Interactomics: Protein Arrays & Label Free Biosensors
Professor Sanjeeva Srivastava
MOOC NPTEL Course
Indian Institute of Technology Bombay
Module 8
Lecture No 40
Challenges in proteomics

Welcome to mooc interactomics course.

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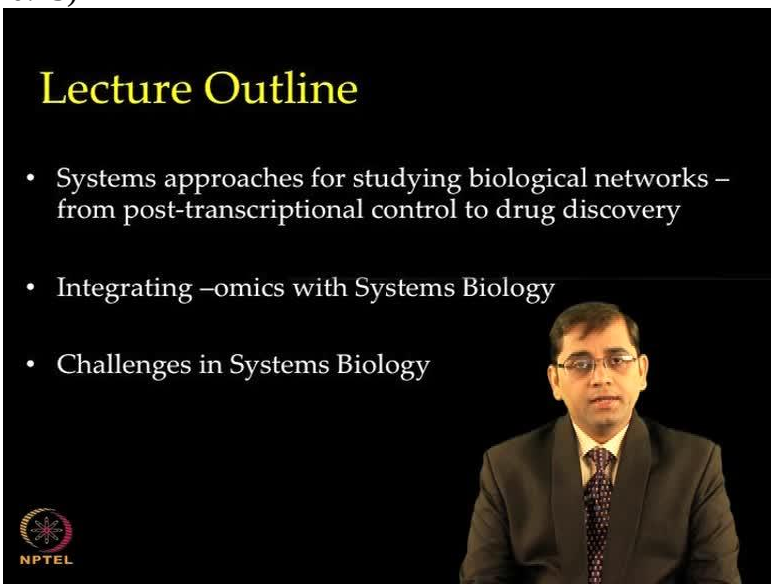

In our previous lecture we discussed various challenges associated with protein microarray work flow and its applicability to interdisciplinary domains of research such as assistant biology. Today we have professor Sarath Chandra Janga from Indiana university and purdue university Indianapolis IUPUI, USA and he would like to discuss with us systems approaches for studying biological networks from post transcriptional control to drug discovery. It is a pleasure to have him with us here to provide you an insight into this promising area of systems biology.

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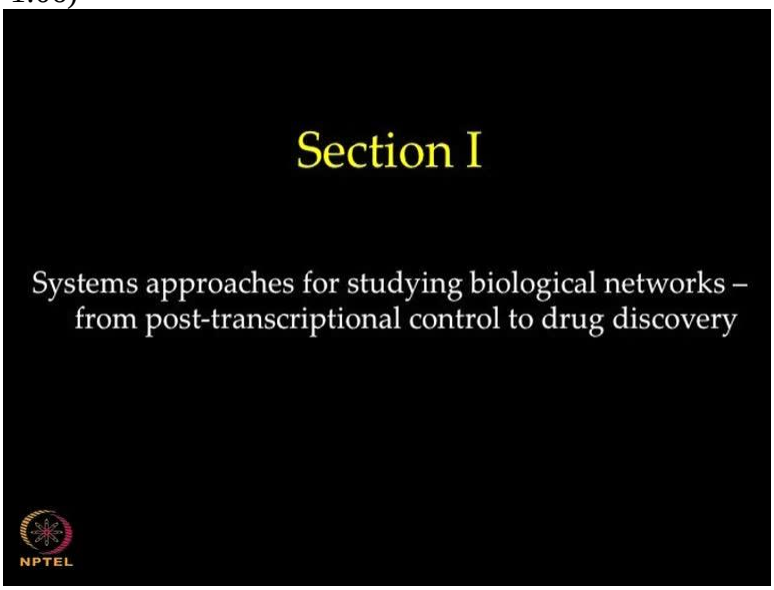

Prof. Sanjeeva Srivastava: So as we have been discussing about need for studying proteomics and systems biology. There are lot of information available at the transcription and translation level and often there is not good correlation between RNA level and the protein level. So today it will be interesting to talk about systems approaches for studying biological networks from post transcriptional control towards the drug discovery. So I have invited professor Sarath for having a discussion and a short talk on this topic.

Professor Sarath Chandra Janga: Thank you Doctor Srivastava, it is my pleasure to be here to talk about some of the work that we have been doing and more generally the principles of regulation and how you can use systems approaches for understanding biological networks more generally. As as some of you might be familiar with the use of the concept of networks is increasingly becoming prominent in not just proteomics but also in genomics data and all kinds of high throughput data so today what we will be talking about is some basic introduction to the applications of networks in a biological systems and how it can be applied to understanding transcription regulation post transcription regulation and as well as to the proteomics data and at large, how this can be used to understand the the drug discovery, how can how it can be applied to the drug discovery concept.

According to the central dogma of molecular biology denying gives rise to RNA through the process of transcription.

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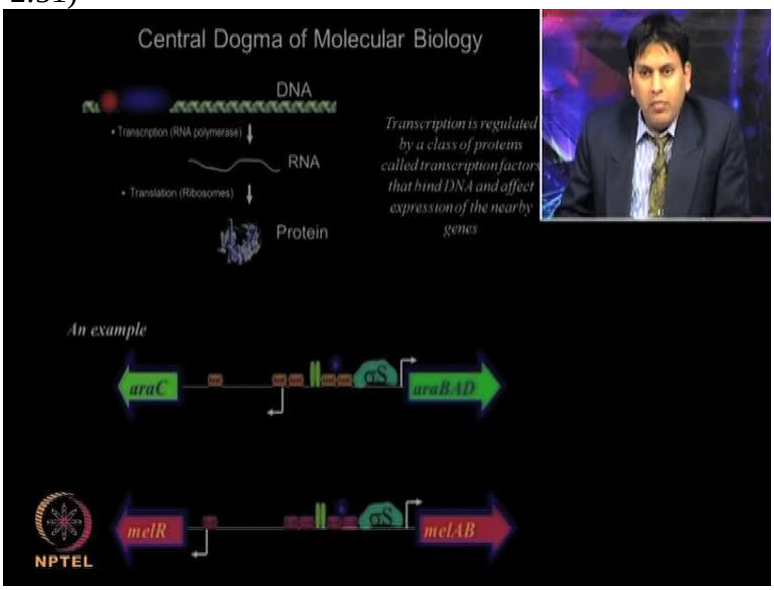

And this process is facilitated by the binding of the RNA polymerase as well as a number of other transcription factors which bind to the upstream regions of the DNA as you can see and control the expression. And RNA can give rise to protein through the process of translation and this is this happens through the process of translation with the help of the ribosomes.

Now in this process the proteins which are produced some of them can be classified as transcription factors which bind to the DNA and some others are classified as RNA binding proteins which can bind to the RNA and control the expression at the post transcriptional level as opposed to at the transcription level where transcription factors bind to the DNA.

