electron acceptor, terminal electron acceptor.

And while doing this electron transfer, some of this carrier molecules act as the proton efflux pump and a proton gradient is created which is responsible for the proton motive force. Now this proton motive force we have seen that is responsible or can be utilized to produce ATP.

But otherwise also this proton motive force could be useful because these protons can be useful in transporting lactose, galactose or a number of other molecules inside the cells, which are otherwise difficult to transport inside the cell because the concentration of those molecules might be low outside, high inside. That means, the gradient of the concentration is on the reverse side.

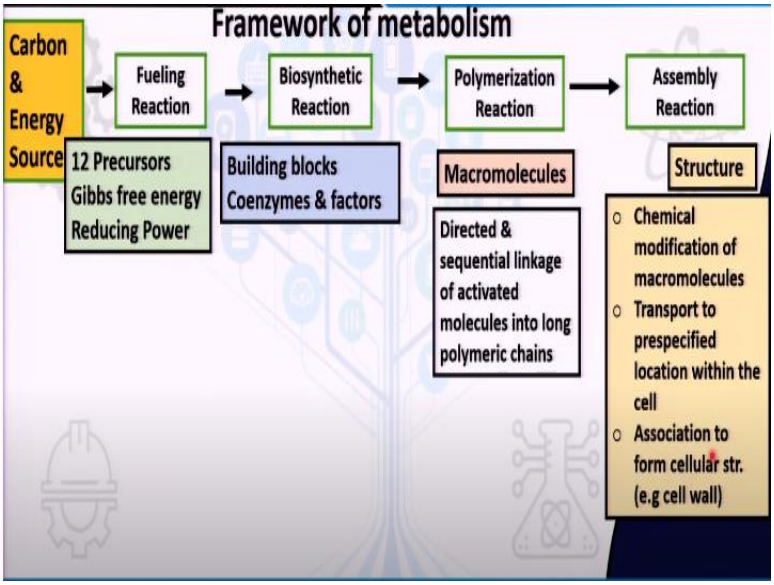
But these compounds or these molecules can be transported, if we can couple up if the cell system is able to couple this proton motive force or the transmembrane gradient with this transport process.

(Refer Slide Time: 14:36)



Now we are going to talk about the framework of metabolism. Now on the basis of the primary function of the overall cell synthesis process the different reactions which are carrying out or which are going inside the cellular systems are classified.

(Refer Slide Time: 14:55)



And it is classified into four distinct types of reactions. So the framework of metabolism constitutes the fueling reactions which are starting from the carbon and energy sources. And then the biosynthetic reactions. Biosynthetic reactions are then connected to the polymerization reactions and the polymerization reactions are connected to the assembly reactions.

Now in fueling reactions, the precursor molecules are produced out of the carbon and energy sources. The Gibbs free energy or the ATP mostly it is the ATP which is

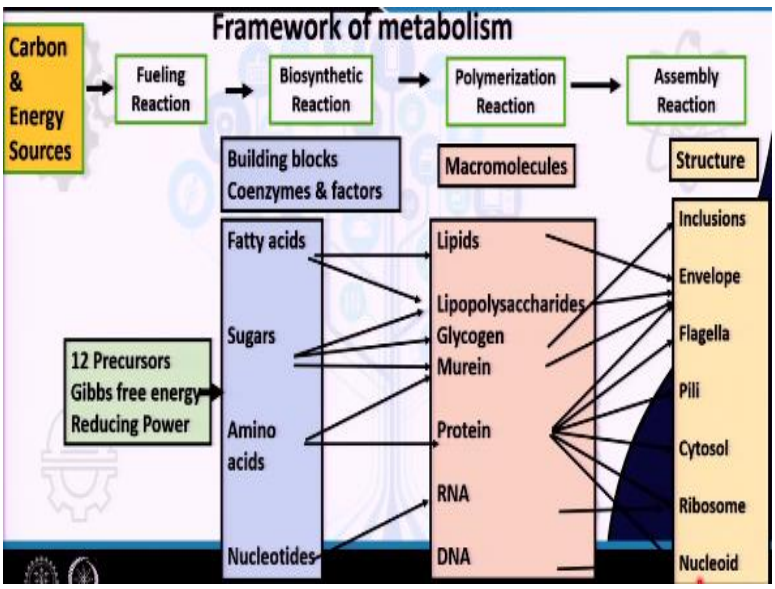
produced, which is useful for all other biosynthetic reaction or energy requiring reaction. And the reducing power like NADH+H+ or FADH2 or NADPH are produced. Now during this biosynthetic reaction, the building blocks are produced.

Building blocks including the small molecules and the coenzymes and other factors which are necessary for enzyme function and other cellular reactions are produced. Macro molecules are produced by the polymerization reaction and these macromolecules are assembled to form the different structures. Now during this polymerization reaction specifically a directed and sequential linkage of activated molecules into long polymeric chain.

We can take an example of the polypeptide chain synthesis like for the proteins we need the polypeptide chains. And these polypeptide chains are actually produced by a very directed and sequential linkage of amino acids. And these amino acids when they are joined by the peptide bond, they eventually form the long polypeptide chain. Now these reactions are highly directed and highly organized.

Now once this macromolecules are produced, the macromolecules need some kind of modifications and transport before they are deployed into different cellular function. So these include the chemical modification of the macromolecules, transport to prescribe location within the cell and association to form cellular structure like the cell wall.

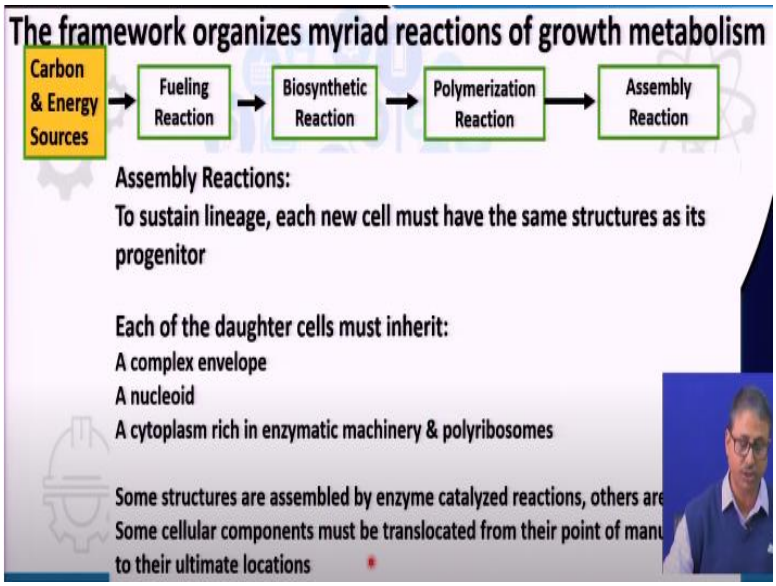
(Refer Slide Time: 17:14)



Now if we look in detail, we will see that these precursor molecules are able to provide the necessary resources, chemical resources and energy resources like the ATP or the reducing power to facilitate all the biosynthetic reactions, which are the production of the building blocks like the different fatty acid, different sugar monomers, different amino acids and different nucleotides.

