Metabolic Engineering
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Lecture - 06
Review of Cellular Metabolism

In today's class on metabolic engineering, we are going to talk about cellular metabolism and we will discuss the broad aspects of cellular metabolism and review it with respect to metabolic engineering applications.

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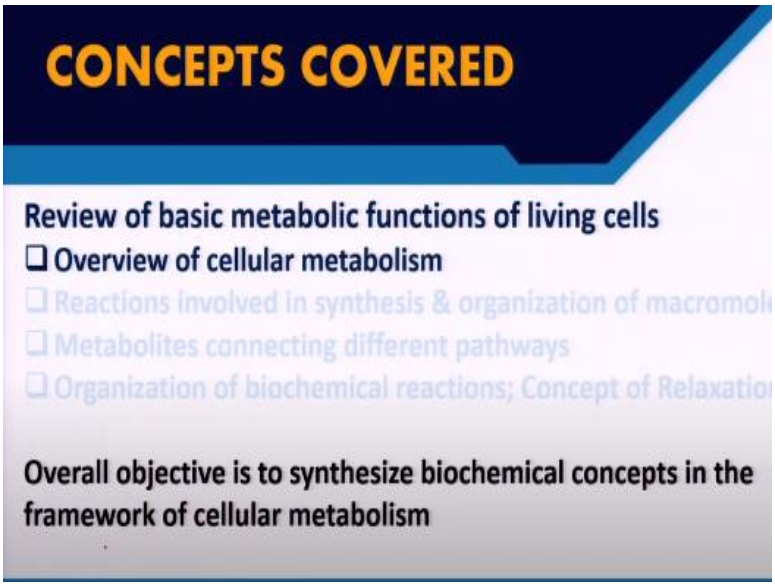

So during this lecture and in continuation with that, we will be highlighting the basic aspects of cellular metabolisms and other components and important parameters and processes within it. Overall objective of this lectures would be to synthesize a biochemical concept in the framework of cellular metabolism.

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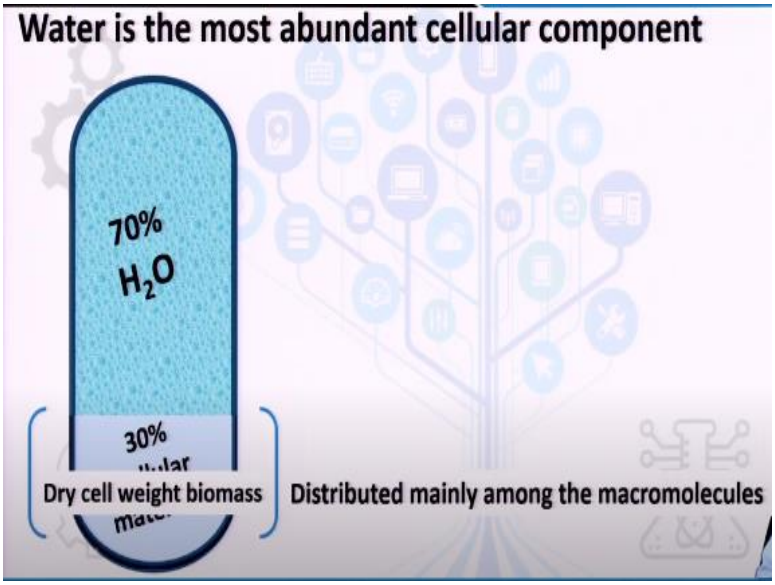

Now water is the most abundant cellular component across all the different type of cellular systems. Nearly 70% of the cellular component is represented by water and rest that is 30% or nearly 30% is constituted by different cellular materials which actually represents the dry cell weight biomass. Now this 30% chemical constituents, they are distributed mainly among different macromolecules.

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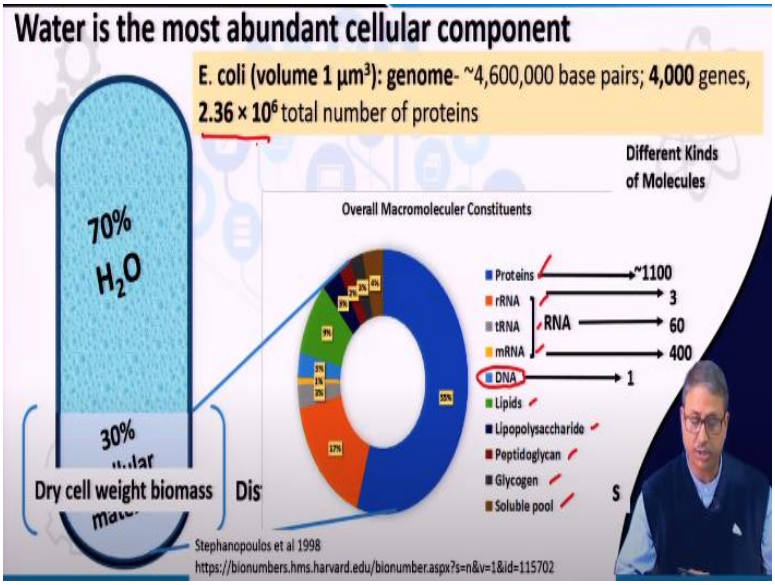

And these macromolecules are mostly represented by a large number of molecules including the protein, different type of RNAs, including the ribosomal RNA, transfer RNA and messenger RNA. DNA, which includes both the chromosomal DNA as well as the plasmid DNA. Lipids, lipopolysaccharides, peptidoglycan, glycogen and different other soluble pools.

Now the diversity and abundance of these molecules, all these macromolecules are pretty abundant. Like if we look into the number of protein molecules, different kind of protein molecules present in an *E. coli* cell that could go more than, up to more than 1000 different kinds and the total number of protein molecules could be as high as 2.36 billion types.

The type of ribosomal RNA compared to other things are relatively less because as we know there are only three principal types or main types of ribosomal RNA so far found in cellular systems, which is only three. Transfer RNAs are quite high up to 60. And messenger RNAs are also significantly high because it is representing the transcripts or the genes which are being expressed at any point of time particularly during the active growth phase of the cells.

Other molecules including the lipids, lipopolysaccharide, etc., generally present in varying concentrations.

