Metabolic Engineering
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Lecture - 04
Essence of Metabolic Engineering - Part C

Welcome back to today's lecture on essence of metabolic engineering part three.

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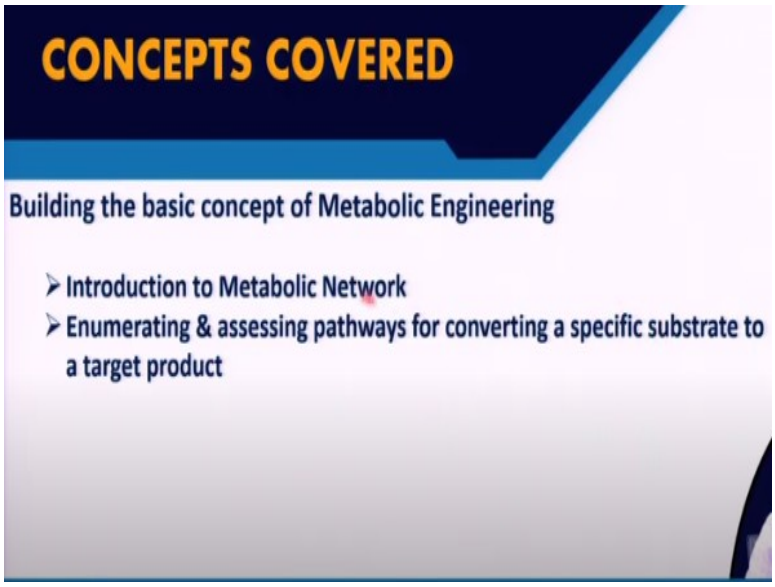

In today's lecture, we would be continuing our discussion about the basic concept of metabolic engineering. Today we are going to emphasize on metabolic network and how the metabolic reactions which are present within biochemical pathways are elucidated particularly, what are the steps of enumerating and assessing the pathways within each of the metabolic processes going on in living system.

And those which are involved in converting specific substrate to a target product. In this reference, I would like to emphasize upon the very definition of metabolic engineering wherein we have seen that metabolic engineering has a very unique attribute and that attribute is targeted development of the cell towards a specific product or a specific cellular properties.

So there must be one or more than one metabolic reaction involved towards the production of a specific target molecule or target compound out of the specific substrate, which is provided to the cell. Now during today's lecture, we are going to

understand how these different reactions which are possibly involved in converting a specific substrate to a target product are enumerated first and then they are assessed.

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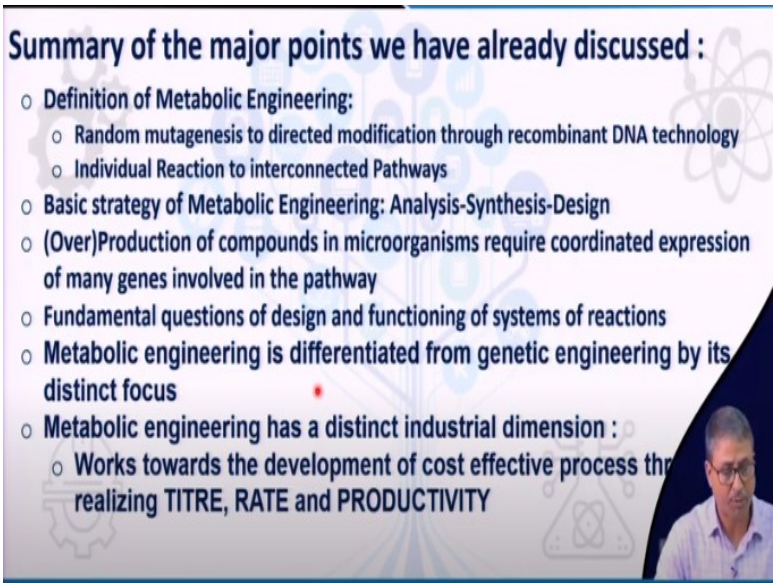

And this will be our continuation towards understanding the broad steps and aspects of metabolic engineering which we are doing during these last couple of lectures.

And we have already defined the metabolic engineering and we have understood the difference between the random mutagenesis to metabolic engineering and how this particular research area or particular subject has progressed out of a very random process towards directed and specific modification of cellular system through recombinant DNA technology based processes.

And we have also exposed to some kind of ideas that how individual reaction centric views are need to be revised and need to be looked into with respect to the interconnected nature of the metabolic pathways. We have understood the three major steps or strategies to be taken in the metabolic pathways which are analysis, synthesis and design.

And what are the major requirement with respect to the microorganism towards the production of specific compounds or overproduction of specific compounds are already discussed. The difference between a routine genetic engineering procedure to metabolic engineering is already mentioned. But with respect to that, I think today's lecture will illuminate us that how metabolic engineering has a unique nation or

unique notion towards developing a particular metabolic process or metabolic pathway.

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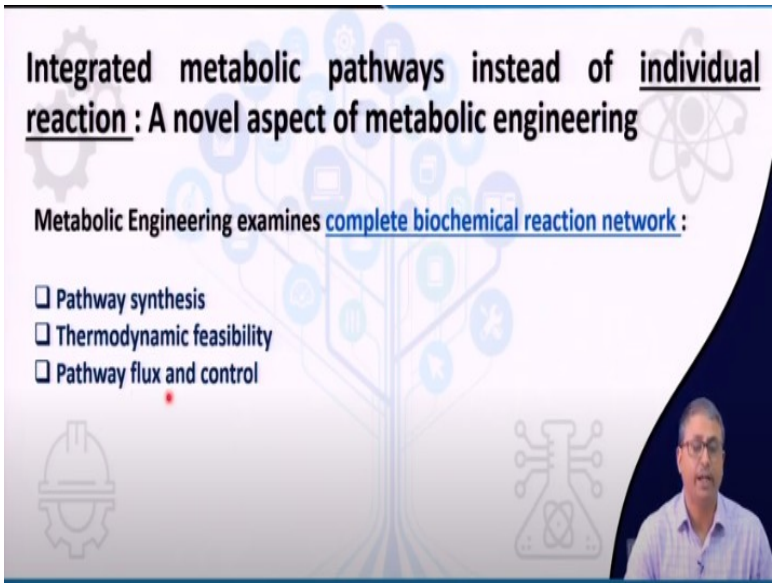

Now while we have discussed with respect to the two processes, which are very important towards development of metabolic systems or any product or process development, those were analysis and synthesis and followed by design. Now with respect to this synthesis part, one novel aspect of metabolic engineering has emerged that the metabolic pathways are always integrated.