Now as an example let us see the case of araC transcription factor in a bacterial genome such as E. coli. This particular transcription factor binds to the upstream regions of araBAD operon which encodes for the enzymes and the transporter responsible for the arabinose from the environment. Now the transcription factor araC not only binds to the upstream of araBAD but it can also bind to itself and control the expression as you can see from the small orange boxes which are shown as a representation for the binding sites.

Now what they suggest is transcription factor can out regulate bind and regulate its own expression or it can also bind to other genes controlling their expression. There are also cases there are many cases actually were transcription multiple transcription factors bind to the

upstream regions as you can see in the case in this case represented with the orange box as well as the blue box so blue circle where other transcription factors bind.

Now in addition to this binding of transcription factors as I mentioned earlier polymerase RNA polymerase also binds shown in with the with the green box green circle green oval box of that so that they can control the expression. Now there are other examples are also shown in this figure with melR regulator also doing something similar.

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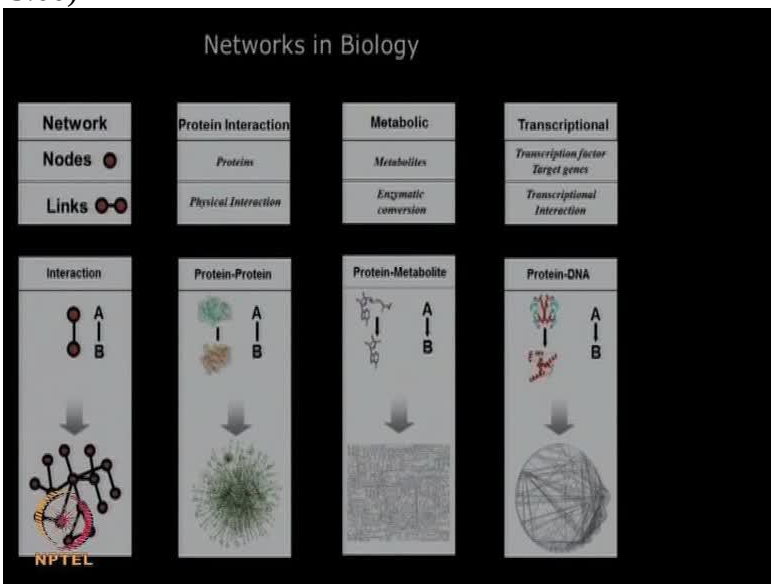

Now this is what we just discussed is an idea of how regulation happens from a biological view point. Now an increasing thing increasing amount of literature now supports the idea of networks in biology. So what exactly are networks? A network simple represent are represented as nodes and links or edges. These nodes can be biological entities and the links or edges are actually the associations between the between these entities.

Now there are number of ways you can talk about the nodes or the entities. So one common form of representation or protein interaction networks where proteins from the nodes and the physical interaction between these proteins forms the edge as you can see in this in the figure below.

You can have a representation of this networks in a fashion that is shown in this figure below. Now an alternate kind of network which is also studied in the literature over the last ten years or so are metabolic networks. In metabolic networks the metabolites form the nodes and the

conversion of one metabolite to the other forms the edge in this case. Now as you can imagine the conversion of one metabolite to the other is actually facilitated by the enzyme.

So the particular protein enzyme coverts a metabolite A to B and when you look at on a global scale and when you are looking at the conversion of number of metabolites one to the other and sometimes one metabolite can give rise to more than one set of metabolites. Such complex set of associations can be called as a metabolic network. Now the third kind of a networks which I will be elaborating in more detail in the next slides are transcriptional networks.

In transcriptional networks, transcription factors form one set of nodes and the target genes form other set of nodes. So as you can imagine what you are actually looking at in this case from a biological view point is the interaction of the transcription factor with the DNA and controlling of the expression of the downstream genes but in the context of networks what we are showing here what we are showing is the transcription factor and the target gene or operon whose expression is control.

Again in this case you can see that the protein A which is a transcription factor controls B but it may or may not be that B is a transcription factor and it also controls A. So that might be a case to case case and case to case specific and may or may not be having a reciprocal interaction. As we just discussed these networks are actually this the concept of network has been borrowed from physics and computer science where often these kind of networks are referred to as graphs.

And graphs are objects which are collection of nodes and entities. The nodes are representing the entities. It could be this entity these entities could be genes, proteins, small molecules, cells, organs at any level you can represent these entities. The interactions are associations between them are the links. Now as I am just mentioned there are different kinds of networks, the protein-protein interaction networks, metabolic networks, transcription networks.

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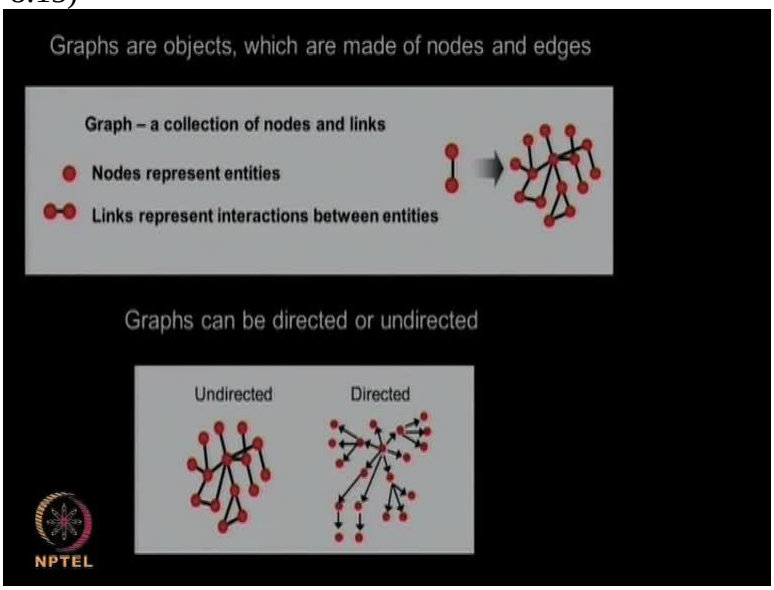

In the case of protein-protein interaction networks what we are looking at often is no directionality in such interactions and these are called as undirected networks. However, there are also directed networks such as transcriptional networks or metabolic networks. In these cases there is a flow of information ie where A controls B which should mean A is controlling A is regulating B. So therefore there is a directionality and these are often commonly studied as regulatory networks.