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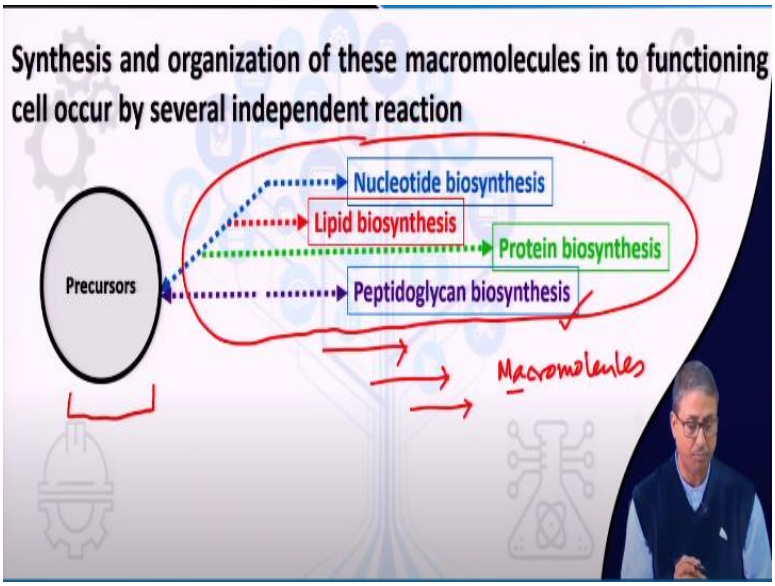

Now synthesis and organization of these macromolecules into a functioning cell occur by several independent reactions. These biosynthetic reactions which are independent to each other include the nucleotide biosynthesis, lipid biosynthesis, protein biosynthesis or the peptidoglycan biosynthesis.

And there may be many other biosynthetic reactions which are leading towards different other macromolecule synthesis as required by the cells. All cells are not

having equal requirement. So apart from the common requirements of different nucleotides, lipid and protein peptidoglycan etc., there might be a number of specialized molecules required by different other cell types.

Now interestingly although all these biosynthetic reactions or the reactions which are leading towards the formation and the assembly and the production of these polymer or these macromolecules these are quite independent. But interestingly, these all biosynthetic reactions are connected to a particular pool of precursor molecules which are small metabolites molecules, which are continuously providing the resources, the chemical and other resources for the biosynthesis of large number of macromolecules.

So the point of interest over here is that there are large number of independent reactions, which are producing different type of macromolecules like the nucleotide, lipid, protein etc. However, all these biosynthetic reactions are originating or utilizing fewer number of small metabolites or small chemical compounds, which are called precursors.

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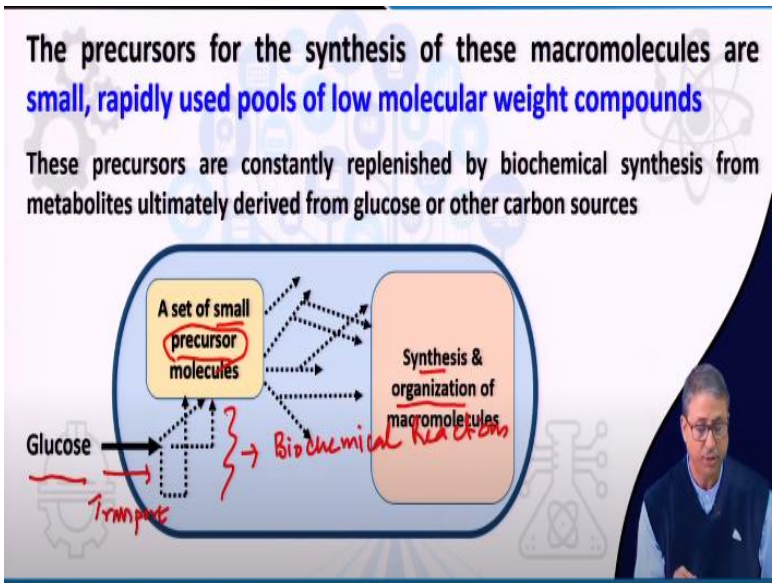

Now the precursor for the synthesis of these macromolecules are small as I mentioned before, rapidly used pool of low molecular weight compounds. Now these precursors are constantly replenished by biochemical synthesis from metabolites ultimately derived from glucose or other carbon sources. Now let us discuss this particular point.

For example, if the cell is utilizing glucose as its carbon source or carbon and energy source that glucose is taken inside the cell that is the glucose transport process. So following the transport of the glucose, a series of reactions, a series of biochemical reactions are taking place and these biochemical reactions are converting this glucose or similar type of complex organic molecules to a set of small organic precursor molecule.

So these are small molecules like two to three carbon molecules or sometimes even four carbon molecules, but generally it is three to four carbon molecules. These are small molecules which are considered or called as precursor molecule, because they are used as the starting molecule or molecule of major importance in initiating the biosynthesis of all other macromolecules.

For example, a large number of reactions which we were discussing in the previous slide, which are basically the independent biosynthetic reactions, these biosynthetic reactions eventually lead to the production of the synthesis of a large number of macromolecules and their organization. So a large number of synthesis and organization related organic processes or biochemical reactions, which are leading towards the synthesis of macromolecules are basically utilizing the small set of precursor molecules.

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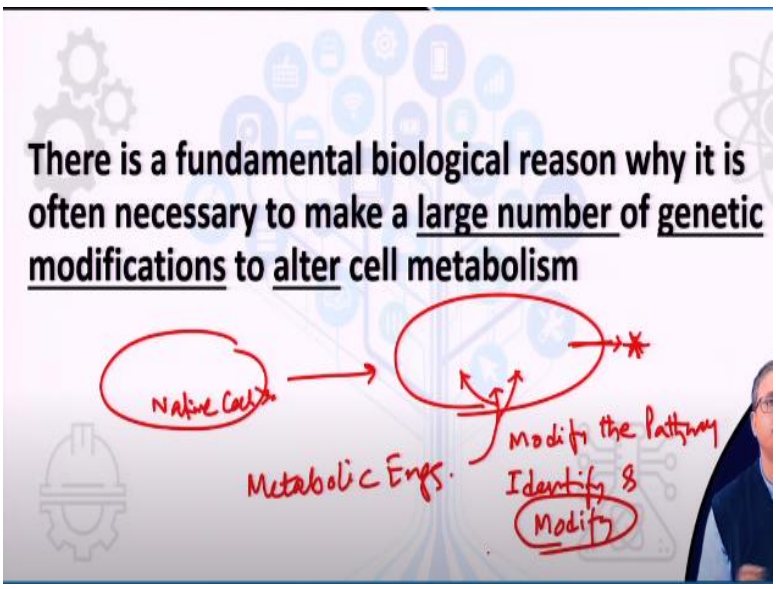

Now there is a fundamental biological reason that why it is often necessary to make a large number of genetic modifications to alter cell metabolism, particularly with

respect to metabolic engineering, when we are trying to improve a native cell, this is the native cell condition, okay. This cell we are trying to improve with respect to production of a particular molecule, maybe the earlier the production was very low.