So the novel aspect with respect to metabolic engineering is this integrated nature of metabolic pathways instead of individual reaction. And this metabolic engineering examines the complete biochemical reaction network.

So it is to be clearly understood that instead of individual reactions, individual reactions or reaction are always very important because these are the reactions which are involved in converting a particular substrate to a particular product and a particular product could be an intermediate or kind of a substrate for the next reaction.

So during this integration of metabolic reactions, we will eventually develop or tried to develop an understanding about the interconnectedness of the metabolic reactions and metabolic pathways.

And during this process, we will emphasize on pathway synthesis that how these individual pathways are actually present and they are synthesized, the thermodynamic feasibility of each of these different reactions and different pathways which are involved in a particular product formation out of a substrate or specific substrate. And we will be also getting an exposure to pathway flux and the control.

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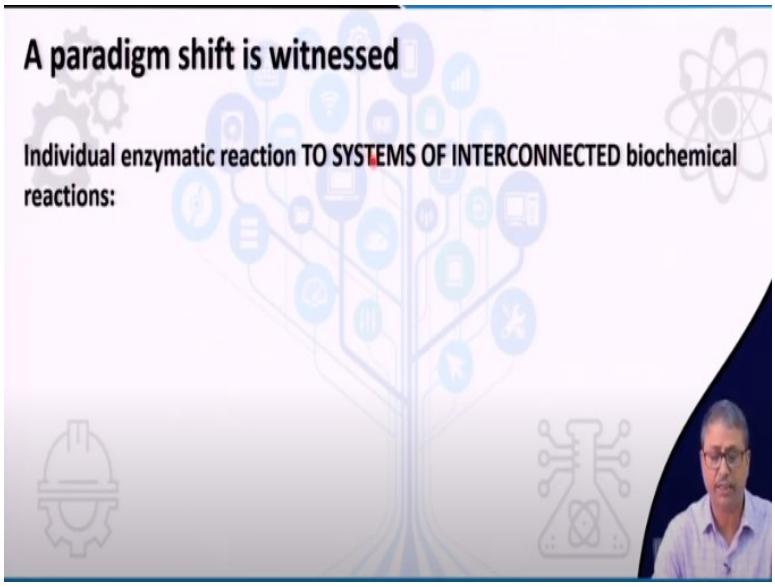

Now as we start discussion about this interconnectedness of metabolic reaction or metabolic pathways, we are witnessing actually a paradigm shift within the concept of metabolic process of metabolism as such within the living system. And this paradigm shift is basically from individual enzyme centric reaction to systems of interconnected reactions.

So from individual enzymatic reactions, which are basically converting a particular or specific substrate to a specific product. So these are the individual reactions and from these individual reactions, individual reactions will still be very important part of metabolic engineering, understanding the individual reactions, the individual substrate, individual product, the enzymes, the genes, the regulatory factors involved in individual enzymatic reaction.

But we have moved forward from the individual enzymatic reaction centric view or understanding towards an understanding of systems level interconnected nature of the biochemical reactions.

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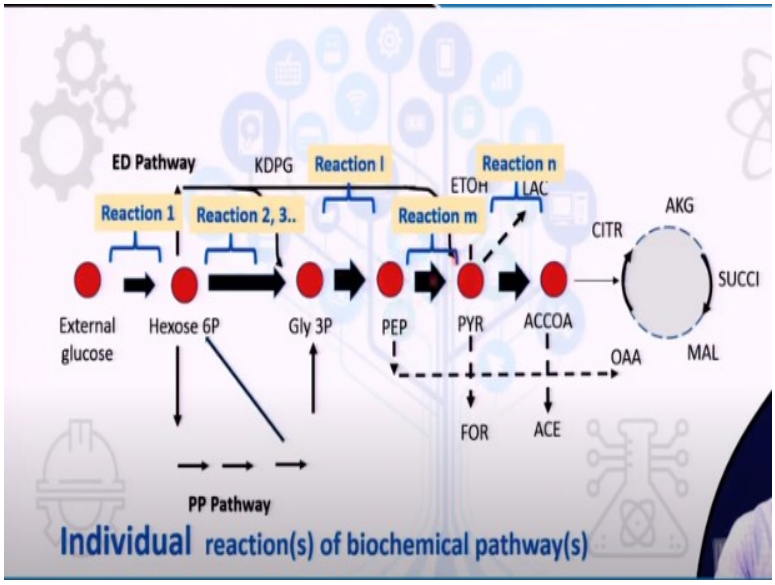

Let us take this example. So here glucose molecule is converted to acetyl-CoA through a part of the central metabolism which is called the glycolytic pathway, the glucose is converted to glucose 6-phosphate and then glucose 6-phosphate to fructose 6-phosphate and then glyceraldehyde 3-phosphate. Glyceraldehyde 3-phosphate is oxidized to phosphoenolpyruvate.

And phosphoenolpyruvate is further metabolized to produce the pyruvic acid. And this pyruvic acid is now subjected to the conversion of acetyl-CoA and acetyl-CoA enters into the TCA cycle. This is the set of reaction or a set of reactions that we see here within these glycolytic reactions. And here we have a set of reactions which are basically the TCA cycle.

Now we should also indicate over here that this particular reaction or set of reactions, which is representing the glycolytic pathway may be constituted by 10 or so reactions and of course, all the reactions are not shown over here in this particular picture, because this is just a diagrammatic representation of the set of reactions.