Now this fatty acid, sugars, amino acid and nucleotides these are the fundamental or the major building blocks for the cellular metabolism, they are then utilized in the polymerization reaction to produce the respective macromolecules. And each of these macromolecules which are produced out of the building blocks are assembled, modified and transported for their designated function including the formation of the inclusion bodies, the envelop, the membrane, the flagella, the pili, cytosolic constituents, ribosomes and nucleoid.

(Refer Slide Time: 18:22)



Now the framework organizes myriad reactions of growth metabolism. Large number of reactions in a simple and one of the most well studied microbial species like *E. coli* we have identified that there are more than 4000 genes in a tiny *E. coli*. And out of these 4000 genes, nearly half of the genes are responsible for producing some enzymes or other or regulating them.

And these large number of enzymes are involved in different reactions, which are happening at any point of time within the cells particularly when the cells are in active growth metabolism. So there are actually two types of metabolism. So one is called

growth metabolism, when is the cell is proceeding towards the growth that is one cell is getting doubled into two cells, cell division is occurring.

The other type of metabolism is called maintenance metabolism. So during the maintenance metabolism, the cell is not actively growing, but cell is trying to maintain its homeostasis and also trying to repair and produce necessary energy and necessary precursor just to replenish the required molecules inside the cell, but the cell is not growing.

Now in case we consider the growth metabolism, the events are more complicated, because as we have just seen that the substrates which are taken by the cell are converted or channeled through a set of well-organized reactions, the fueling reactions to assembly reactions. And these set of reactions are very well organized and very well conserved.

Throughout the evolution we have seen, these reactions are very well conserved. You can imagine that more than 1000 reactions are happening with only 12 or so precursor molecules. So it is absolutely fascinating that a small number of precursor molecule are responsible for producing any kind of macromolecules.

Or any kind of functionally active molecules inside the cell, be a protein molecule, be it RNA molecule, or be it a carbohydrate or lipid molecules. So the kind of control and kind of organized network of reactions responsible for maintaining this framework is truly astonishing. Now within this reaction framework, we will briefly discuss in a different manner.

Instead of proceeding from the source to the structure, source means the carbon and energy source to the structure, now we will be proceeding on the reverse way, like we will go to the left side from the right side, like we will start with the assembly reaction and try to reach to the fueling reaction, because we want to understand what are the requirements and what reactions must happen in the preceding steps, so as to support the final reaction.

So the assembly reaction, which is the ultimate goal of this growth metabolism, because molecules are required, and the molecules are required in a sense that the cells are ready to divide and when the cells will divide, they will produce two cells. So one cell will produce two cells. Now these two cells are called daughter cells. Now these daughter cells must have the same structure as its progenitor.

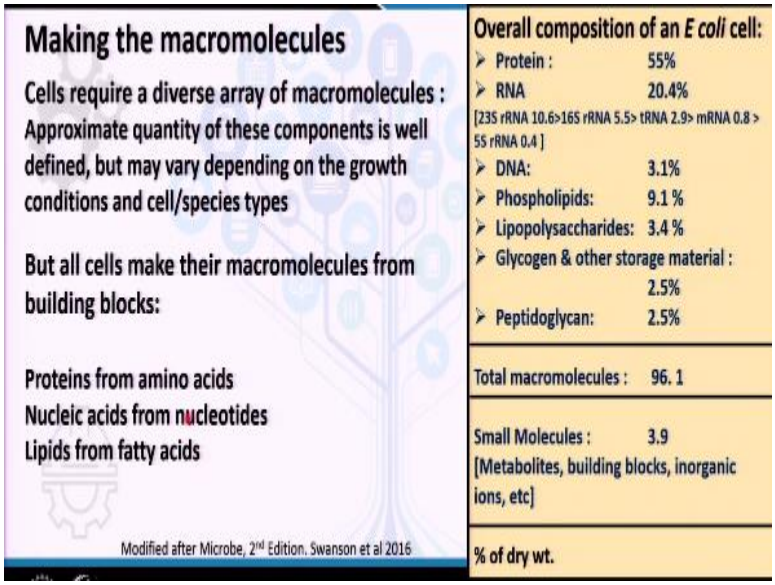
So the mother cell structure and the daughter cell structures must be conserved, must be same. Now how the metabolism is controlling that? Because each of the daughter cells produced from the mother cell must inherit a complex envelop that is the cell membrane cell, cell wall and associated cell wall associated structure, the nucleoid which represent the chromosome.

A cytoplasm rich in enzymatic machinery and polyribosomes. Now it is fascinating to understand that the set of enzymatic machinery present in any kind of cells are highly conserved. So as the cell divides, one cell to two cells and two cells to four cells and so on, it goes on, each of the daughter cells are capable of acquiring the same set of enzymatic machinery as it was there with the mother cells.

Now some structures are assembled by enzyme catalyzed reactions, others are not. So structures which are produced during this polymerization reactions, and which are the part of the assembly reactions are assembled by enzyme catalyzed reaction. So enzyme can very well control their assembly process, but other reactions are not.

And also some cellular components must be transported or translocated from their point of manufacturing to the ultimate locations like in the membrane or in the cell wall positions.

(Refer Slide Time: 23:25)



Now making this macromolecules is a very interesting task. Because cell requires a diverse array of macromolecules. By this time, we have understood this, that cell requires protein, cell requires the DNA, cell requires RNA, multiple carbohydrate molecule, lipid molecules and many other molecules. Now the approximate quantity of these components, all these components in a cell is well defined or a species is quite well defined.

Like for example, in a general *E. coli* strain, which is of medium or genome size, we can see that out of the total dry weight material that is a 30% of the chemical constituents represent the entire molecules or the materials present in the cell, which is also responsible for the dry weight of the cell. Protein is 55%, RNA is around 20% that constituted by 23S rRNA, 16S ribosomal RNA, transfer RNA, mRNA and other RNAs.

DNA is around 3%, phospholipids, 9.1%, polysaccharides 3.4% and all these things. Total macromolecules are around 96.1%. And there are some small molecules which are around 3.9%. Now which is very interesting again is that these list of compounds and their relative distributions are almost consistent and conserved within a particular species, particularly when they are growing under a conserved or a kind of standard condition.