Now you want to produce a large concentration of or large amount of the particular metabolite. Earlier it was there, but the concentration was very low, we want to produce a large concentration. Now for this, we intend to do many things. So what we intend to do? We intend to actually modify the pathways, modify the pathways. Now how do we modify the pathways?

For modification of the pathways we have learned in our earlier lectures that we need to identify the reactions and following identification of the reaction we can target that how we can plan to modify them, identify and then modify. So identify the reaction and modify the reaction. Now why a large number of genetic modifications are required.

So why the modifications which are done by genetic engineering is often very exhaustive? That means large number of modifications are required, because there are large number of control mechanisms within the metabolic process that is going on in any particular cellular system.

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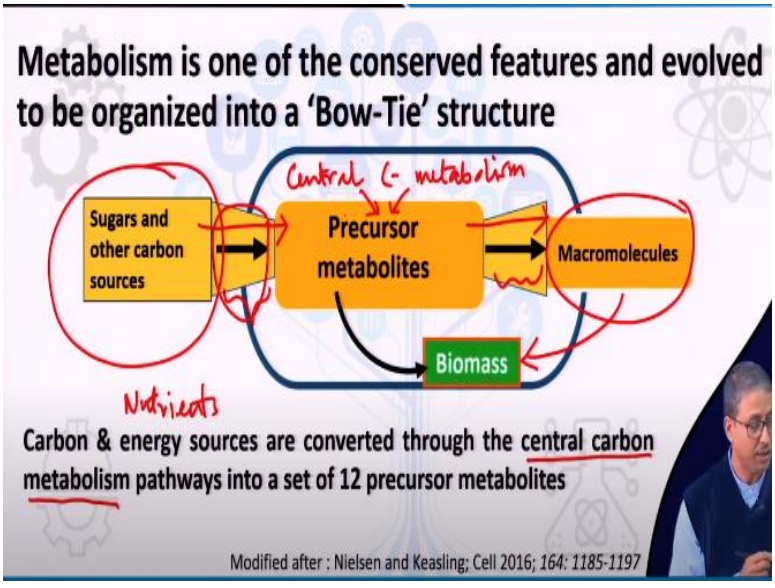

So metabolism is considered to be one of the conserved features over the period of nearly 4 billion years evolution or at least 3.5 or 3.6 billion years steady evolution,

where we can see the how the living system has evolved. They have tried to maintain or evolved and then maintain a very specific structure within this metabolic processes, which is called bow-tie structure. So what is this bow-tie structure?

In this bow-tie structure, the cellular metabolism try to utilize the sugar and other carbon sources which are the nutrients basically. So the nutrients are utilized by the cells and during this course of utilization, this process of reactions which are basically the metabolic reactions they produce or enable the cell to produce a large number of small metabolites which are considered as the precursor metabolites.

Now these precursor metabolites enable the cell to produce the macromolecules through a series of reactions, which are called biosynthetic reactions. So the nutrient molecules which are mostly the complex organic molecules and other substrates or other energy resources, which are transported and consumed by the cell, utilized by the cells.

And during this process of utilization, a large number of metabolic reactions, metabolic pathways are involved and that metabolism ultimately leads to the production of a number of precursor metabolites which are small carbon molecules and these carbon molecules are facilitating the production or the synthesis of the macromolecules.

Which are basically the protein nucleic acids, lipids, carbohydrates and all other molecules, which are necessary for the cellular function, cellular structure etc. And this synthesis of molecules, macromolecules are connected to the synthesis of the biomass. So it is kind of a natural process within the cellular system that the cells they need to grow and as they grow, they actually produce more amount of biomass.

So the production of the biomass is intrinsically connected to the synthesis of the macromolecules. And the synthesis of the macromolecules is connected to the precursor metabolites. And the precursor metabolites are being continuously produced by the sugar and other molecules which are provided as basic nutrients.

Now the carbon and energy sources are converted through the central carbon metabolism which is very commonly used term in metabolic processes, the central carbon metabolism that constitutes the transport of the glucose and then subsequent breakdown of the glucose through the glycolysis or Embden-Meyerhof-Parnas pathway.

Or which is connected to the pentose phosphate pathway and also the ED pathway allowing these cells to produce a large number of precursor molecules that we are going to discuss shortly. Now as these carbon molecules which are or energy molecules, which are transported inside the cell and utilized by the cell through a number of biochemical reactions, and these set of reactions, which are proceeding over here is basically the central carbon metabolism.

So central carbon metabolism, this central carbon metabolism enables the cell to produce this precursor molecule. So these precursor molecules are produced by the central carbon metabolism.