But the point also is to be highlighted that some of these metabolites are intermediate products like hexose 6-phosphate like fructose 6-phosphate or fructose 1,6 bisphosphate or even glucose phosphate, glucose 6-phosphate. These are also involved in other pathways like the hexose 6-phosphates could be the substrate for the ED pathway, which is very one of the very important pathways in microbial system or the other very important pathway which is the pentose phosphate pathway.

So these glycolytic reaction which is supposed to be discussed or emphasized only with respect to conversion of glucose to acetyl-CoA or towards the pyruvic acid, which ultimately connecting it towards the with the TCA cycle.

But eventually we understand that this is not a single pathway, rather it is a simple example of interconnected pathways where the ED pathway and PPP these are the two important pathways connected over this hexose 6-phosphate and the TCA cycle or the citric acid cycle, which is a complex set of reaction pathways are connected to these glycolytic pathways.

So this itself gives us a kind of sense of initial sense of or initial view of the how the metabolic reactions or metabolic part of set of metabolic reactions could be interconnected between each other.

But the major point of interest is this that while we try to investigate these particular reactions or this particular pathway, we might consider these individual reactions like reaction 1, reaction 2, 3, 4 or reaction l or reaction, these are arbitrarily named as l, m or n. So we can investigate or we can study the importance, the process, the stoichiometry, the synthesis, the thermodynamic feasibilities the enzymes all possible aspects of individual reactions.

Of course, we can do that if we are interested to know how this process is actually happening. And moreover, from metabolic engineering or a product development point of view, if we are interested to produce more pyruvate in the system, we might be interested in reactions which are directly involved in pyruvic production like this reaction where phosphoenolpyruvate is converted to pyruvate.

So this particular reaction might be very important in order to understand the pyruvate production inside the cell. And pyruvate production inside the cell might be an important objective of any metabolic engineering process development. But what is the paradigm shift because, we were talking about the paradigm shift with respect to the interconnected nature of the metabolic reactions.

Now we may consider that okay this reaction is our target reaction, we are trying to improve upon this reaction. We want to make this reaction more efficient and try to make or we are hoping that we will be making more pyruvic acid out of the glucose which is taken up by the cell. So one can do that and actually, we were doing this for a long period of time.

And as I have mentioned during the early days of metabolic engineering when the metabolic engineering as a scientific term or scientific field or research field was not developed; so maybe early 70s particularly. So we were discovering these pathways and investigating all the details of the pathways.

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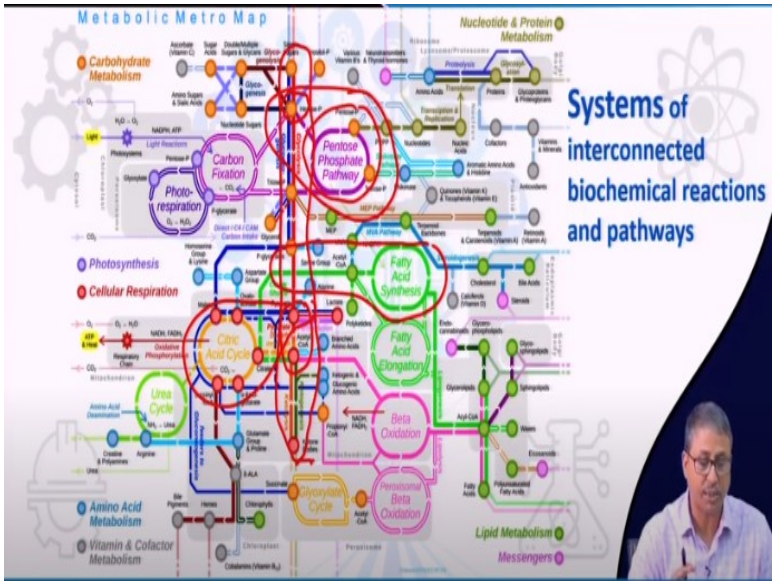

But we need to understand that these type of reactions, which we are talking about that pyruvate to acetyl-CoA. So pyruvate to acetyl-CoA if we try to consider over here, so for example, if I want to show you the pyruvate to acetyl-CoA, so this could be like, this part of the reaction is only the pyruvate to acetyl-CoA reaction. But is it enough?

So if we just highlight this part, that pyruvic acid to acetyl-CoA this is what we were targeting. But, if we look carefully, that we will find that this is actually a small part of a very complex and interconnected set of metabolic reactions that starts from the glucose uptake where the glucose is taken up.

And then we have a number of associated pathways which are connected to this hexose phosphate which is formed from the glucose molecule which is taken up because we know very well that following the uptake of the glucose either by the PTA system that is the group translocation system or by direct transport of glucose. The glucose is phosphorylated immediately.

So these phosphorylated hexose sugar might be used or must be used by other pathways like the pentose phosphate pathway particularly, or it will also process through this main reaction series of the glycolytic reaction. So the point over here is that instead of focusing only on these or we can continue focusing on this particular reaction, which is basically the point of interest or the reaction of our interest.

But we must understand or try to understand that this reaction is part of an entire set of reaction, which is basically this entire glucose uptake to complete metabolism of the glucose and also it is connected to a number of associated reactions including the pentose phosphate pathway and fatty acid synthesis reaction.

And of course, we have the citric acid reaction and then amino acid synthesis and all those are also present. So the from the individual reaction centric view to kind of interactive or inter connected nature of the reactions, this is one of the major developments happened, which is considered as the paradigm shift towards understanding the metabolism of the cellular system.