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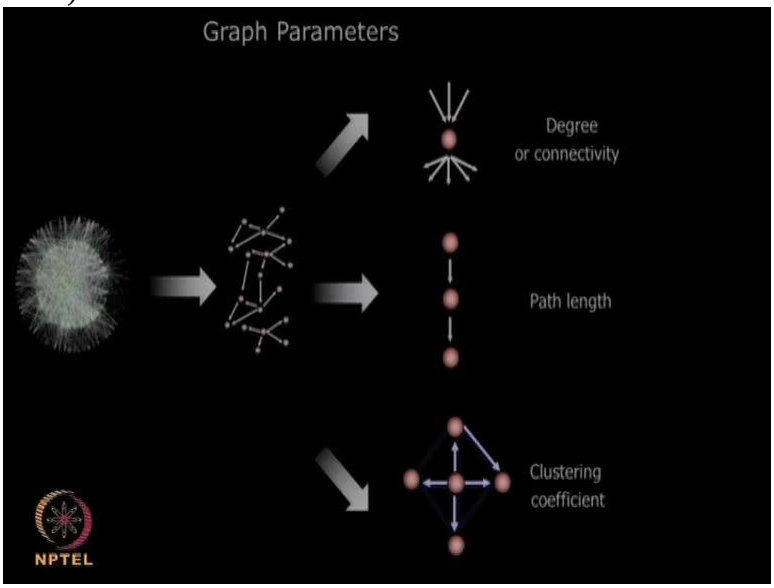

And we will talking in more detail in the next slides. However before we get into the more specific observations about the properties of these networks one set of common properties which are studied when you are looking at biological networks are degree, path length and clustering coefficient. Now often when you look into a network as such you do not have a clear understanding of the properties of the different nodes.

But when you look into the specific aspects such as in this case shown in this case as degree what it tells you is how many connections of particular gene, protein or node has in your network. So what we can say from the first example on the top is the degree of the node is 8, that means it is connected to 8 other proteins. On the second property is the path length what it is showing in this case if you cal is that the number of edges that you need to travel from one node to the other.

So if I ask you what is the path length between that first and the bottom node in this figure, you would say the path length is equal to 2. The third kind of property which often studied is a clustering coefficient. Clustering coefficient tells how often the neighbors of a given node are connected to what you would see in a completely connected graph. Let us let us look at more detailed examples.

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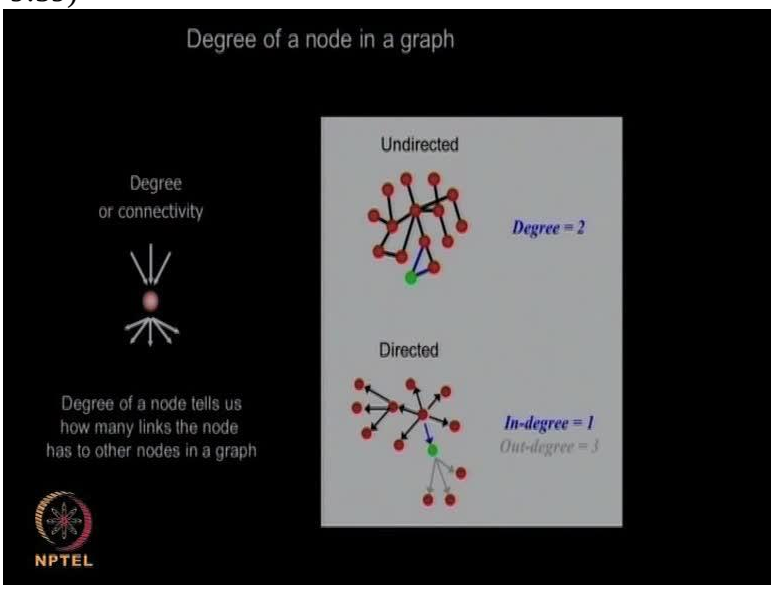

For instance if you are studying the degree of a node in a case of an undirected network such as in the examples shown in the top. The fluorescence node that is shown fluorescence color has a degree equal to 2. On the other hand a directed node example shown at the bottom has a degree equal to 4 because it is connected to 4 other nodes. However, what you can also say is there is an in degree and out degree and in degree is the number of incoming connections of a particular node.

So the green or fluorescent node here has in degree of 1. It also has an out degree equal to 3 because it is directing 3 other nodes shown in red color out there, so it is out degree equals to 3. Now you can also extend this idea of undirected and directed graphs and ask what is the path length of a node. Now as I mentioned the path length is referred to as the number of edges that one need to travel between two different nodes that you are interested. In the top network that you are seeing the path length between the two green or fluorescent nodes is equal to 2 as well as equal to 1.

Because the path that you can take can be different than the shortest path that you are looking at, however almost often unless otherwise specified when you are talking about the path length between two nodes it is a shortest path length. So the two fluorescence nodes have a path length equals to 1. However if you are asked what are the all the path lengths you would say there it has two different paths, one with a path length of 1, the other with a path length of 2.

In the undirected networks your definition of path length essentially does not change. So in the examples in the example that you see at the bottom the path length between the two fluorescent nodes is equal to 2.

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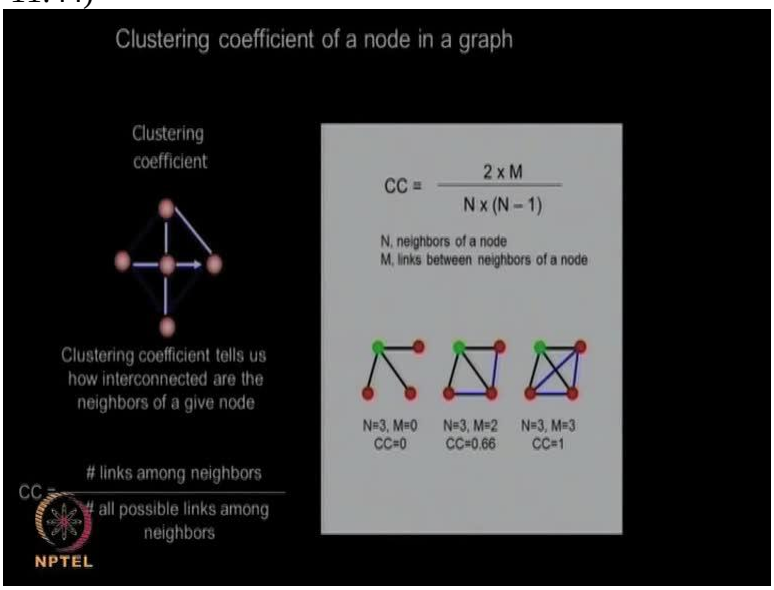

The other property that I was referring to previously is the clustering coefficient of node and clustering coefficient refers to the number of connections between the neighbors of a given node of interest to what you would see in a completely connected graph. Now let us look at an example, in this figure that you see there is the first example the fluorescent node the green node has three connections three red dots are connected to it.

However if you ask the number of connections between the red dots it is zero, there are zero connections between the red dots. But if they were fully connected, you would see that they will have three edges between them. So the clustering coefficient of the fluorescence node right now is 0 upon 3. Let us look at the second toi network, in the second toi network the fluorescence node has a clustering coefficient of 2 upon 3.