But if we change the growth condition, if we change the growth conditions or the cell or species types, this ratio or this distribution may vary. Because in some other

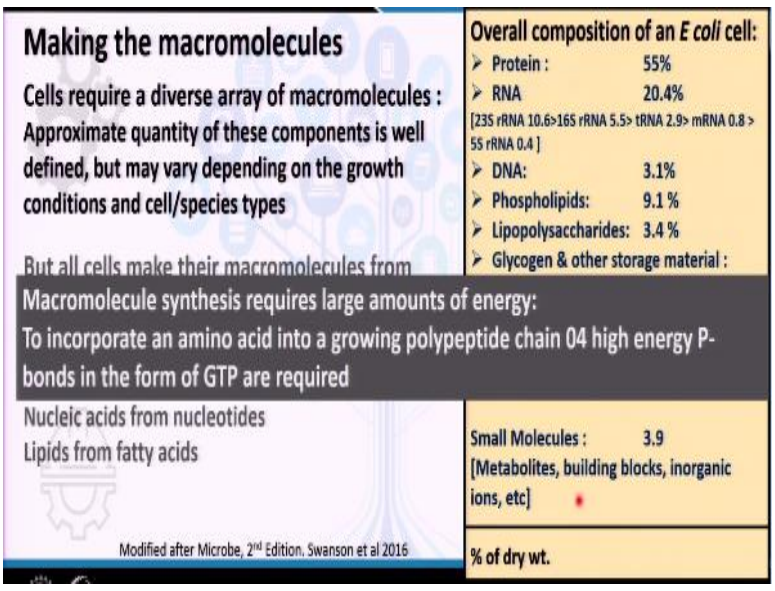
environments, the transcription rates might be varying or the number of ribosomes involved would be involved would be maybe different, the kind of lipids the cell required to produce might vary.

So eventually we may expect a variation in cellular constituents if we change the growth condition. With respect to metabolic engineering, this is one of the very important points that although the overall composition of a particular cell or a species type is relatively well defined and is conserved, but it varies depending upon the growth condition.

And if we change the types like E. coli one particular strain to E. coli another species if we take or another strain if we take, we may expect a variation in the constituents and also their types. But all cells, but all cells make their macromolecules like this molecules from building blocks. This is highly conserved.

So the portion of these like protein percentage may vary depending upon the growth condition or the RNA or the phospholipid percentage may vary depending upon the growth condition, but all of them would be producing these macromolecules from their building blocks. Like proteins would be produced from amino acids, nucleic acids from nucleotides or lipids from the fatty acids.

(Refer Slide Time: 26:40)



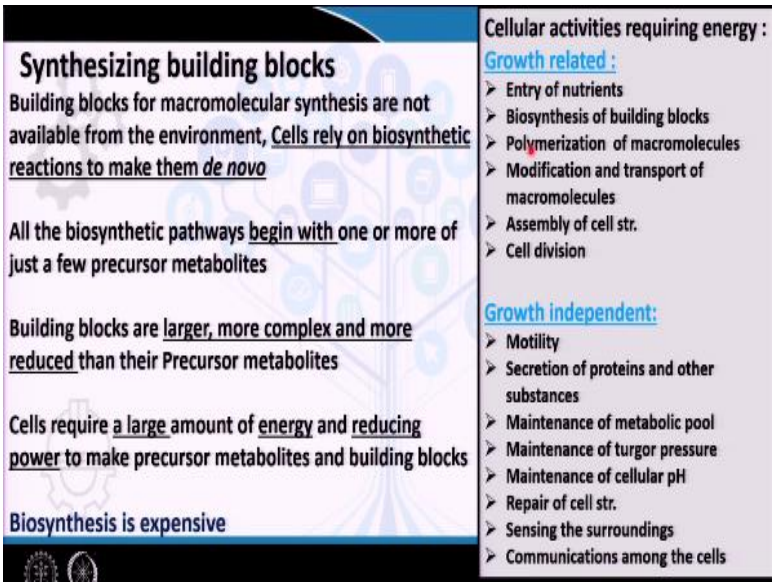
Now the one of the most important message from this part is that macromolecule synthesis requires large amounts of energy. So synthesis of all these macromolecules

which are responsible for all the structure and function of the cell are actually produced by the synthesis reactions, which are biosynthetic reactions. And these biosynthetic reactions require large amounts of energy.

This energy is in the form of both ATP as well as the reducing power. As an example, to incorporate an amino acid into a growing polypeptide chain 4 high energy P bonds in the form of guanosine triphosphate are required. Just to incorporate one amino acid into the growing polypeptide chain that means a polypeptide chain which is continuously being polymerized.

And more and more amino acids are integrated into that, incorporation of a single amino acids, single amino acid into the growing chain of polypeptide requests 4 energy rich P bonds coming from guanosine triphosphate or ATP equivalent.

(Refer Slide Time: 27:53)



Now next is the synthesizing the building blocks. Now building blocks for macromolecular synthesis are not available from the environment. So building blocks are the small molecules which are responsible for synthesizing the macromolecules like the nucleotides, the DNA molecule the protein molecule, so all the macromolecules are synthesized from their building blocks.

And these building blocks are not available in the growth medium or in the environment. So cells rely on biosynthetic reactions to make them de novo. So we need to feed the cells in order to enable them to produce these or biosynthesize these

molecules within the cell itself. Now all these biosynthetic pathways begin with one or more of just a few precursor molecules. That we have noted down earlier.

That a few precursor molecules are responsible for producing all these biosynthetic pathways. Now these few molecules, there may be 12 precursor molecules are to be produced form the nutrients which we are providing to the cells. Now the building blocks in comparison to the precursor molecules, the building blocks like the amino acid molecules or the nucleotide molecules, which are the building blocks.

Now these building blocks are larger, more complex, and most importantly, they are more reduced. More reduced means, during their production, they must be provided with electrons. So we need to supply electrons to them during their synthesis process. So the building blocks are large of course, complex molecules. But they are most importantly more reduced than their precursor metabolite.

So these precursor metabolites are produced from the nutrients. Now these precursor molecules are converted to the building blocks and the building blocks are polymerized to produce the macromolecules and macromolecules are assembled. Now when we consider that the building blocks are more reduced compared to the precursor molecules, that means the conversion of the precursor to building block would be a energetically expensive reaction.

And that is why cells require a large amount of energy and reducing power to make precursor metabolites and also to the building blocks. Because both these set of reactions that is precursor metabolites to building blocks are energy expensive reaction, it is truly very expensive reactions, lot of ATP molecules.

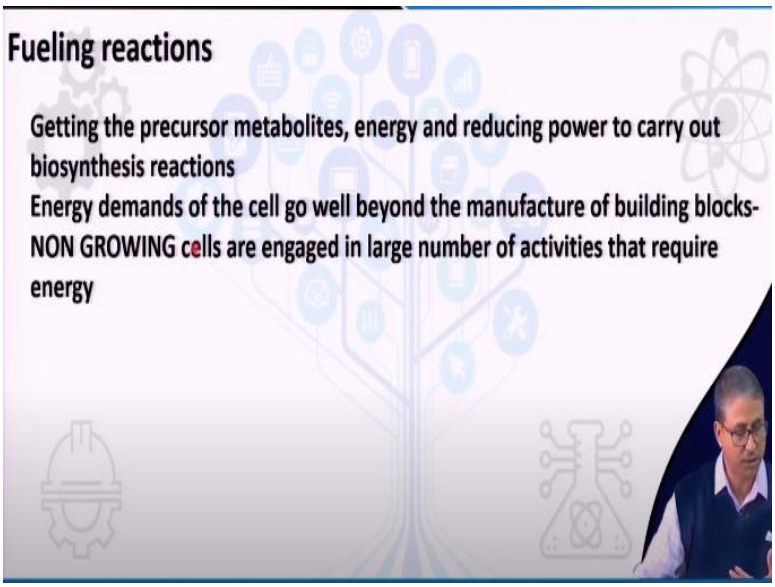
As we have seen that incorporation of a single amino acid into the growing polypeptide chain might require up to or require up to 4 high energy P bonds. But at the same time synthesis of the precursor metabolites are also expensive. Cell needs to spend some energy for producing the precursor metabolites.