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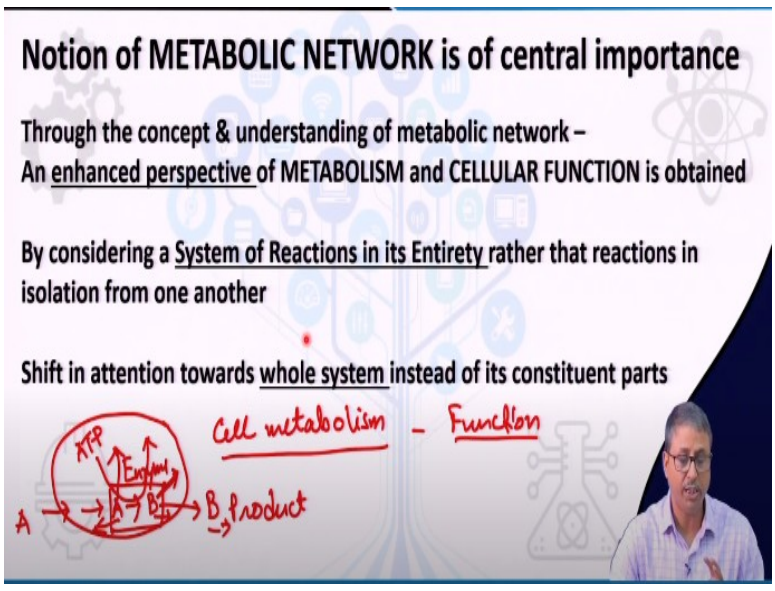

Now as we try to develop this, the notion of metabolic engineering has improved or modified. The notion of metabolic engineering, which is having a central importance towards ultimately developing the metabolic processes or so.

So here through the concept and understanding of metabolic network, so from the interconnected nature of the reactions, which we initially discussed very briefly that from individual reaction to interconnected nature of the reactions and then interconnected nature of the metabolic pathways an enhanced perspective of metabolism and cellular function is obtained, okay.

So basically instead of considering the entire process as individual reaction centric understanding of the metabolism to entirely a different understanding will emerge when we look into the interconnected nature of the cellular function. Now by considering a system of reactions in its entirety, so that is one of the most important outcome of this interconnected nature of the metabolic reactions or metabolic pathways.

That is the system of reactions in its entirety. So we might we may be interested in studying one particular reaction. But so let us take an example that within a cell, we were starting a particular reaction where A is converted to B, okay. And we might be interested into the product, which is B. So we were interested in this, this is our product. So we were interested in this B.

So we were studying this particular reaction and the enzyme which is involved in this. So this is when we are studying this we are sometimes ignoring all these steps which are upstream and downstream connected and might be involved in utilizing the substrate which is the A or the product which is B or different cofactors like the NADH or FAD or maybe the energy like ATP and other things.

So we are not considering all those things, when we were considering only a particular reaction. So about the cell metabolism, that how the cell is, about the cell metabolism or its function, we are going to have an entirely new set of view or new set of understanding when we try to see that how the substrate A is actually taken up by the cell.

And what are the other demands of the substrate A apart from the enzyme which is catalyzing the reaction A to B. So there might be some competing enzymes sitting over here who are also interested in this particular substrate A, and also there might be some other utilities of this product B.

Instead of releasing it outside there might be some other enzymes who would be interested to convert B and produce certain other products which are utilized by the cell. So when we look into the entirety of the system, like all the reactions, which are involved in the specific process that we are interested in, we actually reach a point which is called systems of interaction.

So that is a very important understanding which is emerging out of this kind of analysis at these kind of thoughts. Now as soon as we try to emphasize more on the systems of reaction, we shift towards a whole system based studies or analysis instead of the constituent parts.

Now if we look into the timeframe of these kinds of discoveries and the evolution in the understanding of these thoughts, we would be very happy to see that at the same time when we are about to change our notion or about to change our understanding about how the cell metabolism or cell function should be investigated or looked into, there is a whole lot of change going on towards the omics based concept.

Like the entire genome of the organisms are getting sequenced, and the entire transcript or the mRNA pool or the entire set of proteins, it is a proteomes are being analyzed. And the entire set of metabolites which are produced by the cells are being analyzed. So the methods and concepts are also developed during the same period of time, when we are trying to understand the whole system based understanding of the metabolism or metabolic processes within the cell.

So our intention towards getting a better understanding about the metabolism in its entirety is found to be highly supported or in synergy with the developments that happen in the whole genome sequencing as well as the analysis of the data. And also

as we will discuss maybe shortly about the flux analysis and other mathematical modeling of the entire set of processes happening inside the cell.

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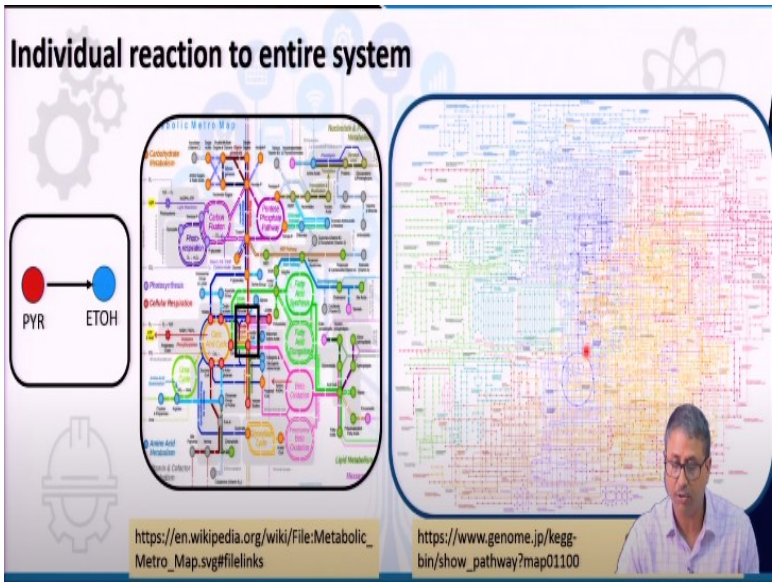

So ideally, we are moving from an individual reaction, centric approach or the individual reaction to an entire system. So originally we were interested maybe interested in reaction like these that pyruvic acid to ethanol because ethanol was a very important product of microbial system for its multiple uses.