In the third case the cluttering coefficient of the fluorescence node is 3 upon 3 which is completely connected. So the clustering coefficient is equal to 1. Now more generally the formula can be brought up and can it can be written as if there are m number of interactions between the neighbors of a node of interest and there are n number of neighbors of a given node of interest, then it can be written as m upon n into n - 1 by 2.

So that would be defined as a clustering coefficient of that particular node. And when averaged the clustering coefficient of node on whole network scale it gives you an essence of modularity

of the network, the higher the average clustering coefficient the more likely is the network clustered or it can be decomposed into a specific modules. Another property that is of great interest in understanding biological networks is a scale free structure.

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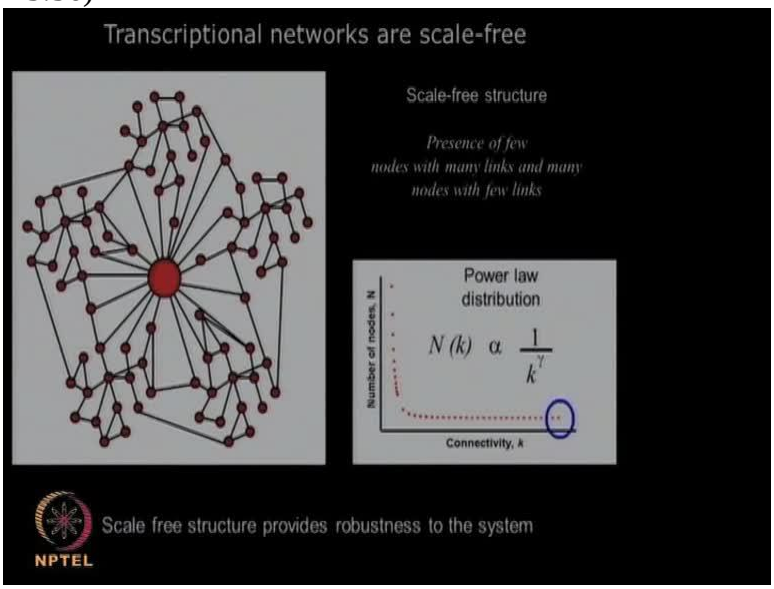

And while lot of biological networks are documented and shown to be scaled free, transcriptional networks are also documented to be scale free structures. So what exactly are scale free networks? Scale free networks correspond to the structure of a network where there are few nodes which are highly connected. For instance in the figure to the left in the network figure that you see to the left there is a big red dot big red node which is highly connected.

So but there are not many such highly connected nodes and there are many nodes which are very poorly connected. So in other words a scale free structure refers to network structure were there are few nodes which are highly connected and most nodes are poorly connected. Or more mathematically if you plot the connectivity of a node versus the number of nodes with a given connectivity you should see a power low distribution.

Or otherwise if you plot the log plot of the connectivity versus the number of nodes with a given connectivity you should see a negative slope of gamma as shown in this figure where gamma lies between 2 to 3. That is when you can call the structure to be scale free and the and the distribution to be a power low distribution. Now what is so special about this scale free

structure? Scale free structures have been postulated to provide robustness to the biological system.

Now what exactly is robustness? So robustness is the ability of a complex system a complex system such as a biological system to maintain its function even when the structure of the system changes significantly. Now let us look at example.

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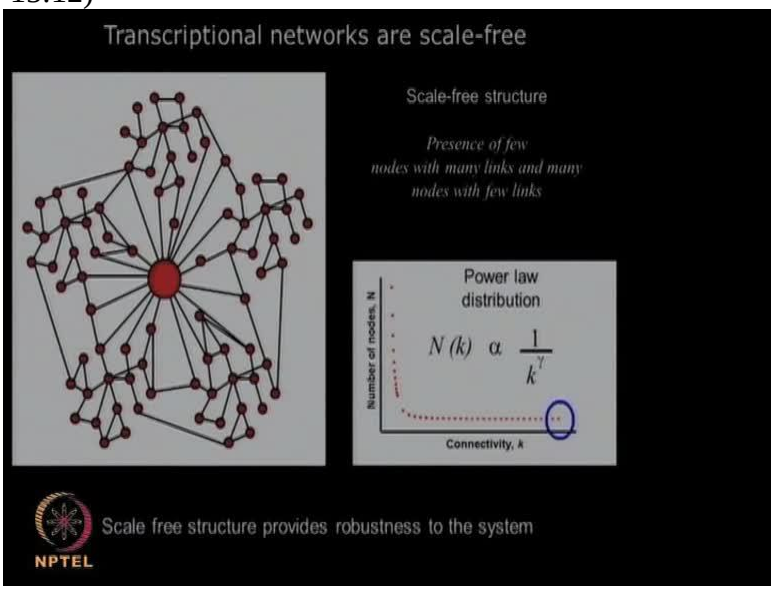

So in the network figure that you see, if you randomly put up any of these nodes you are likely to affect a small fraction of the network. However, if you target the highly connected node that is the central node which is highly connected you are going to disrupt a major fraction of this network suggesting that these highly connected nodes can be vulnerable to be the drug targets so if you are trying to inhibit the growth of a pathogen, you are likely to target this highly connected nodes because you are more likely to be able to crumble the biological system of the pathogen.

So and this has been increasingly gaining attention as a method of targeting drugs to this kind of these classes of proteins.

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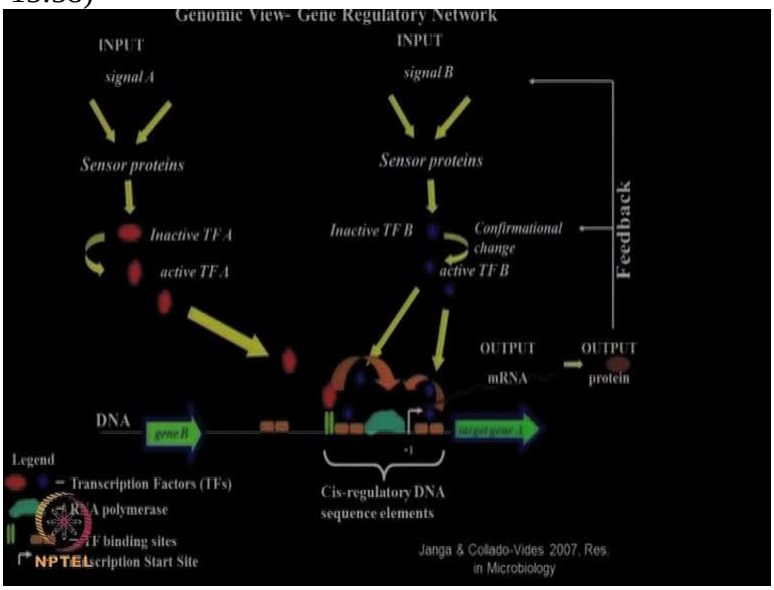

So as mentioned earlier we have been talking about regulation of a single transcription factor, but in the contexts of networks regulation is much more complex and what we are referring to is a combinatorial or regulation by many different transcription factors. Let us look at a specific scenario. So the slide the slide shown here shows a typical regulatory system in a bacterial bacterial organism. What you usually have is a set of signals which are sensed by the cell and these cell signals are sensed by sensor proteins.