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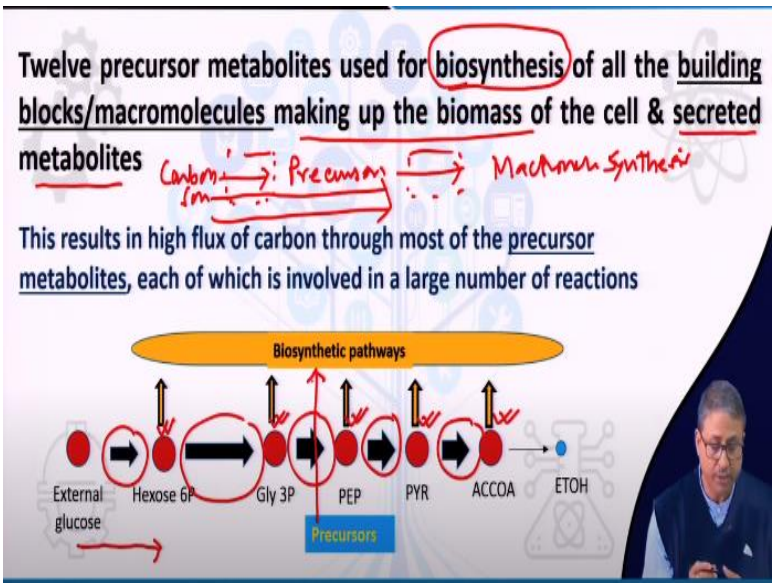

Now we have identified 12 major precursor metabolites, which are produced during this carbon metabolism or central carbon metabolism and are used for biosynthesis of all the building blocks. So used for the biosynthesis of all the building blocks. So in the on the one side, the precursor molecules are produced or precursor metabolites are produced. And on the other hand, these precursor metabolites are used for the biosynthesis of the building blocks of the macromolecules.

Like as we mentioned before, the DNA molecule the protein molecules, the RNA, different type of RNAs, the fatty acid, lipids, carbohydrate molecules, glycolipids and phospholipids and all kinds of macromolecules which these with the cellular system, they require to build their structure and for their growth.

And that is how the biomass, continuous growth of biomass is achieved and the cells are able to secrete some of the metabolites also. So sometimes these secretion of the metabolites are very important from a metabolic engineering point of view because some of these secreted metabolites are point of interest, because they might be having different industrial importance.

Now this particular event that on the one hand, we have a set of biochemical reactions leading towards the production of the precursors. So these are the carbon sources, carbon sources and the precursor are feeding towards the macromolecular synthesis. This results in high flux of carbon through most of the precursor metabolites.

As we discussed briefly in our earlier lecture about carbon flux or metabolic flux that is the rate of flow of metabolites. So these results, what results, this chain of reactions that one side we have these biosynthetic reactions or the reactions which are leading to the synthesis of the precursors and then connecting this precursor to the macromolecular synthesis, we found that there is a considerable amount of flux flowing towards the synthesis of the precursor.

For example, if we use the same schematic diagram, which shows the processing or the metabolic reactions happening after the glucose is transported inside the cell and up to its conversion to pyruvic acid and acetyl-CoA and acetyl-CoA to under fermentative condition the ethanol may be produced. So we can identify that there is a high rate of flux. That means the flow of, rate of flow of the metabolites are pretty significant during this entire stretch of the central carbon metabolism.

Because, all these molecules are actually representing the precursor molecules. Now why the flux is so high? The flux is so high because the concentration of these precursor molecules or the potential precursor molecules need to be maintained in

such a way so that they can feed to the biosynthetic reactions appropriately. So we are going to discuss about briefly about the balance between these two.

So one way we have the substrate. The substrate is converted to the precursor. And in the other way, the precursor is getting converted towards the biosynthesis of the macromolecules. So these are two distinct set of processes going on inside the cell and these two set of processes are highly coordinated and naturally, a high metabolic flux or particularly the carbon flux is necessary in order to maintain the balance appropriately.

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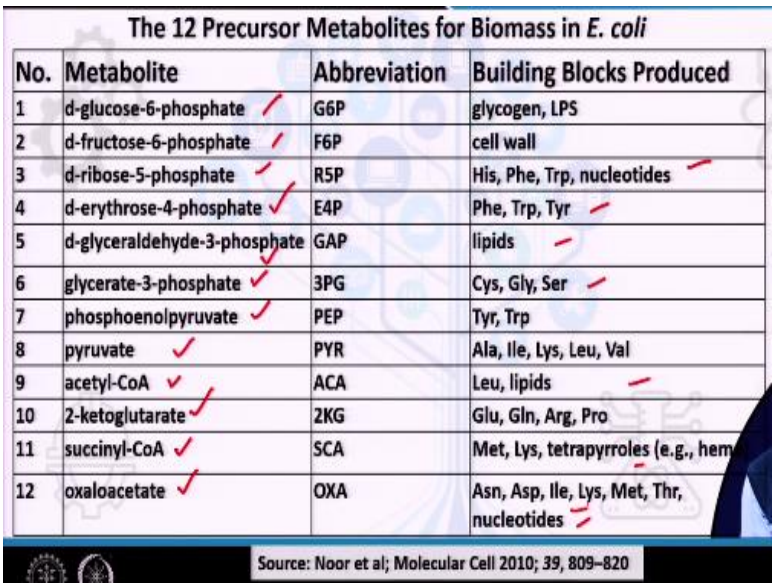

Now this is the least of the 12 precursor metabolites in within E. coli cell, which includes the hexose phosphate like the glucose 6-phosphate, fructose 6-phosphate or the pentose phosphate which is produced in the pentose phosphate pathway, erythrose 4-phosphate. Glyceraldehyde-3-phosphate which is a very important branch point also where the glyceraldehyde 3-phosphate is metabolized.

Phosphoglyceric acid, phosphoenolpyruvate and pyruvic acid, the three important metabolites within the glycolytic cycle. Acetyl-CoA, where the pyruvate is converted to acetyl-CoA. We have seen the formation of this pyruvic acid to acetyl-CoA under aerobic and anaerobic conditions earlier also. 2-ketogluterate, an important component of the TCA, succinic acid and oxaloacetate.

All these are produced from the TCA cycle or the citric acid cycle. Now all these 12 metabolites which are small molecules, and often they are produced within the central carbon metabolism, which is which is a basically combination of the Embden-Meyerhof-Parnas pathway as well as the pentose phosphate pathway and ED pathway and also the TCA cycle. So number of biochemical pathways are interconnected.

And of course, we have discussed earlier the importance of this interconnections within the or interconnected nature of the metabolic process within the cellular system. Now here in this table, we are also able to identify that how all of these precursor molecules or the small molecules are feeding the biosynthesis of different macromolecules.

Like the glycogen which is present in lipopolysaccharide or within the cell wall, the different type of amino acids and nucleotides, other amino acids lipids, the amino acids and different type of lipids and tetrapyrroles or the heme group and different other amino acids and nucleotides.