So from that we understood that this ethanol, pyruvate to ethanol production could be a part of this glycolytic reaction, which is highly interconnected. So we may be interested to interconnected pathways, but very soon with the help of all these omics technologies and metabolic flux analysis as well as the modeling tools, we came to know that this particular reaction which is over here, might be somewhere here only, and you can see this is the TCA cycle.

So we need a very complex and integrated metabolic jungle you have only the small part that you are interested or we are interested in order to maybe producing more ethanol or maybe producing more pyruvic acid. So this change in our approach that we are still interested in producing more ethanol or more pyruvic acid, but the reaction which is going on is actually a part of a very big and very vast or robust systems of metabolic systems of a pathways or systems of multiple reactions. So this is considered as a paradigm shift.

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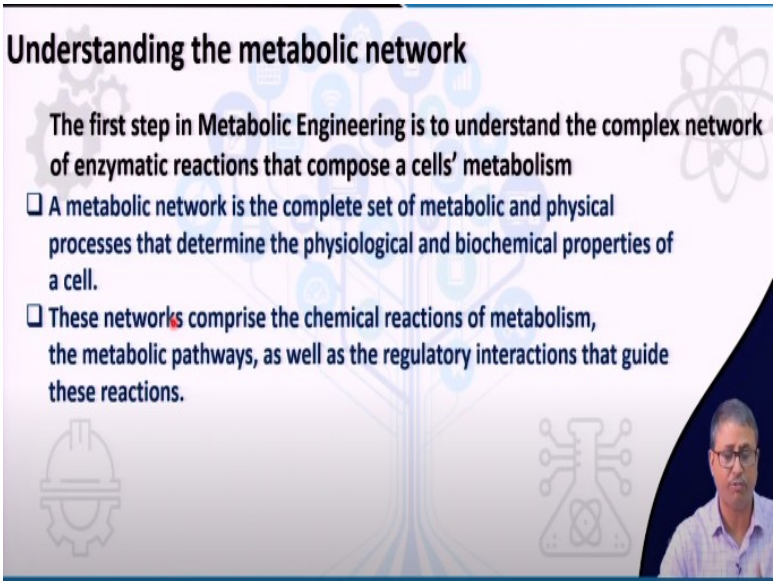

Now as soon as we realize that there is a kind of a great deal of change in our understanding from individual reaction centric view towards some kind of an entire system based view of the cellular metabolism, the metabolic network concept evolved. In fact metabolic network concept was there before that.

But our understanding about metabolic network was improved significantly, once we realized that this interconnected nature of the reactions and with the advent of all the omics based and all the flux analysis tools and the mathematical modeling that also will be discussed in the other lectures of during this course, the metabolic network concept improved significantly.

Now the first step in metabolic engineering is to understand the complex network of enzymatic reactions. So once we fix our target that this is our target, this is our product or this is our property that we would like to improve with respect to a particular cell or particular system. The first step would be to understand the complex network of enzymatic reactions that composes cells metabolism.

Like in the previous slides, we were just visualizing some of the complexities or some of the intricacies of the reactions, multiple reactions and multiple pathways. So we need to understand the complex or complex network of enzymatic reactions or the complexities of the reactions. Now a metabolic network is the complete set of metabolic and physical processes.

So it includes all the metabolic and physical processes that determine the physiological and biochemical properties of a cell. So when we try to build a network, the network might be with respect to a particular product formation, but ideally, it should include all the other properties including all the other metabolic processes, which are actually connected directly or actively or passively or indirectly, to this particular reaction.

And the physical processes which are actually influencing or likely to be influencing this particular reaction, for example, the solubility or the pH or the uptake of the glucose or the oxygen concentration of the medium. There are many other parameters. As we proceed, I think we will be able to discuss all those points thoroughly.

So the point over here is, the metabolic network is actually is a kind of another level of understanding where all the metabolic and physical processes that determine the state of the cell, the physiological state and biochemical properties, these are the different dimension of the cellular properties, okay.

So one biochemical properties may be assays by biochemical assay like assaying different metabolites, different enzymes etc. But the physiological status could be monitored by growth and other cellular activities. Now these networks, these metabolic networks that we would like to build should comprise the chemical reactions of metabolism.

So individual all the reactions which are taking place, so they will be included in the while building the metabolic networks, metabolic pathways or reactions, the pathways as well as the regulatory interactions, all the regulators, the promoter, the repressor and there are cofactors and other associated environmental factors, which might be influencing or influencing these metabolic reactions are to be included because they are guiding these reactions.

So the networks will basically build on the reactions, the individual chemical reactions. The reactions are joined to form the pathways. So the pathways and the regulatory interactions. It is very important because the pathways are not to be

considered only in isolation like only the substrate and the product and the enzyme. The enzyme will only operate under some ambient condition.

And the solubility of the product, the transport of the product, the role of different cofactor, role of energy substrates, role of reducing power, all these factors, including the expression of the related proteins like the genes, which are controlled by different promoter and other gene regulatory system must be included.

So a metabolic network is a kind of representation or a level of understanding where we get a complete set of the information integrated into that.

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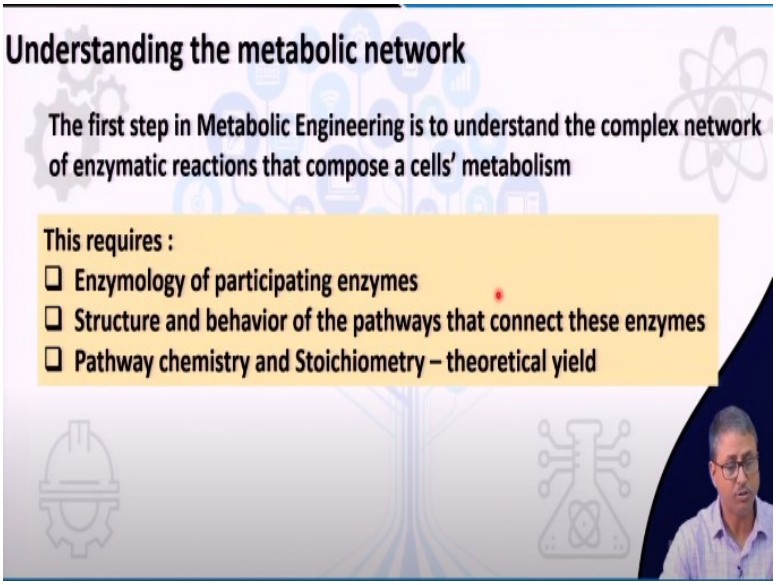

Now when we wish to get this complete set of information and we try to build the metabolic network we require the enzymology of participating enzyme. So all the enzymes which are involved, there may be 10 enzyme, maybe 20 enzyme, this maybe 200 enzymes involved. So enzymology of participating enzymes must be elucidated, must be studied. Structure and behavior of the pathways that connect these enzymes.