These sensor proteins could be transcription transporters or this could also be (())(16:36) kinases and once this sensor proteins sense the signals from the exterior or even sometimes interior of the cell they can cascade the information to transcription factors. The transcription factors upon receiving these signals can change from active to inactive or inactive to active state.

And when this happens because of multiple sensor proteins, these transcription factors can change the confirmation and bind to the upstream regions. And shown at the bottom is a stretch of DNA where this transcription factors combine in a combinatorial fashion often and control expression of the target gene or operon. As a rule of thumb, if transcription factors bind to the upstream regions in the upstream of transcription starts site shown as + one that is where the transcription actually starts you often or stimulating the polymerase and enhancing the expression.

However, when you bind to the downstream of transcription start site you typically repress the expression of the target gene thereby blocking the transcription by the polymerase shown in theoval shaped polymerase symbol in dream. So based on these principles and together with the intercule with the transcription factors and the polymerase, your transcript is produced.

And once transcript is produced you can have mRNA and protein levels regulation which is not what we will be talking immediately now but all these levels together contribute to provide feedback and this is typically a system simple regulatory system that you encounter in bacterial organisms. But more complex systems more complex eucorytic gene regulation is much more complex and beyond the scope of our current discussion.

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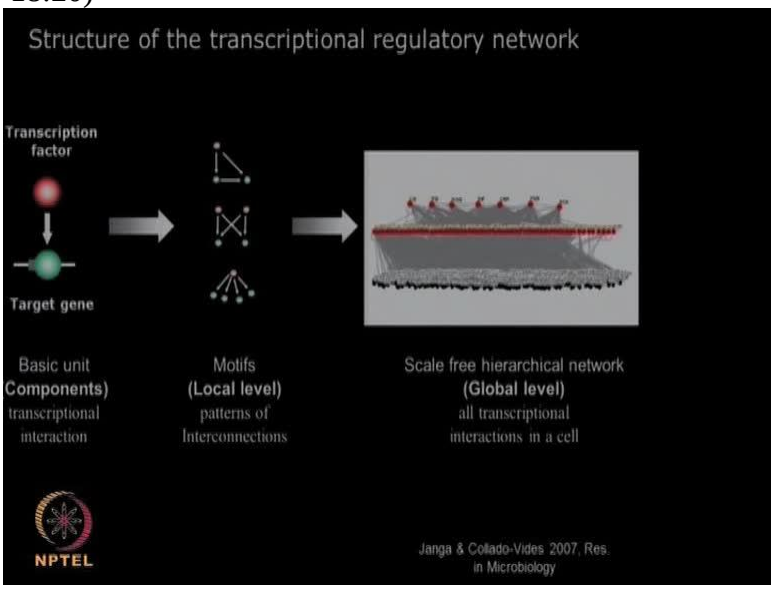

As discussed in previous slides a the basic unit of regulation is a transcription factor and a target gene who's expression is being controlled. Now on a different scale if you increase if you put together all the set of regulatory events between transcription factors and the target genes or operons, you construct a global transcription regulatory network.

And as I mentioned earlier this network is scale free structure scale free network, but in addition to this it is also hierarchical structure wherein what we are actually referring to in a hierarchical structure is there are set of transcription factors which are able to regulate a large numbers of

genes and there are set of genes other transcription factors which are also controlled by this global transcription factors shown at the top of this network structure.

And both the top layer and the second layer all of them together regulate the set of genes which are not essentially encoding for the protein coding which are not essentially encoding for the transcription factors. So in a way there are transcription factors which are at the top of the system, there are transcription factors which are controlled by this top layer and there are subsequent layers and the number of layers in such a hierarchical structure depends on the complexity of the system.

Now in between the top and the bottom layer the in between the left most figure of the basic unit and the right most figure there are set of substructures or sub graphs within the regulatory network which we call as motives. Motives are the set of sub graphs which occur more often than expected by chance and there were three kinds of regulatory motives that are identified in regulatory networks, one is the feet forward loop where there is there are two transcription factors the first transcription factor regulates the other two genes, the second transcription regulates the target genes.

The second kind of motive is multiple input module where there are two different transcription factors, both of them regulate two different target genes. The third is a single input module where a single transcription factor regulates a set of target genes. Now each of these set of regulatory motives have been shown to have specific functions and which would be beyond the scope of our current discussion.

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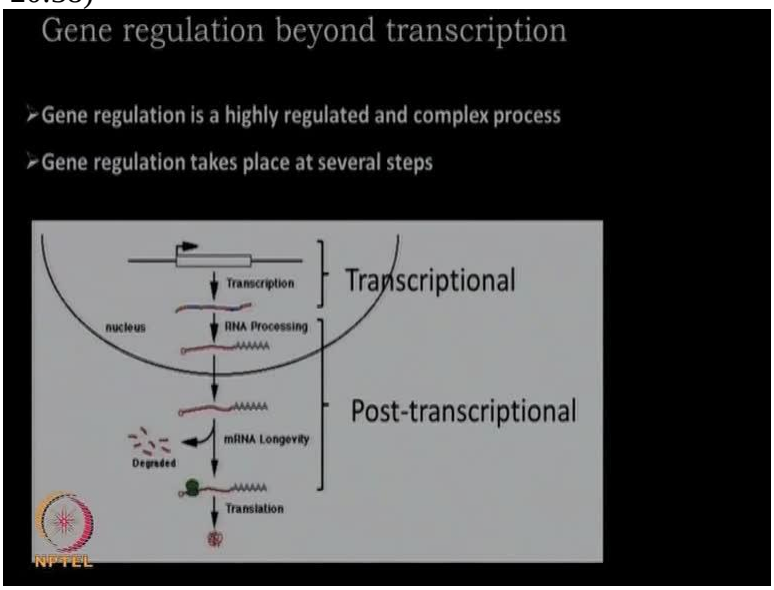

Now although the idea of regulation of gene expression the level of transcription has been documented for several years and then we have extensive understanding very little is known about the regulation of gene expression beyond transcription and it is only been recently been appreciated about the role of regulation at the post transcription level. Now most of this evidence for the reason why post transcription regulation is becoming important is coming from the lack correlation between mRNA and protein pools in moral systems.

Now there is now enough evidence to such is that these post transcriptional processeses are actually controlled by a class of proteins called RNA binding proteins among non-protein coding components such as micro RNAs non-coding RNAs. So RNA binding proteins are now known to be involved in controlling the RNA processing, RNA longevity as well as in the translation.