Now the major cellular activities which are consuming or requiring energy are distributed as growth related and growth independent. In growth related the entry of

nutrients or transport of the nutrients, biosynthesis of the building blocks, polymerization of the macromolecules, modification and transport of the macromolecules to designated locations and assembly of the cell structure and eventually cell division.

So these are the major events which would be requiring or which require a high amount of energy input and this energy is provided by the catabolic reactions or the energy harvesting reactions, where the energy is harvested during the cellular metabolism.

(Refer Slide Time: 31:34)



Now fueling reactions are the first set of reactions, which are responsible for managing the entire framework of the reactions. And without this fueling reaction, neither the precursor metabolites nor the energy or no reducing power would be available to the biosynthetic reaction. And that is why we discussed this particular segment of the framework in a reverse direction.

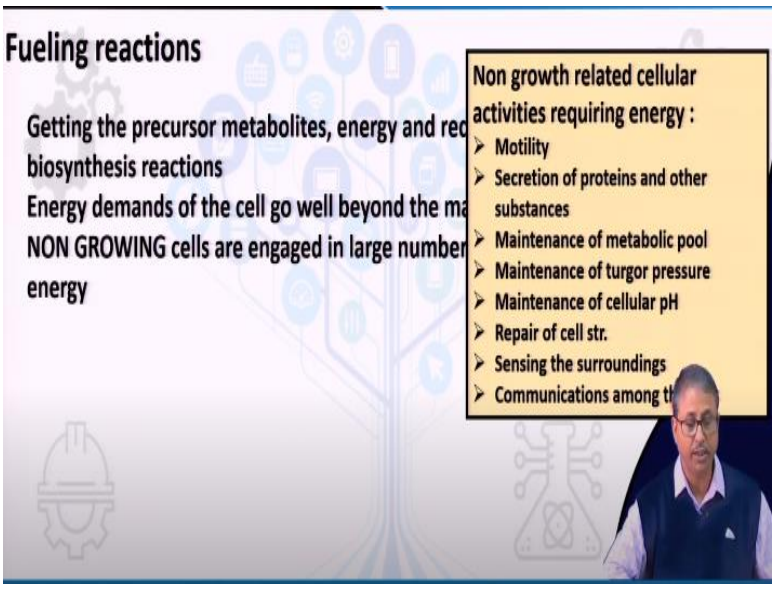
That we started with the assembly process, we realized the requirement and then we moved to the biosynthesis and biosynthesis to the fueling reaction. Now fueling reaction means the responsibility of fueling action is to provide the precursor metabolites. Because precursor metabolites would then feed the, into the biosynthetic reactions and the biosynthesis of required monomers will be produced and these monomers will be assembled to the polymeric macromolecules.

Now these precursor metabolites are produced from the fueling reaction. The energy in the form of ATP or ATP equivalents are produced. And the reducing power which is the carrying the electrons within them like NADH+H+ or the NADPH or FADH2, these are all produced during this fueling reactions. And these are essential requisites for the biosynthetic reactions.

Now energy demands of the cell go well beyond the manufacturing of building blocks. Because we might think that our cells are not growing because we might be running our bioreactor or the particular cell is maintained in a condition where the cells are not allowed to grow much. So cells are not allowed to grow that means the growth metabolism is slow. The cells are not growing vigorously.

So we may think that then the energy requirement would be low for the cell, but actually that is not true. During the condition, when the cells are actually not growing, which is referred as non-growing state of the cells, the cells are still engaged in a large number of activities. A large number of activities are going on inside the cell. And those large number of activities which are not responsible or not connected to the growth of the cells are energy requiring.

(Refer Slide Time: 33:52)



And some of these non-growth related cellular activities requiring energy are the motility, secretion of proteins and other substances, maintenance of metabolic pool, maintenance of turgor pressure, maintenance of cellular pH, repair of cell structure,

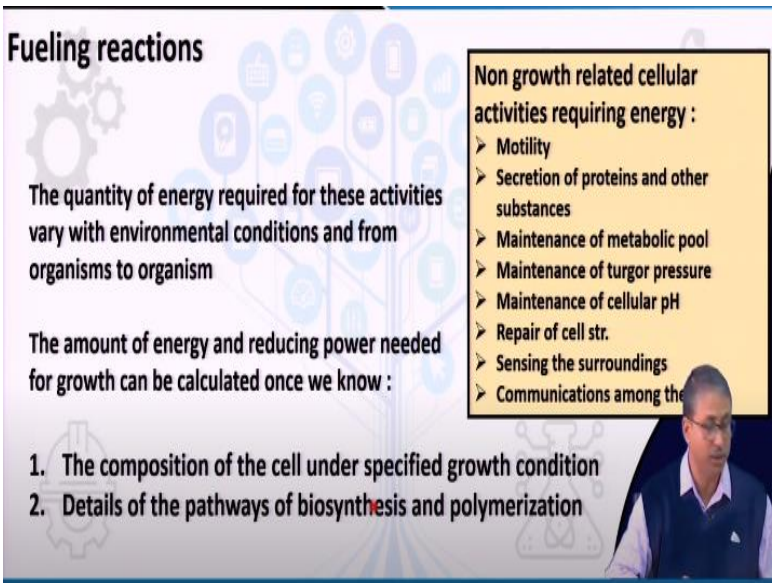
sensing the surroundings and communication among the cells. All these events are very essential for the cells, cells wellbeing.

So when again in context to the metabolic engineering when we used to engineer a cellular system so as to produce the desired compound or to so as to endow the cell with a desirable property, we need to understand the requirement of the energy and the nutrients are not only for the designated purpose that we think. We need to understand what cell think about themselves.

Because the cell might not be growing because we are not allowing them to grow or we have not created such condition. That does not mean the cell would be will be not requiring energy. Cell will be still be requiring energy in order to maintain very essential physiological process like the maintaining the pH, maintaining the turgor pressure, maintaining the metabolic pool, DNA repairing and other repairing mechanism.

And very importantly the communication among the cells. Because cell to cell communication through different chemical signals are very important for maintaining a overall homeostasis within the system.