Almost all the macromolecules required by the cell or any kind of cell not only by E. *coli* cell, it maybe eukaryotic cell or it may be a prokaryotic cell have been found to be produced by using independent biosynthetic reactions. Now point to be noted over here is that for biosynthesis of different macromolecules there are very specific and dedicated biochemical reactions.

These are called biosynthetic reactions like amino acid biosynthesis, nucleotide biosynthesis, lipid biosynthesis or carbohydrate biosynthesis. Now these independent biosynthetic reactions are truly independent, but somewhere they are all connected together by the by sharing a fewer number of small metabolites at their starting compound or the precursor compound.

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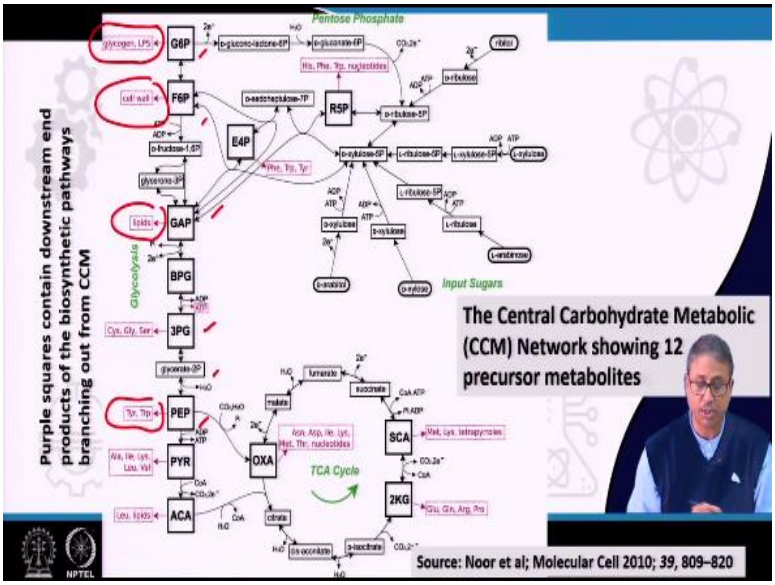

So in this picture, I think we are able to see that how the central carbohydrate metabolism or the central carbon metabolism network which we have, which clearly indicates the present or presence of the different kind of the precursor molecules within itself. And you can see that also that how the different biosynthetic reactions which are marked as this the purple color are actually progressing utilizing these precursor molecules.

Like glucose 6-phosphate is feeding towards the glycogen or lipopolysaccharide. Fructose 6-phosphate is feeding towards the cell wall biosynthesis. GAP is feeding towards the lipids. Phosphoglycerate is leading towards the cysteine, glycine and serine production. Phosphoenolpyruvate is feeding the tyrosine and tryptophan and so on and so forth.

Even the TCA cycle intermediate oxaloacetic acid, succinic acid or the alpha ketoglutarate they are also enabling production of different amino acids and tetrapyrroles. Here we have the pentose phosphates, the ribose 5-phosphate which is involved in the production of the nucleotides.

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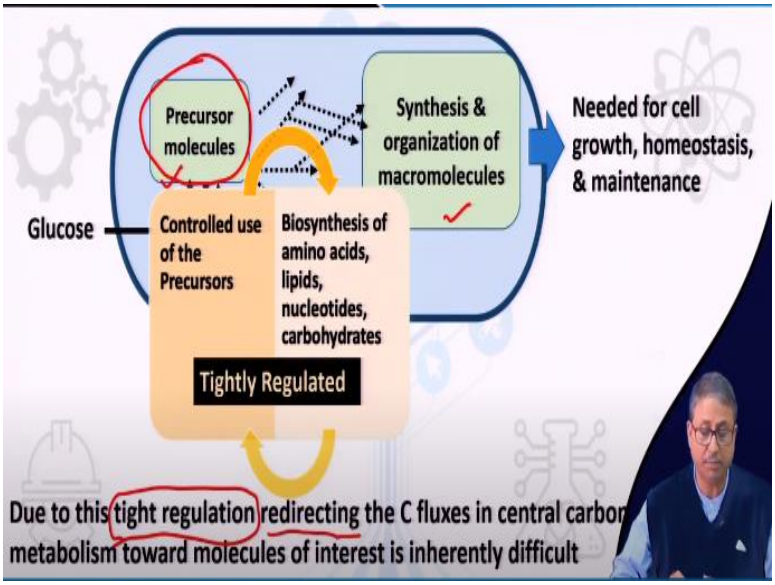

Now we have understood that there are two distinct processes, but both the processes are actually interconnected. So one process is basically the synthesis of the precursor molecule and the other process is the synthesis of the organization of the macromolecules.

Now the synthesis and organization of the macromolecules are absolutely required for any type of cell for cell growth, maintaining the homeostasis and other maintenance functions. Now the point of further interest is these two set of reactions, these two set of processes are highly balanced. Balanced because the controlled use of the precursor, because precursors are having high demand.

These precursor molecules are having high demand. What is the demand? Demand is from the biosynthesis reaction sites. The biosynthetic reactions are always looking forward to use the precursor molecules. So there must be a balance between the use of the precursor and the biosynthesis of amino acid, lipid, nucleotides carbohydrate etc.

That means, it should not be so that if we have more amount of precursor molecules, more amount of biosynthesis will be naturally happening there. So cell generally uses a very tight control mechanism by which the requirement of the biosynthesis of the macromolecules the requirement for this is recognized by the cells and the cell generally, they use this signals that what is the kind of requirement of the macromolecules to control the biosynthesis of the precursor as well.

So these two processes are tightly regulated. The use of the precursor, that means if we have more amount of precursor, it may so happen that a cell is growing on high concentration of glucose. So the cell would be transporting glucose and producing. It maybe thought that cell will be transporting glucose inside the cell and large amount of precursor molecules will be produced.

So the large amount of precursor molecules will lead to production of a large number of macromolecules. But essentially it is not going to continue for a long period of time, because cell will soon realize that we may not the cell may not have that much of requirement of the precursor metabolites because of the control mechanisms which are imposed to that.

And due to this tight regulation, so this about the regulation we will learn later that how these processes are regulated inside the cell. So due to this tight regulation, redirecting the carbon fluxes into central carbon metabolism toward molecules of interest is inherently difficult.