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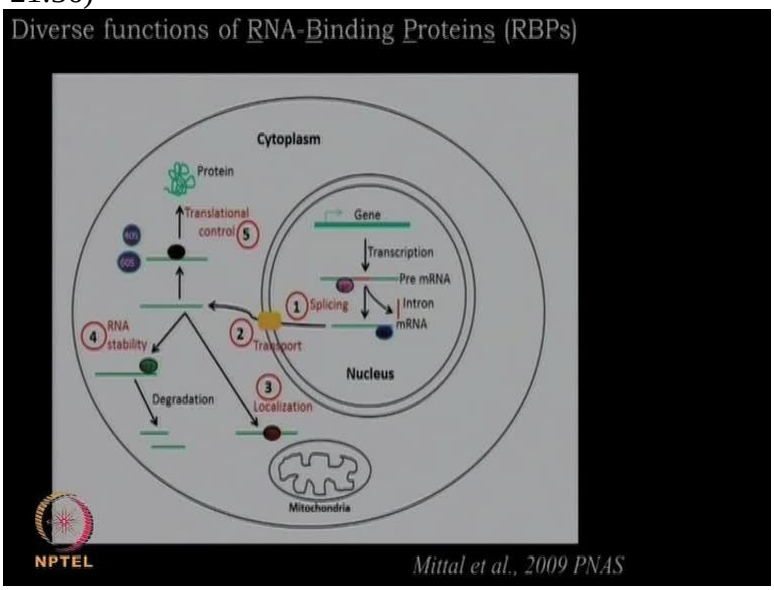

Now in particular as soon as a gene is transcribed and pre mRNA is produced splicing associated RNA binding proteins bind to the pre mRNA and convert into mature mRNA by splicing out the introns. Now the produced RNA not necessarily only mRNA is needs to be exported from the nucleus into the cytoplasm and this is carried out by class of RNA binding proteins which can be termed as transport RNA binding proteins shown with number 2 in the figure.

RNA binding proteins have also been implicated in the specific sub cellular localization of this transcripts. RNA binding proteins are documented also in controlling the stability of the transcripts thereby promoting or degrading the expression of these transcripts. As it is expected RNA binding proteins a number of them are associated with the ribosomal proteins to control the regulation of expression at the translational level.

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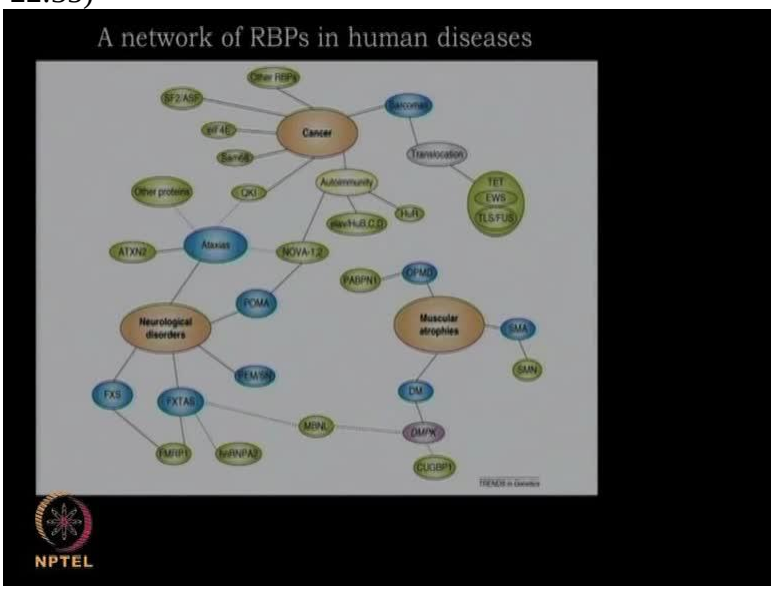

Now a other aspect of regulation understanding regulation at the post transcriptional level is that number of RNA binding proteins are involved in human diseases major class of human diseases such as cancer, muscular atrophies and neurological disorders. In this network diagram shown here the major class of diseases are shown in orange while the sub types of diseases which are sub which can be sub classified are shown in blue and the specific RNA binding proteins which are been documented are implicated in this disorders are shown in in green.

Now let us take a specific example of a muscular atrophy called myotonic dystrophy. In this particular kind of disorder a CUG repeat binding protein called CUGBP1 binds to the three prime untranslate region of a DM protein kinase and because of this sequestration of this CUG repeat binding protein on to the trinucleotide repeat expansion in the three prime untranslated regions this particular disease phenotype is observed.

Another example of a misregulation of an RNA binding protein happens in OPMD which is another kind of a muscular atrophy. In this particular kind of disease a there is GCG repeat expansion in the exon 1 of an RNA binding protein or which is a polya binding protein called PABPN1. Another example we can observe which is which is heavily documented in the literature is a brain specific splicing factor called nova whose misexpression is known to cause a disease called poma which is a sub type of neurological diseases.

So what I am trying to arrive at here is that if there is a change in expression of either RNA binding protein or any of its targets it can be associated to a disease phenotype.

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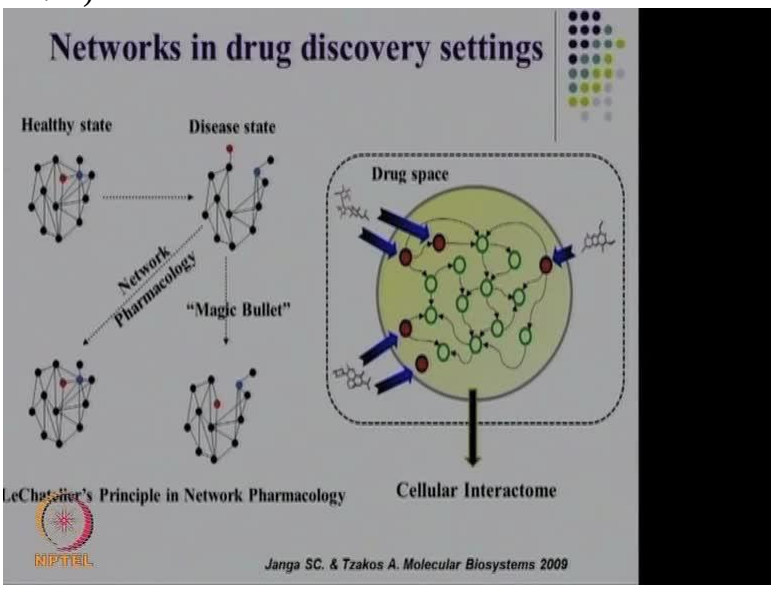

And all the studies basically suggest that it is not just the effect of single gene or protein, it is rather a combination of different set of genes and proteins which contributes to the disease phenotype. Now while this observation is not very new while we knew that this is common for a number of complex diseases what we have still been not able to achieve is the able to cure diseases for this complex diseases.