(Refer Slide Time: 35:19)



Now the quantity of the enzyme, energy required, how much energy is required by these activities vary with environmental conditions and from organism to organism. It is obvious that if a cell is under stress or under altered pH condition or altered osmotic

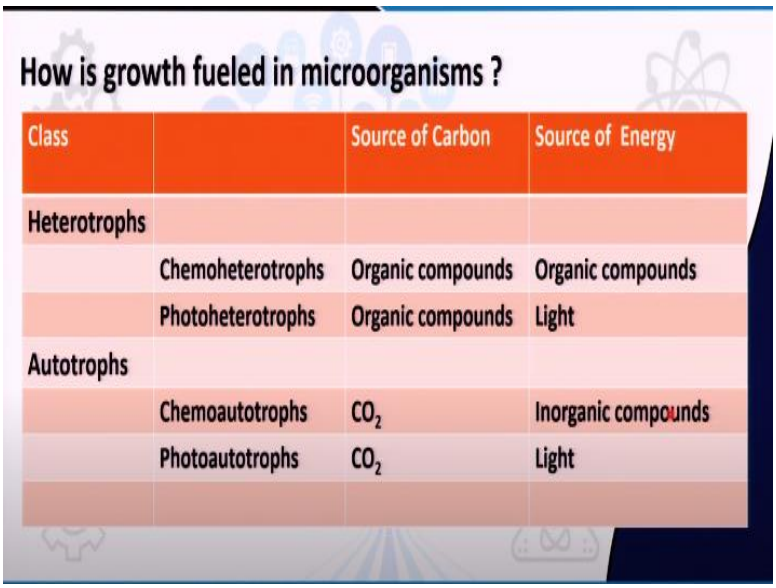
pressure condition, then it would be require more mechanisms to or implementation of mechanisms to maintain that. That is called the homeostasis.

So when such situations are there, so cell might be requiring even more energy to spend to maintain and to achieve a kind of homeostasis. Now the amount of energy and reducing power needed for growth can be calculated once we know two very important things. So the amount of energy and reducing power which is subjected to the growth condition or the environmental conditions in which the cells are exposed.

But they can be determined by determining the composition of the cell under specified growth condition. The specified growth condition is a very important term over here. So if we change the growth condition like from the medium to pH to temperature to the oxygen diffusion, any kind of environmental change might lead to a change in composition of the cell materials and hence, the energy requirement of the cells.

Now details of the pathway of biosynthesis and polymerization, that information is also required. So in fact during the process of all the metabolic pathway assessment and including the flux, MFA flux analysis and all those things, we analyze this biosynthetic reactions, biosynthetic process, particularly how the carbon flux is processing within the metabolic reactions.

(Refer Slide Time: 37:03)



Now how is growth fuelled in microorganisms particularly? This is very well-known fact but we want to just quickly revise this in case of heterotrophic organisms which are relying on complex organic compound as their source of carbon. So there could be chemoheterotrophs and or could be photoheterotrophs. The chemoheterotrophs the source of organic compounds while the source of energy is also organic compound.

So in case of chemoheterotrophs like for example *E. coli* or yeast, it is basically the carbon substrate which is we provide like yeast extract or maybe the lactate or acetate or the glucose molecules, these organic molecules provide the source of carbon and also the source of energy. However, for the photoheterotrophs the carbon source is the organic compounds like the glucose or other carbon compounds, organic compounds, but the source of energy is the light energy.

So they harvest the light energy and they utilize the carbon from the organic carbon. In case of autotrophs there are also two types of autotrophs, the chemoautotrophs and photoautotrophs. The chemoautotrophs they use source of carbon. In case of autotrophy is obviously carbon dioxide because they rely on fixing their own carbon. So in both the chemo and photoautotrophs it is the carbon dioxide which are which is there as a carbon source.

However, the source of energy is different in case of chemoautotroph. The source of energy is the inorganic compounds like the ammonia or the water or even iron or sulfur compounds, which are highly reduced compound if we oxidize or the cell oxidize them and get the electrons out of them and those electrons are used in the cellular metabolism. In case of photoautotrophs, it is the light energy.

So light energy is utilized to actually derive the electrons from water or H_2S or other molecules.

(Refer Slide Time: 39:09)

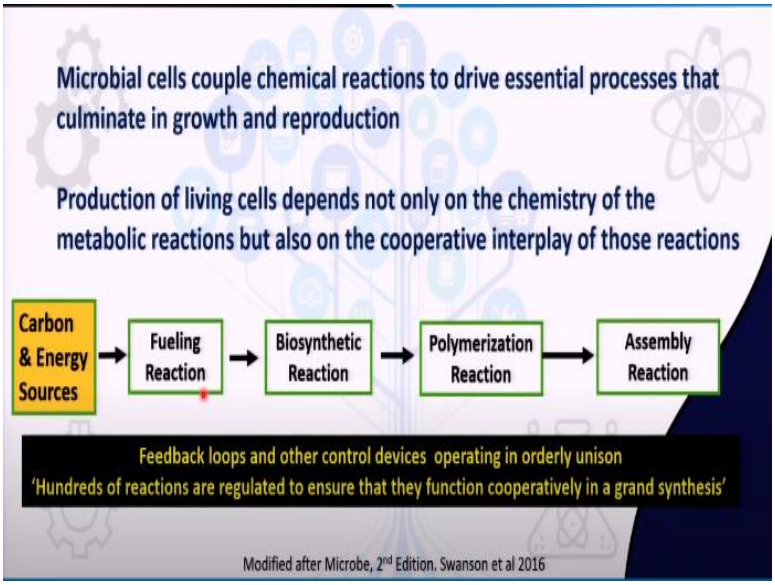
Now two interesting facts are there with respect to the growth metabolism which is connected to this framework of metabolism. Metabolism is diversified to process specific nutrients available in the environment, yet all lead to conserved internal order and chemistry.

What I was referring to earlier that over the evolutionary period microorganisms, mostly microorganisms and then other eukaryotic organisms too, they try to maintain this framework of metabolism starting from the nutrient sources like the carbon energy sources to the fueling reactions up to the assembly reactions. Now the cellular system can begin with any of the starting materials.

Like in case of heterotrophs, no matter whether in the medium you have glucose or succinic acid or maltose or yeast extract, the end products will be always the same, the same set of molecules which the cell actually requires. Obviously, the kind of secondary metabolites or other metabolites which the cell might not be using for its own purposes.

Sometimes in metabolic engineering, we try to exploit that properties of the cells that is different. But otherwise, for the regular compounds, the framework is very well designed and it is like a kind of a funneling reaction. Like all different kinds of substrates can be accommodated and the cellular system will be able to run smoothly using those different set of substrates.

(Refer Slide Time: 40:46)



Although there are certain organisms which are called picky type of organisms or they are very selective about their nutrients, but apart from that, generally the heterotrophic microorganisms are very versatile with respect to their nutrients and nutritional sources. The second very interesting point about this framework is that the cells couple chemical reactions to drive the essential process.