So how these enzymes are involved in connecting particular substrate to a particular product and then a particular product might be a substrate of another enzyme. So the entire map of the reactions, how the reactions are interconnected, the pathway chemistry and the stoichiometry that is the, it is connected towards the theoretical yield of the products, everything need to be elucidated very clearly.

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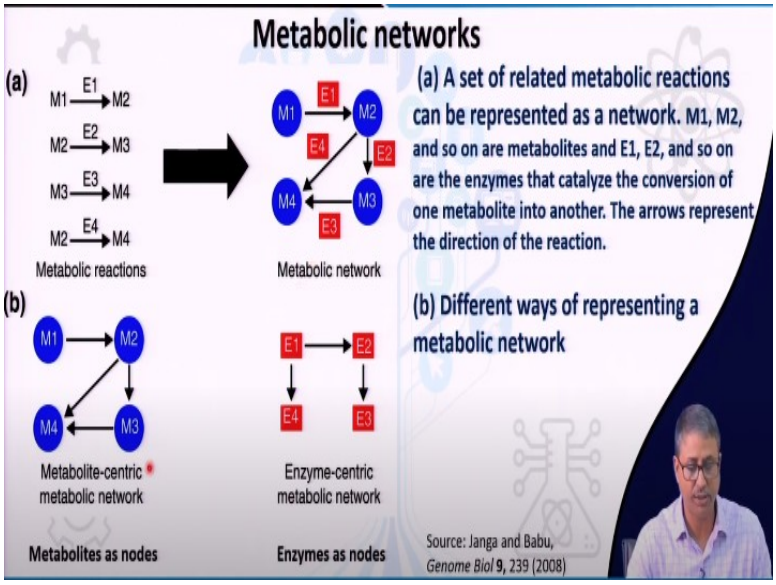

Now here if we present a very simple representation of this metabolic network development, where you can see a set of related metabolic reactions can be represented. So these are the individual reactions like M1 is converted to M2. Substrate M1 is converted to product M2. M2 is the substrate for another enzyme E2 converting it to M3. And thereby M3 to M4 and M2 can be also converted to M4.

So there are four enzymes which are catalyzing four metabolic reactions in a given system. So this is just an example of how the metabolic networks are actually developed or built in a very simple manner. So we can see that these reactions or these individual reactions can be connected like M1 to M2 and M2 to M3. And M2 can also be converting to M4. And M3 can also be converting to M4.

So this is a simple or simplistic view of the network with respect to four particular metabolites or four particular products and intermediates and substance representing a metabolite. Now this type of networks can be represented in multiple ways. So we are just going to present here two very general and very simple ways of representing the metabolic work. One is the metabolite centric metabolic work.

Now metabolites are all the kind of chemical compounds which are taking active role in the process like the substrate, the intermediate and the product. So these are called metabolites. So it could be substrate, it could be product, it could be intermediate.

So if we build a metabolite centric metabolic network, so this would be like this or we can also build an enzyme centric metabolic network which would maybe appearing like this that E1 is connected to both E2 and E4 because it is interconnecting actual two type of reactions. And then E2 to E3.

So you can see that how the network is different from this to this although they are all or both of them are actually discussing or presenting the data which is broadly presented over here. So metabolic networks can be presented with respect to different aspects like metabolite centric, enzyme centric gene centric because the genes are responsible for producing these the enzymes, the regulator centric and you can even incorporate all the factors who are involved in a particular metabolic reaction.

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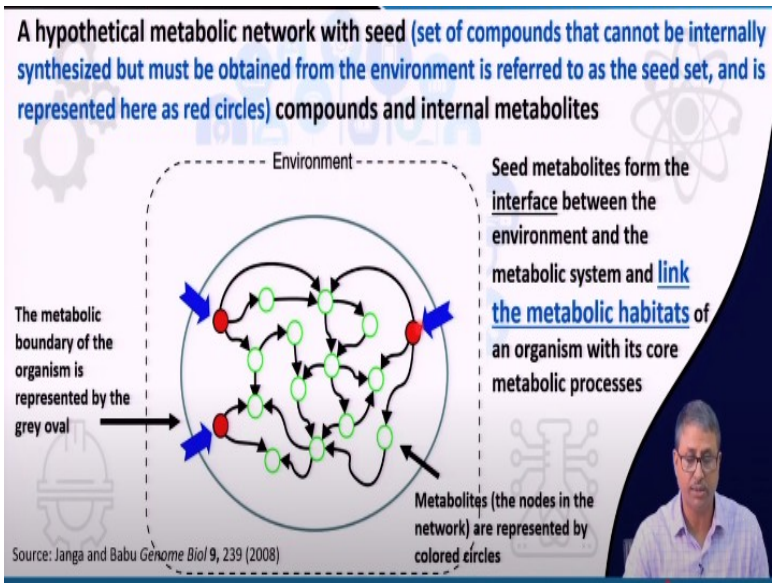

Like in this case, you can see a hypothetical metabolic network with some of the seed compounds and internal metabolites. Now the seed compounds are marked over here like the red dots. These red dots are seed compounds, because they cannot be internally synthesized, but must be obtained from the environment.