Now let me introduce to you the traditional notion of how drug discovery is usually happening in most places. Let us represent the health state of an individual with a network of interactions shown in this figure on to the left. Now at this state could be studied as a perturbation such a network where some of these nodes are actually not properly connected compared to the health state.

Now according to the idea of (())(25:18) the magic bullet approach suggest that the conversion of disease state to health state should involve one or most likely one particular drug which is non-promiscuous and specific to a particular drug target so that you have minimal off target effects. Now often such magic bullet approach can be only yield only a semi recovery to the from the disease state.

Now what network pharmacology or network medicine approaches are trying to revet is use a combination of perhaps promiscuous drugs but which do not cause negative side effects which do not cause side effects are lethal and can still convert the disease state into health state as close as it is to the original one.

Now how would you achieve such an approach? To understand this particular idea let us look at a network representation of how the different entities in the cell are interacting. In the figure to the right you can see that a number of drugs each of them can be could have been different nodes. Now all of these nodes are actually interconnected to each other because we are looking into the cellular context and there are protein-protein interactions, there are metabolic interactions, there are also regulatory interactions.

But relation of one cannot be seen in isolation it has to be seen in the context of other perturbations. Now a combination of these perturbations is going to yield phenotype which we hope can be treating the complex disease. That is the concept behind this idea of network pharmacology.

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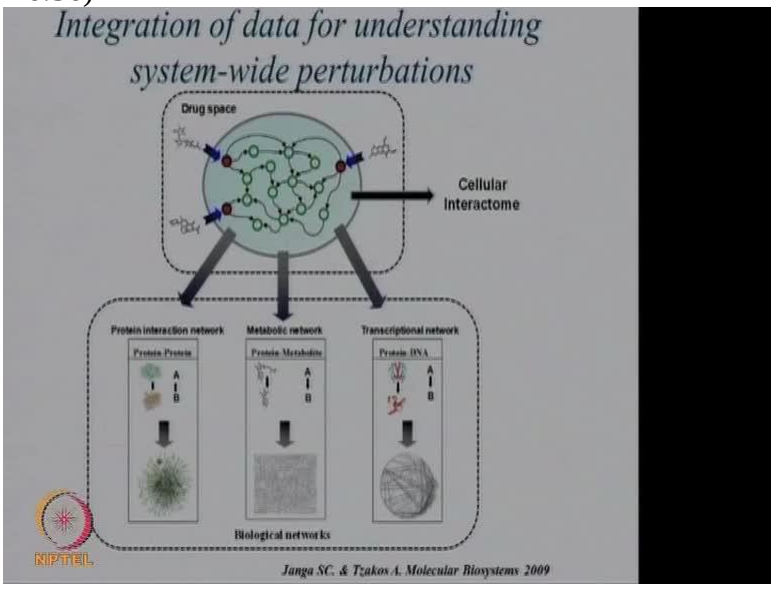

Now how do we achieve such bigger goal? So usually when you have such kind of a complex problem complex phenotype you have to put together data such as knowledge on current metabolic network in the human genome, knowledge on the transcription network, knowledge on the protein-protein interaction network and knowledge on the post transcriptional network and together with the current knowledge of the drugs and the targets and the target pathways one can start looking at how these perturbations can be studied in the context of specific disease and what particular drugs can be used to identify potential new therapies for existing diseases.

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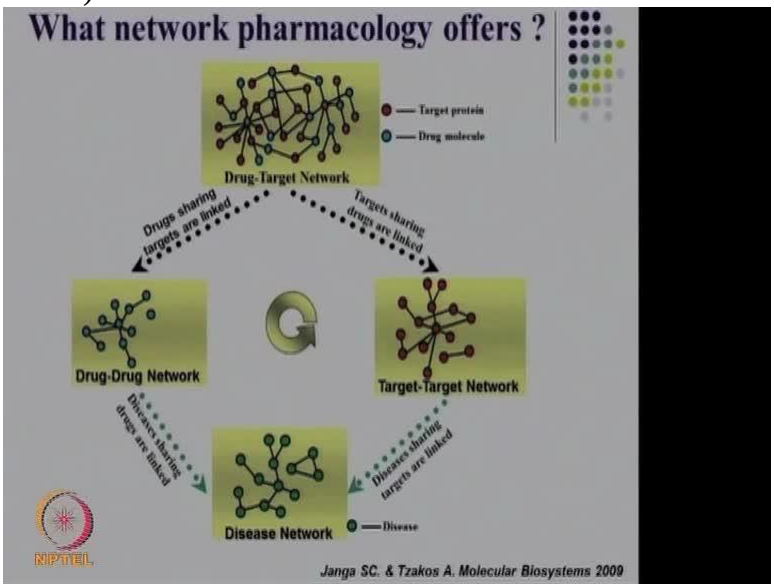

An alternative set of approaches which are being used in the context of drug drug discovery is that if you have a target a drug target network for all the approved drugs in the literature one can start understanding what are the drugs which are sharing the targets. Can we use the the drugs which share the targets as alternatives to existing drugs? What if there is a resistance acquired for a particular drug? Can you compliment the current drug with another drug which is having the same set of targets? Or one can start studying the set of drug-drug relations. If their drugs are sharing the targets can we start studying what are the profiles of the two drugs which are linked? Are they similar in the structure? Are they similar in the final phenotypes? Or what are the common principles of these drugs which are connected to each other?

Likewise one can also study disease-disease associations by linking any pair of drugs which are working which are used for the same disease. Likewise one can study target-target network to

construct a disease-disease association network. So this is these is some of the ideas which were the field is moving to understand or to even repurposed existing drugs for novel therapies.

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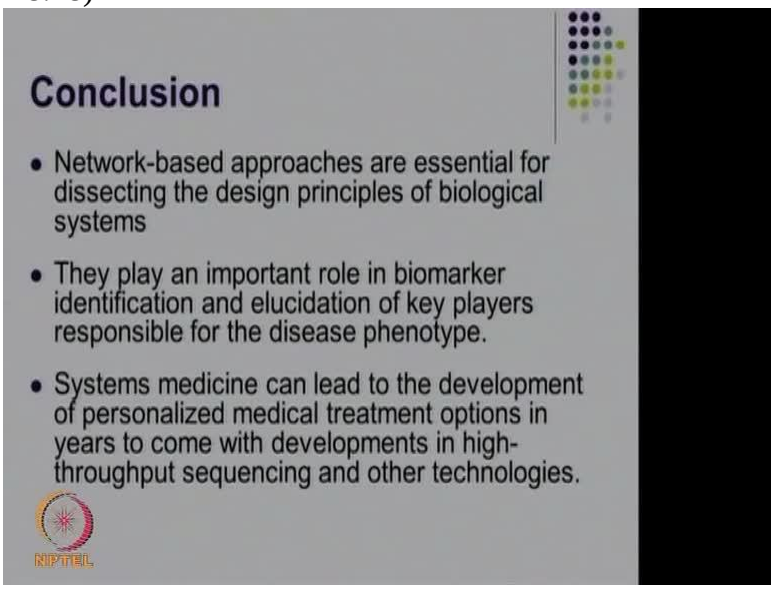

So to conclude what we have tried to cover in the past set of slides is that the network based approaches are essential and powerful paradigm for dissecting the design principles of biological systems. They play an important role in biomarker identification and even in the elucidation of key players responsible for the disease phenotype. systems medicine can lead to the development of personalized medical treatment options in years to come with development in high-throughputs sequencing and other technologies which can yield lot of data in a very short time.