So there is a these kind of a natural tendency or a kind of intrinsic mechanism of carbon flux within the central carbon metabolism, which we have we have seen in our previous slide where we are looking into the carbon flux and carbon metabolism where the 12 precursor molecules were produced.

So this is kind of a fixed and almost nearly conserved mechanism or conserved process where the carbon flux is steadily moving towards the precursor molecules and the precursor molecules are ready getting ready to be utilized by the macromolecular synthesis process. Now these two events are tightly regulated.

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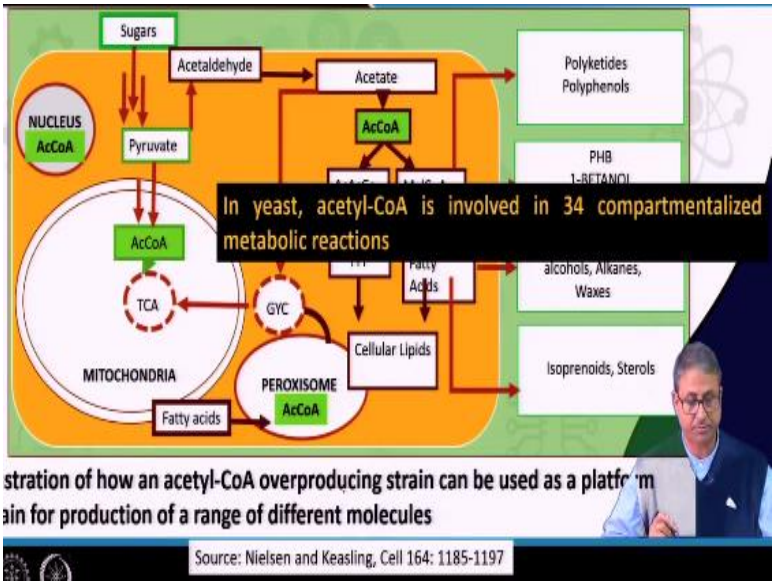

Now in this picture, we are basically trying to illustrate that how a particular metabolite which is involved in a biosynthesis of multiple macromolecules are compartmentalized and how their production could be controlled. So in yeast for example, acetyl-CoA is involved in 34 compartmentalized metabolic reactions, metabolic different type of reactions and these reactions are compartmentalized.

Compartmentalized means they are happening in a particular organelle of the cellular system. So this is yeast we are talking about. So in yeast which is basically an eukaryotic organism. So within this eukaryotic yeast, we have the macromolecular or within the cell we have the membrane bound organelles, and within these organelles, different types of specific set of reactions are happening.

Like as you can see here, that part of the or the major part of the acetyl-CoA metabolism through TCA cycle is happening inside the mitochondria. Similarly, externally derived fatty acids, externally transported fatty acids are processed to produce acetyl-CoA within the peroxisome. And peroxisome or acetyl-CoA could be produced inside the cytosol by the metabolism of pyruvic acid through acetaldehyde and acetate and that acetate can give rise to the acetyl-CoA.

So that means the acetyl-CoA could be produced inside the yeast cell within several compartments and in each of the compartments they may be exchangeable in different forms like this paroxysmal acetyl-CoA may be converted to some form and then it

will be ultimately converted to or feeding towards the TCA cycle like malate and the malate will be utilized within the TCA cycle.

So they may be interconnected but not directly interconnected. So they are highly compartmentalized and this actually indicates the kind of regulation cell imposes.

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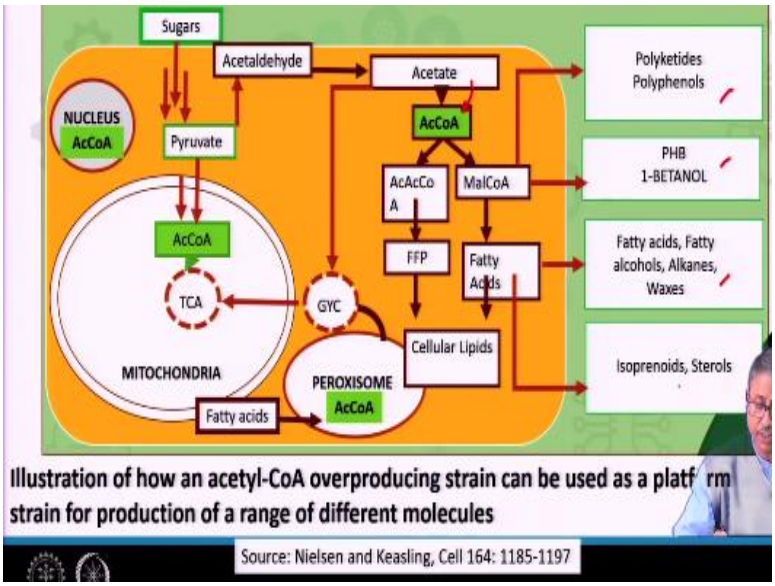

Because each of these precursors or each of these important metabolites, they are having very important and specific roles towards production of individual types of macromolecules like as you can see over here that this malonyl-CoA can lead to the production of polyketides and polyphenols or the PHBs.

Similarly, the fatty acids which are produced out of these malonyl-CoA could be involved in fatty acid biosynthesis, production of wax and different alkenes and also the different isoprenoids and sterols.

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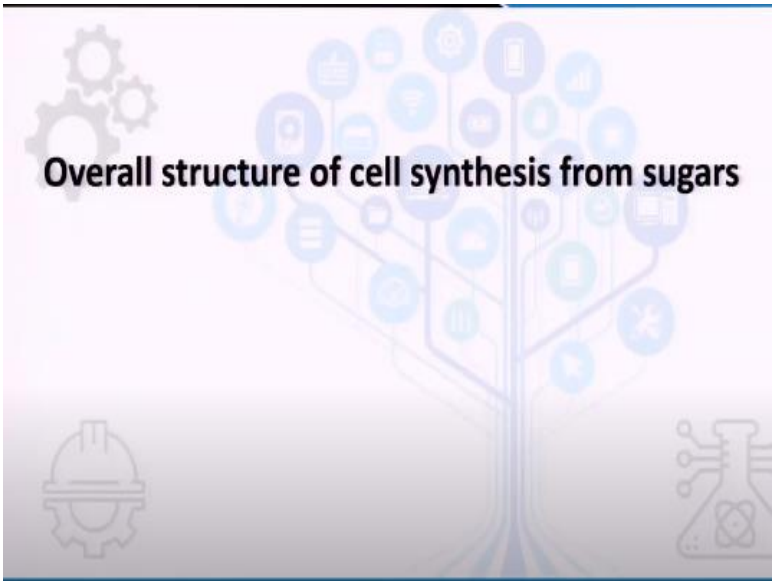

Now overall structure of cell synthesis from sugars.