So basically they are the different type of molecules or compounds which are transported from outside of the cellular environment. So the environment plays a role over here because the environment must be supplying these important molecules who are part of the cellular network.

Now here you can see multiple things that the metabolic boundary is well defined in this case, because it has to be defined that how far we are going to consider this boundary. So this is the metabolic boundary of the organism, which is represented by these gray the oval shaped ring. And then, we see that these are the red dots, which are the external molecules not synthesized by the cells but taken up or transported inside the cell.

And they play a very important role as you can see, some of them could be substrate in fact. And then the green dots are the metabolites that is the products or intermediates or maybe the substrate for other reactions are represented by the colored cycle, the green color cycles. So you can see how a particular red dot which is actually externally derived molecule is fueling or supporting a number of other reactions and how these reactions are interconnected.

So this is just a very simple view of the metabolic networks. So the truly the metabolic networks if we try to incorporate more reactions and more processes going on inside the cell might be more complex as well. Now when we incorporate the seed metabolite, or seed metabolites, there might be multiple metabolites which are externally acquired and not synthesized internally.

And additional benefit is obtained, which is basically the link the metabolic habitats. Like these metabolites are not synthesized within the cell. They are taken up or transported inside the cell. So they form a kind of interface between the environment and the metabolic system.

So basically by incorporating this seed or these externally derived, externally produced molecules or metabolites, when we include them in the metabolic network, we are able to see or understand how a particular cell metabolism will be dependent on external environment because external environments are supposed to supply these seed metabolites.

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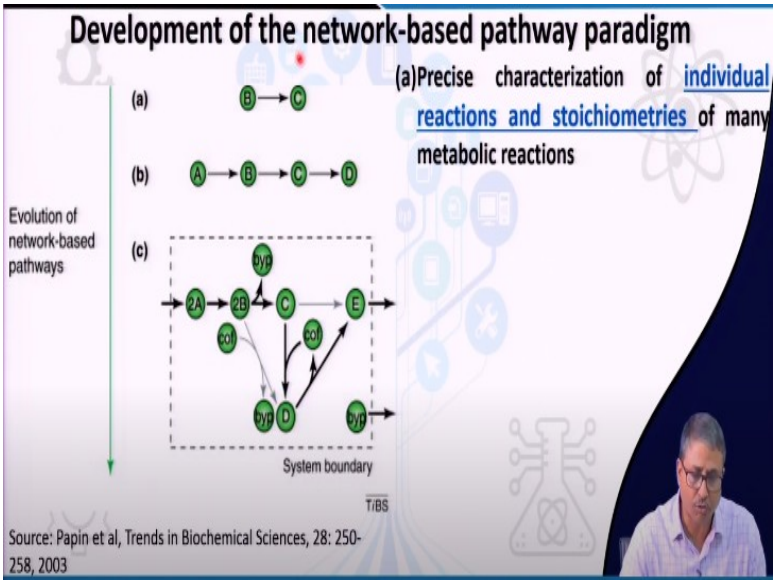

And finally, we are going to briefly talk about the development of the network based pathway paradigm because we have been talking about these individual reactions, and we want to go up to this the interconnected nature of the network. So during the process of revealing the individual reactions, precise characterization of individual reactions and stoichiometry of many metabolic reactions are first achieved.

So individual reactions are identified, individual reactions are characterized, their stoichiometries are determined and they are thoroughly understood including the enzymology of individual reactions.

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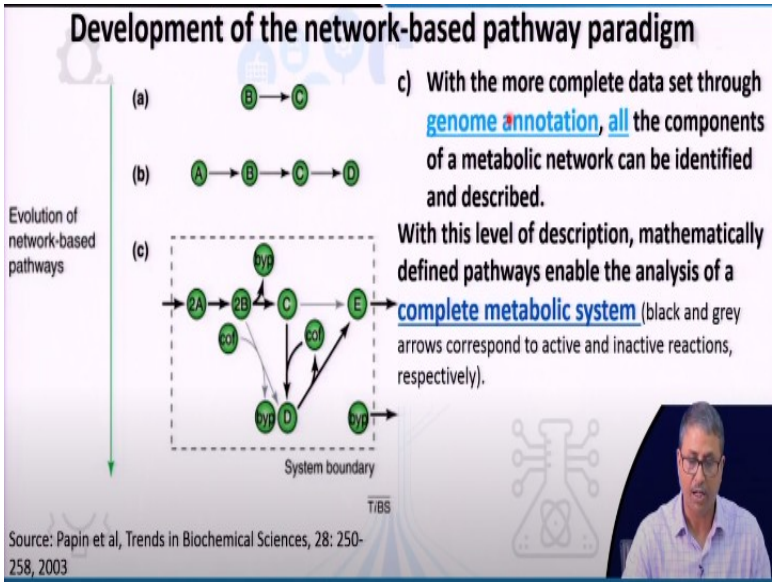

And thereby, we are we actually catalogue or we prepare a list of all the reactions or multiple reactions with shared metabolites like here you can see that there are actually

two more reactions. So one is the B to C. But there are two reactions which are sharing the metabolites like A to B. The B is shared between these two reactions. And then C to D, where the C is shared between this first reaction and this last reaction.

So we prepare a catalogue of all reactions which are possibly sharing the metabolites. And then, as we see that during these traditional pathways, all the traditional pathways like the glycolysis, pentose phosphate pathway TCA cycle, we have been successful, maybe decades ago, that these, the entire stage of metabolic reactions or the metabolic pathways are well elucidated.