So there are, I mentioned there is a large number of chemical reactions involved in this framework okay, which actually culminate in growth and reproduction or maintenance of the healthy cell type. So cell phenotype or cell physiology will be maintained in a healthy state if the cell is not at least not divided. Now production of living cells depend not only on the chemistry of the metabolic reactions, like the kind of reactions.

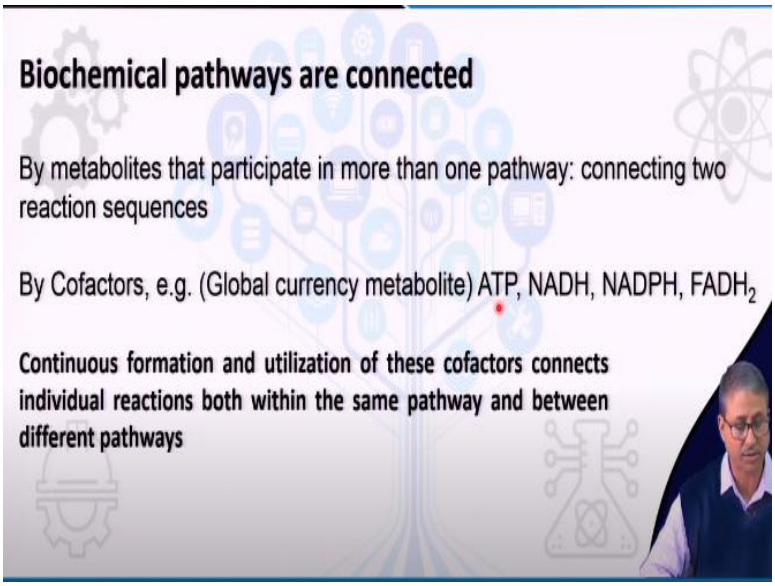
There are large number of reactions going on inside the cell, but also on the cooperative interplay of these reactions, this is another very interesting thing. So while the first interesting thing about the framework was the acceptability of different substrates. You can start, the cells can start, particularly the heterotrophic organism, they can use any of the different kind of organic carbons.

And then still achieve the similar set of complex well-structured compounds, which are required for the cellular function, but at the same time, a strong cooperative interplay of numerous reactions going on the cells. Because here we are not representing the reactions, which are just showing the broad flow of processes.

So these arrows are not to be considered as reactions, these are only the flow of the framework that the fueling reactions is now feeding the biosynthetic reactions. But here we can have few 100 reactions operating on that. Now how these reactions are regulated, coordinated as per the requirement of the final products like the cell is trying to divide or cell is not trying to divide, cell is planning to maintain the non-growth condition.

So feedback loops and other control devices operating in very orderly manner, okay. We will be talking about that in another lecture. And it has been found that hundreds of reactions are regulated to ensure that they function cooperatively in a grand synthesis. The grand synthesis is the synthesis of the required molecules, and only the required molecules which are essential for the cellular function and cellular growth.

(Refer Slide Time: 43:14)



Now the next one that we are going to talk today is that biochemical pathways are connected, because we have already talked about the metabolites and we have talked about ATP and reducing power. So biochemical pathways within a metabolic setup can be connected or interconnected by participation of intermediates.

Many intermediates like hexose phosphate or pyruvic acid or glyceraldehyde 3-phosphate for example or oxaloacetic acid or ribulose 5-phosphate or different other such small molecules, they help the cell to engage or connect multiple pathways with each other. And there might be a question like how these are actually selected that

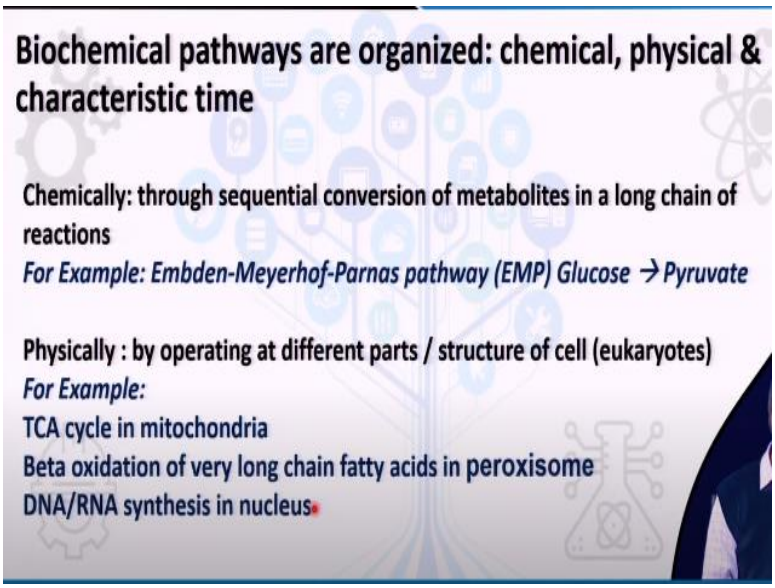
which metabolites will be involved in more reactions and which metabolites would not be involved in more reactions.

So this is that is not the purview of today's lecture. But the second important connecting compound is the cofactors including the ATP and similar high energy currencies, NADH, NADPH and FADH2. Now continuous formation and utilization of these cofactors like continuously the ATP molecules are produced and they are also consumed, because in some of the other slides, we have seen that some reactions are thermodynamically not favorable.

So they might be requiring the input of ATP to make them favorable, make them proceeding towards the desirable site. Some reactions might be reducing reactions or requiring the electrons from NADH or FADH2 or NADPH.

Now on the one hand, these are continuously being produced by the fueling reactions and the other side these are continuously being consumed by the different biosynthetic reactions or assembly reactions or even some of the activation type of reactions which are there in the catabolic set of reactions itself.

(Refer Slide Time: 45:11)



Now the organization of the biochemical pathways. So there are we are mentioning that there are many pathways inside the cellular system like a tiny *E. coli* might have more than a thousand or so, close to thousand reactions going on and there are a large

number of pathways. Pathways mean we have metabolic pathways, we have defined a set of well-organized sequence of reactions by feasible and observable events.