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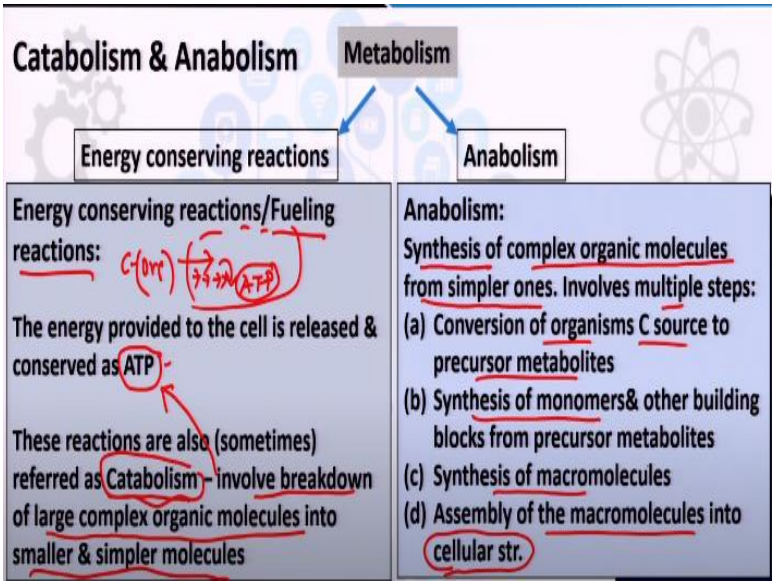

So during this part of the discussion, we are going to briefly introduce ourself to the metabolism, which is the sum total of all biochemical reactions happening inside the cells. And it is basically composed of two distinct types of metabolic reactions which are very commonly referred as catabolism and anabolism. So metabolism is fundamentally of two types.

So one is the energy conserving reactions. We are not calling it in the name of catabolism because of some specific reasons that we are going to mention shortly. So one is the energy conserving reactions where basically energy is produced by the cells and the second one is the anabolism, where the synthesis of the macromolecules are

taking place. Now this energy conserving reactions are also called as fueling reactions.

Because they fuel the metabolism. They fuel the metabolism how? They fuel the metabolism by the production of the energy currency of the cell which is the ATP. So the energy provided to the cell is released and conserved in the form of ATP during this energy conserving reaction or fueling reaction. That is why it is called the fueling reaction. So basically cell is supplied within energy source, which is we can take an example the carbon source which is basically the organic carbon.

So this organic carbon is taken by the cell and inside the cell, we have a series of reaction which facilitate the production of, for example the ATP. So this ATP is very useful energy resource for the cell. So cell cannot get the energy directly from the glucose unless it is metabolized, unless it is a process through a series of reactions. So that series of reaction which is actually a couple of reactions joined together.

We are talking about those couple of reactions which are joined together as metabolic pathways. So when the glucose or similar organic carbon sources are metabolically processed within a cell through specific metabolic pathways, the energy which is actually stored within the organic carbon molecule as a chemical energy is converted or released in the term of ATP or similar kind of energy rich molecules, which are more usable by the cells.

Apart from energy resource like ATP, other resources are also produced, we are going to talk about that soon. Now these reactions are called often or sometimes as catabolism. The word catabolism basically involves breakdown.

So whenever we have breakdown of large complex organic molecules into smaller or simpler molecules, and through that breakdown of large complex molecules to smaller molecules yield ATP, then the terminology is perfectly matching with the catabolic process or catabolism. Because catabolism is a type of energy solving reaction or a type of fueling reaction.

Or generally it is the energy conserving reactions, where the breakdown of large complex organic molecules are achieved to produce smaller and simpler molecules.

Now within the anabolism, synthesis of complex organic matters or organic molecules are achieved and these complex organic molecules for example, the different type of nucleotides or the synthesis of the DNA itself or the different other type of carbohydrates or fatty acid molecules or the reproduction of the entire large larger complexes are achieved from simpler compounds or simpler ones, okay.

And this anabolic type of reactions or anabolism involve multiple steps like the conversion of organisms carbon source to precursor metabolites. Like the organism is taking the carbon source and trying to metabolize it and producing ATP and other resources. But those other resources need to be converted to precursor metabolites. All intermediate metabolites may not be precursor metabolites.

So one of the first step towards the anabolic reaction is the conversion of the organism carbon sources to precursor metabolites and from that precursor metabolite or number of precursor metabolites synthesis of the monomers and other building blocks are achieved. The next is the synthesis of the macromolecules from the monomers because once the monomers like the amino acids or the nucleotides are produced, then the synthesis of the macromolecules by polymerization reactions are achieved.

And finally, once the polymers are produced, then the assembly of the macromolecules are required because the macromolecules which are produced are to be converted or to be structurally organized to form the required or functionally or structurally active molecule. So assembly reactions actually enable the cells to produce the required cellular structure require the molecules in proper structural configuration.