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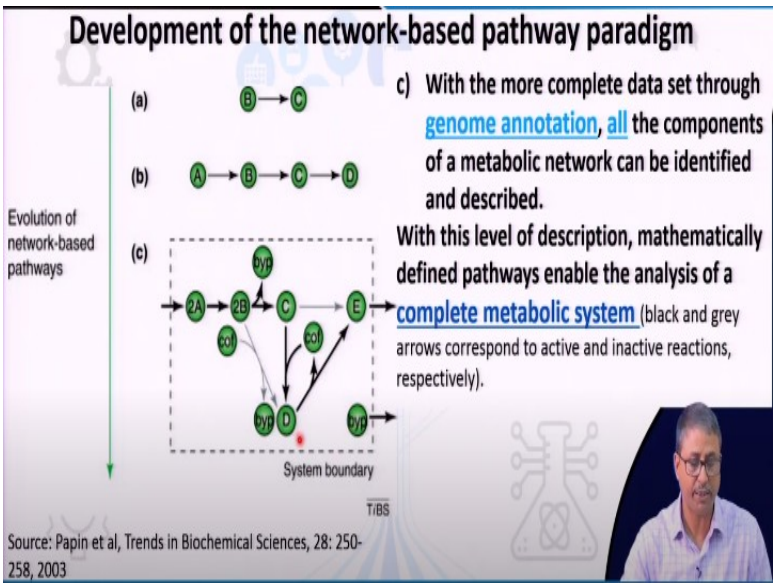

However, as we try to get more data, maybe through the omics, or mostly through the omics based approaches like as we go for genome or bacterial or microbial or other cellular systems whole genome sequencing, we are able to identify all the genes and all the enzymes that the organism is potentially able to produce and identify all the regulatory factors that which might be controlling the process.

Now all these components of out of this the genome centric data or genome based data, all these components of a metabolic network can now be included. First there are to be identified and they are to be included. And when we do that, so initially we were building this reaction, which were basically done by characterizing the shared metabolites.

But now we are able to include all the components which are possibly involved like you can see here that B has 2A and 2B. Might be because from the genetic or genome based information or the genome sequence based information. We could identify that actually A has multiple form, so first it is 2A. And then it is isomerized or maybe change to some other form where it is now 2B.

And 2B is actually converted to C and in between there is a byproduct which is also formed. In our other instances like when we are studying these, we were actually not able to find out that this byproduct would be forming. So it is only because of the study which is based on the entirety of the process, we are able to identify that there could be a kind of a byproduct which is also forming.

Now with this level of description, which is also parallely coupled or supported by mathematical modeling etc., so we are able to analyze the complete metabolic system. So like this in this case, the simple schematic diagram represent the same reaction like A to D. Now here you can see that how actually A is converted to D and what are the requirement of cofactors, what are the requirement or the potential reactions where the different byproducts are produced.

And how the different substrates are actually processed and how they are actually interconnected.

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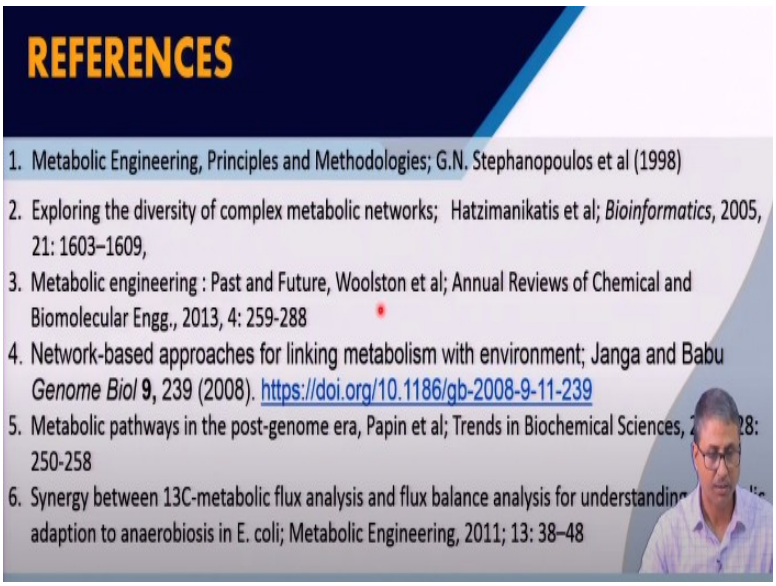

Okay, so during this particular part of this lecture, we have covered the following literature and we of course, followed the textbook which is the metabolic engineering textbook, but some additional reference papers are also used.

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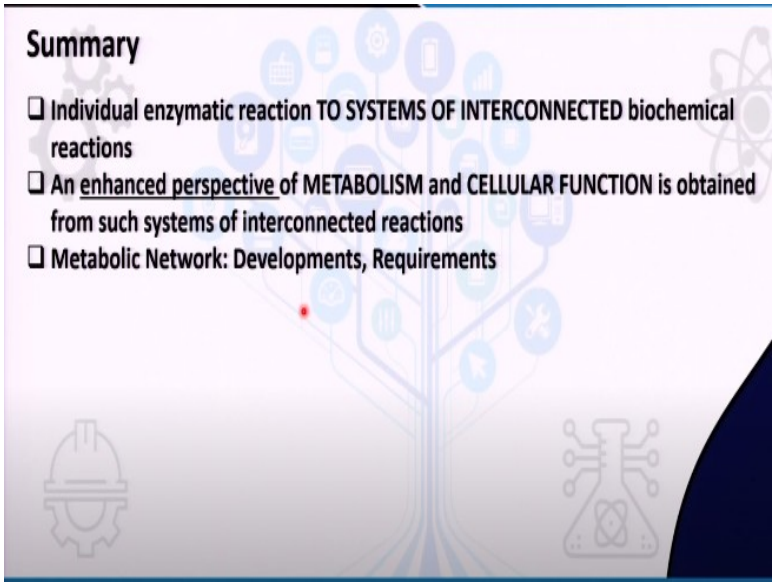

And in summary, in this lecture, we have discussed the individual enzyme reaction to systems of interconnected biochemical reaction and thereby an enhanced perspective of metabolism and cellular function is obtained from such systems of interconnected reaction. And finally, the metabolic networks which are basically understood in a very fundamental or preliminary levels and their developments that how from reaction to the networks are progressed.

And some of the requirements before producing these or before coming into this metabolic networks are also presented. Thank you.