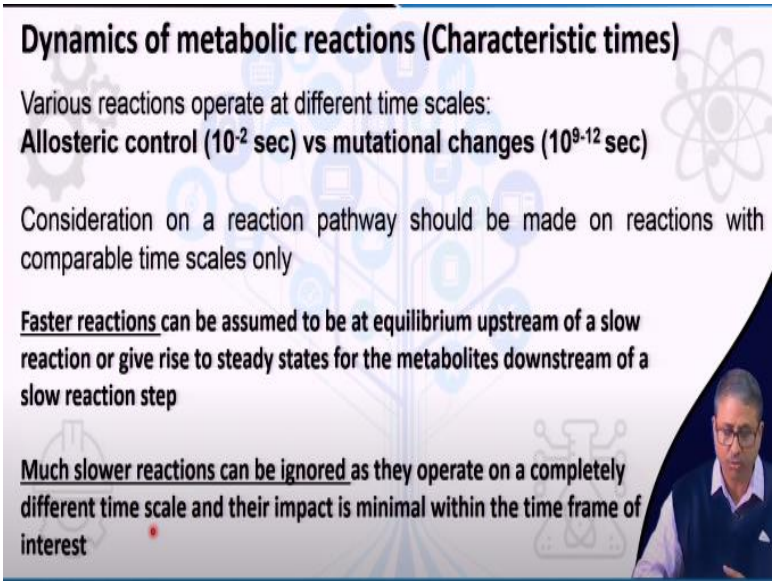
So these pathways are generally organized because in metabolic engineering, we need to organize them in order to understand and in order to modify them appropriately. So these reactions, biochemical pathways are organized based on their chemical properties, they are based on their physical properties and also based on their characteristic times.

So chemically through sequential conversion of metabolites as we generally see in case of a biochemical reaction series, that a particular substrate is converted to a particular product, which is an intermediate and then this product is converted to another product, why because these two reactions are interconnected and so like glycolytic reactions or pentose phosphate pathway.

These are called chemically organized reactions like pentose phosphate pathway itself is a chemically well-organized reaction and Embden-Meyerhof-Parnas pathway is another very well organized set of reactions. So there could be chemically the reactions are organized. Physical organization of the chemical reactions or biochemical pathways can be understood best when we look at the fact that the different set of reactions are operating at different parts of structure within the cells.

Particularly in case of eukaryotic organisms like yeast we can understand that for example, the TCA cycle operates in mitochondria, beta oxidation of very long chain fatty acids are happening in the peroxisomes. Or DNA and RNA synthesis are happening inside the nucleus. So set of biochemical pathways are very well organized with respect to their physical location, some reactions are happening in particular location within the cell itself.

(Refer Slide Time: 47:34)



The third and most important category of organization is organizing the reactions based on their characteristic types. Now before we define the characteristic times we need to understand that different reactions although there are numerous reactions as we have been talking within the cells, there are numerous reactions operating. Now different reactions are operating at different timescale.

Some reactions are very fast like an allosteric control is very fast or nutritional changes, which are very slow. So there are reactions with all kinds of variable timescale. Some reactions are fast, some reactions are slow some reactions are very fast and so on. So we are going to discuss briefly about that and then we will define the characteristic times.

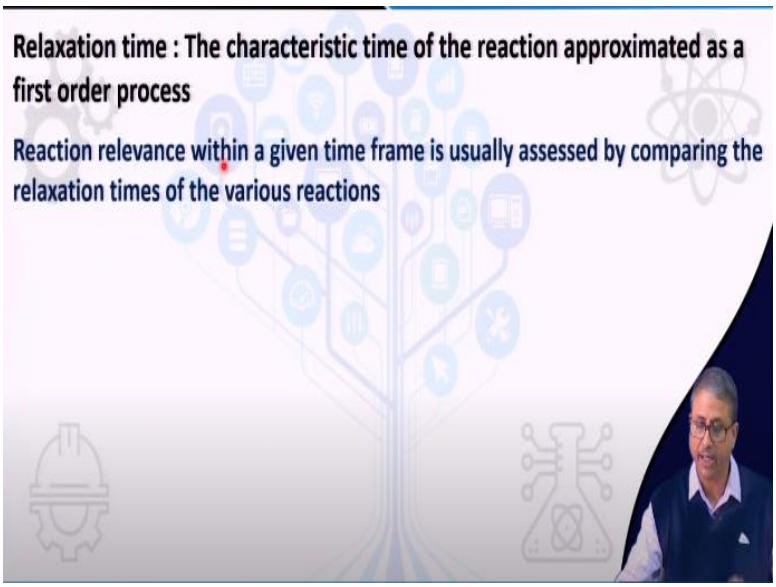
Now when considering on the reaction pathway, we should focus or select the reactions with comparable timescales only. So when I when we are looking at an entire stretch of reaction system or a cellular metabolism, we should make selection about selecting the reactions or the particular reactions based on their characteristic time and we should consider the reactions with comparable timescale only.

Now the faster reactions which are like in this case the allosteric reaction is much faster than the mutational reactions. Now faster reaction can be assumed to be at equilibrium upstream of a slow reaction or give rise to steady state for the metabolites downstream of a slow reaction step. And much slower reactions.

Much slower reactions, reactions like mutational change if we consider with respect to enzyme control can be ignored as they operate on a completely different timescale. Like when we are expecting that the due to the change in reactive configuration or the change in the environmental condition there might be change in enzyme activities, the control mechanisms or the kind of enzyme synthesis etc., might be altered or changed.

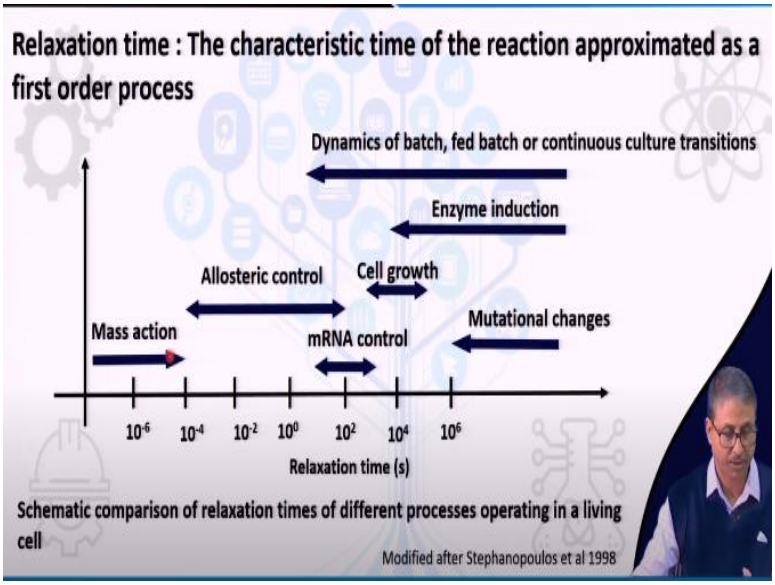
Now during that particular consideration, we should actually ignore changes like mutational changes for example, which are truly very slow and may not be included in this analysis.

(Refer Slide Time: 50:01)



Now the relaxation time, which is actually the present the characteristic time of the reaction. The characteristic time of the reaction approximated as a first order process is called relaxation time. For relaxation time of a given reaction is basically the characteristic time of the reaction approximated as the first order process. Now reaction relevance within the given time frame is assessed by comparing the relaxation time of the various reactions.