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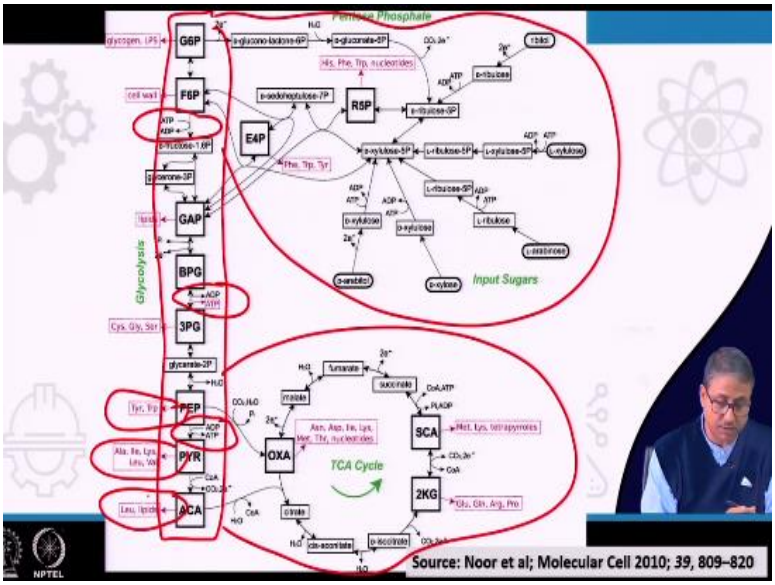

So if we look at the same metabolic map where we were observing the central carbon metabolism, so this is the part of the central carbon, entire stretch of the central carbon metabolism coupled to the TCA cycle and also coupled to the pentose phosphate pathway. If we briefly look into this, we will be able to get a kind of a brief understanding about how these.

So this stretch of reaction you can assume that partly it is the catabolic reactions, because the energy is continuously generated. So it is oxidized and the energy is consumed and energy is generated over several reactions. And also you have the number of metabolites produced. Now each of these metabolites could be utilized by the anabolic reactions, where you have the complex macromolecules are to be synthesized.

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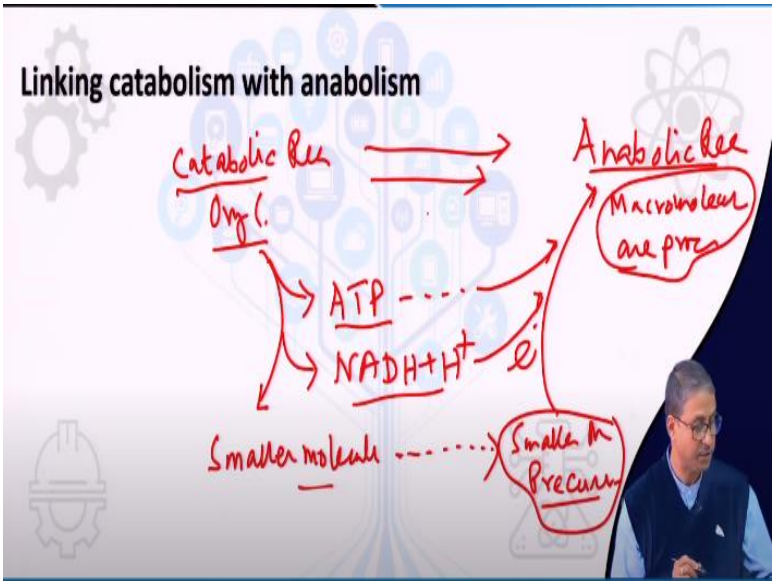

Now these catabolic reactions are actually linked tightly with the anabolic reactions, because on the one hand the catabolic reactions like if we write the catabolic reactions which is basically the organic molecules are processed to produce the smaller molecules. But during this process, we are producing the ATP and also we are producing the reducing equivalent which is basically the NADH+H+.

Now the anabolic reactions, the anabolic reactions, they are utilizing these smaller molecules, many of the smaller molecules, which are going to be used as the precursors. So they are converted into precursor molecule and these precursor molecule they are utilizing these reducing power because they need the electrons and also they need the energy from this ATP and ultimately, the complex macromolecules are produced.

So from smaller precursor molecules we can produce the complex macromolecules through the anabolic reactions, which require the supply of the small molecules, supply of the energy and supply of the reducing power of NADH H+ which are provided by the catabolic reaction. So essentially catabolic reactions are intrinsically connected to the anabolic reactions.

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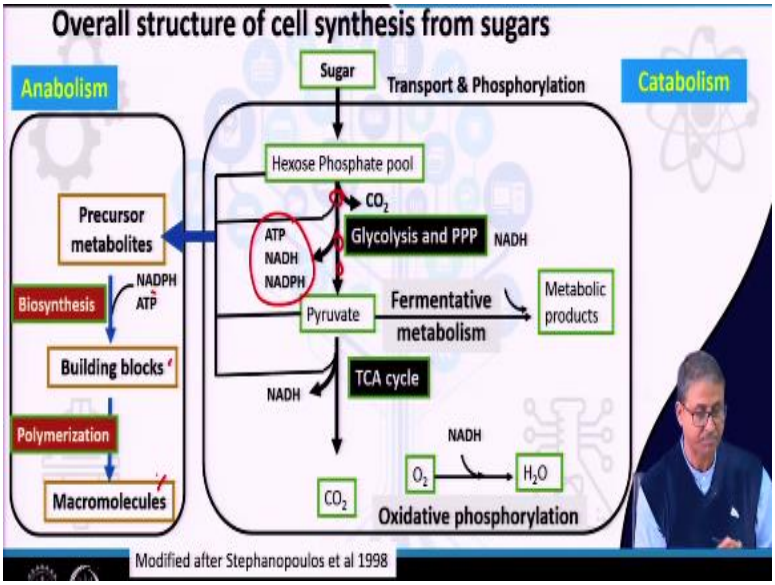

Now overall structure of the cell synthesis because we are more concerned about the synthesis of the macromolecules because that is often one of the major interest in metabolic engineering point of view.

So we have seen these catabolic, set of catabolic reactions where a number of biochemical reactions including the glycolysis, pentose phosphate pathway, the TCA cycle is enabling the production of these major metabolites which are the substrate for the anabolic reaction and also the energy resources like the ATP and the reducing equivalents.

And these set of reactions, the whole bunch of catabolic reactions are actually connected to the anabolic reactions, because the precursor metabolites are synthesized out of the many metabolites into metabolic intermediates, which are being produced over here. And with the input of the energy and with the input of the reducing power, which are produced from the catabolic reactions, the building blocks are produced and then building blocks are assembled into the different macromolecules.

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For today's class, we have basically used the metabolic engineering our textbook and a few review papers by the review paper of Nielsen and Keasling published in Cell that is engineering cellular metabolism and partly we are also going to use the Prescott's microbiology book by Willey et al. Thank you.