(Refer Slide Time: 50:28)

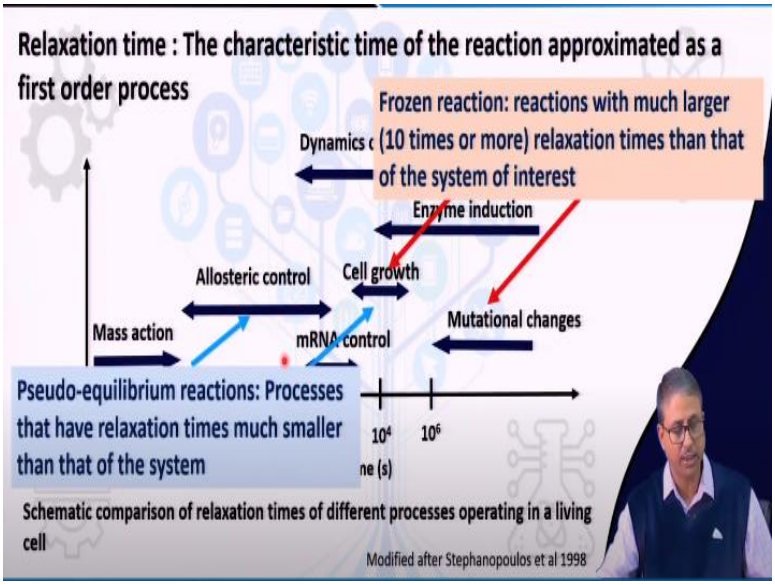


For example, here we see that schematic comparison of relaxation time of different processes operating in a living cell. Now with respect to the x axis is the relaxation times and as you can see changes like the mutation, the enzyme induction, they are particularly the mutational changes are pretty slow taking 10 to the power 6, 10 to the power 9, 10 to the power 12 seconds, very slow process.

Compared to cell growth like cell growth we can have 10 to the power 3 to 10 to the power 5 or so. That is the timeframe within which the cell growth will be completed, few hours for example. Allosteric controls or the enzyme controls are relatively faster depending upon the type of the controls and type of the enzyme mechanisms it can go up to less than a microsecond to a second only.

mRNA control, how the mRNA production transcription is controlled, that is a matter of only an hour or so within that or less than an hour within that the mRNA are produced or controlled. In comparison to the cell growth or mRNA control, mass action or the diffusion of the molecules inside the cells are pretty fast. Like they are less than microseconds or so.

(Refer Slide Time: 51:47)

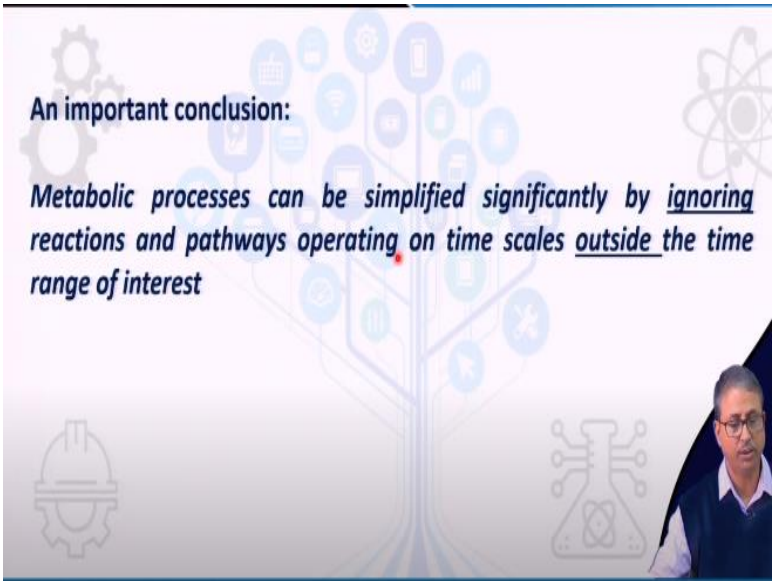


Now when we are comparing different reactions, there could be some reactions which are on absolutely different timescales and they are referred as in this case, frozen reaction. Reactions with much larger, much larger in the sense 10 times or more relaxation time than that of the system of interest. Like if we are considering cell growth with respect to that mutational changes are surely the frozen reaction.

Because they are operating at a much larger 10 times or more timescale. So with respect to cell growth, mutational changes can be considered as frozen reactions and they may not be included, they may not be included within the study. On the other hand, there could be pseudo-equilibrium reactions, because these reactions are having relaxation time much smaller than that of the system.

We were referring to this earlier. So compared to cell growth, the allosteric controls are actually pseudo-equilibrium reaction because they are having significantly lower relaxation time compared to the cell growth.

(Refer Slide Time: 52:52)

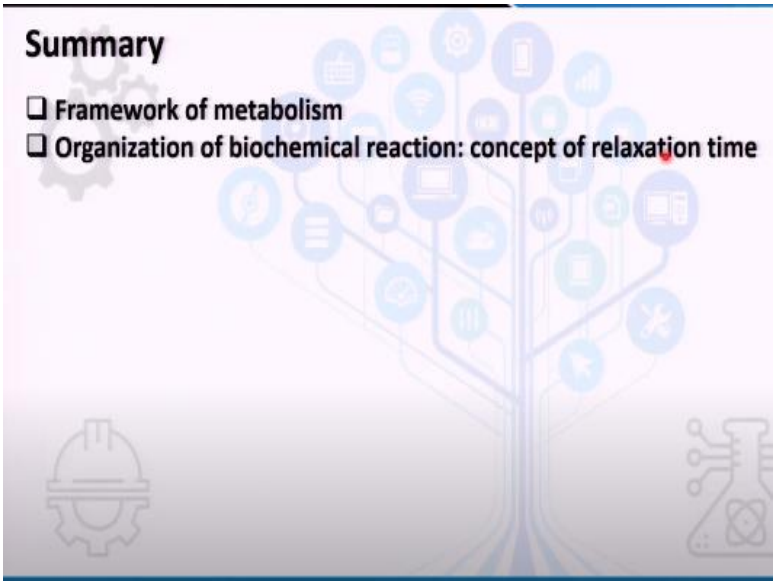


Finally, the conclusion remark out of this characteristic time segment is that metabolic processes can be simplified. We know that there are a large number of metabolic reactions and metabolic processes are happening inside the cell. So that can be simplified significantly by ignoring reactions and pathways operating on timescale outside the time range of interest.

If we are, for time being if we are working on the enzyme regulation, we may ignore even this, even the partly the cell growth also. If we are considering the mRNA expression, however, cell growth will be very relevant because the timescales are overlapping. But the mass transfer or the diffusional factors might be on a kind of a pseudo-equilibrium. So we may not consider them, may not include them.

So when we have large number of reactions to investigate and then understand that how they are working together so we may use the scheme like we can use the chemical organization or the physical organization or the relaxation time based concept to simplify the overall metabolic process.

(Refer Slide Time: 54:04)



So in today's class, we have highlighted the framework of metabolism emphasizing on the different components and how these important parts of the framework are interconnected and interdependent, and the usefulness of this growth metabolism or the energy supply during the non-growth metabolism as well. Organization of the biochemical reactions and the concept of relaxation time is also covered. Thank you.