So that clinical relevance can be achieved for based on this kind of techniques application of these network based approaches in the context of clinical settings.

Prof. Sanjeeva Srivastava: Thank you very much Sarath for giving very nice talk and giving some of the basic concepts as well as illustrating how systems level network studies can be employed for reducing various type of problems including in the drug discovery as well as in the pharmacology and it could be extended for even biomarker discovery and many other applications, so thank you very much.

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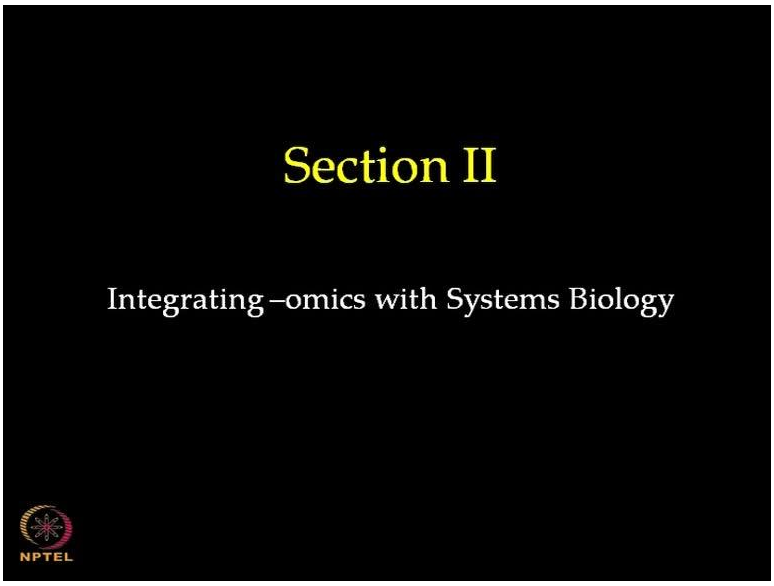
Points to Ponder:

- Systems biology is a discipline for integrating data from various high-throughput omics domains to find holistic information on functioning of a system.
- Biological networks are an essential tool to visualize the molecular working in a cell.
- These networks can describe simple molecular interactions to complex clusters with high degrees of connectivity.

Points to Ponder:

- Networks from such systems biology approaches not only help in understanding sub-cellular functions, but also help in drug targeting/ discovery



After this stimulating discussion with Doctor Janga, let us try to understand how to integrate omics approaches with systems biology.

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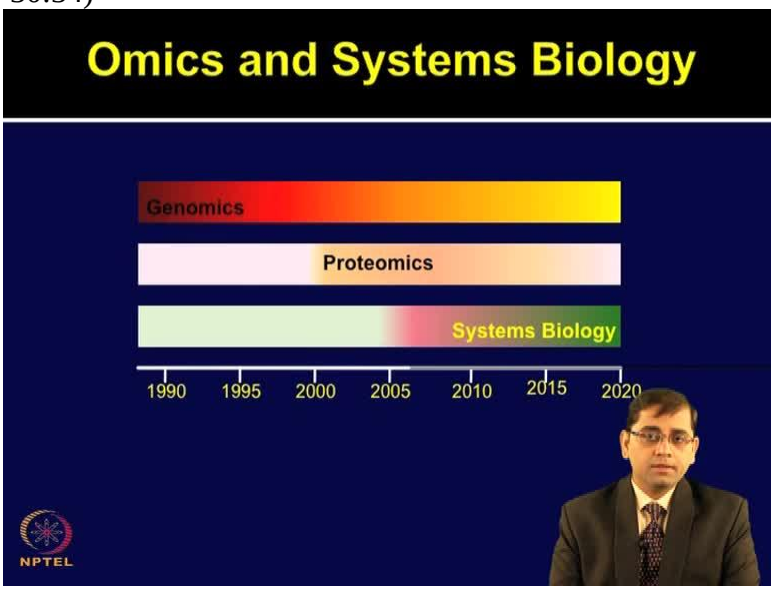

The genome sequencing projects in genomic era from 1990's to 2000 accelerated the phase of omics research. From 2000 onwards proteomics field also got accelerated and new methodologies, new tools came to study proteome. The data derived from genomics, transcriptomics, proteomics, metabolomics and other omic approaches have now brought the integration of these datasets in systems biology field.

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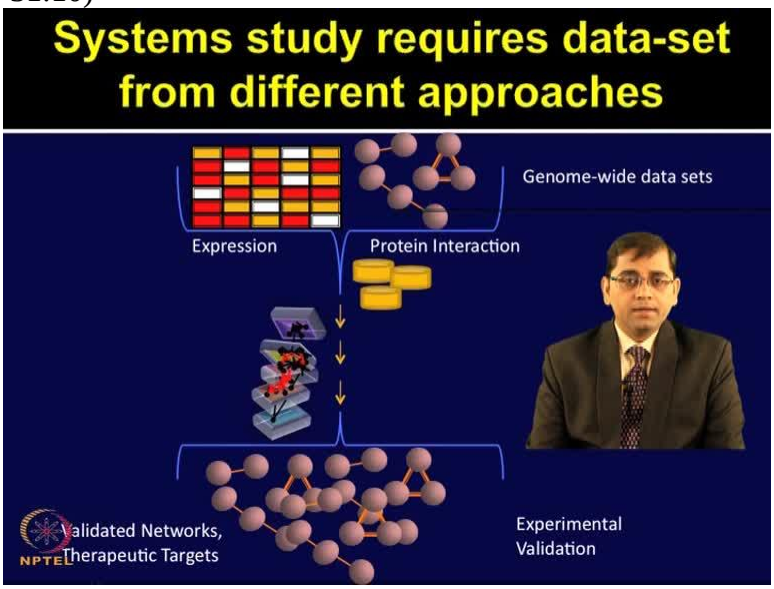

The systems study requires obtaining data sets from different approaches and analyzing then together. For example, as shown in this slide the genome-wide datasets can be derived at the genome level and looking at the expression of different transcripts or at the proteome level one could look at different type of protein interactions. These data sets can be stored in clinical databases and also it can be mined from literature.

The integration of orthogonal datasets can be used for validating the networks and derive it to identify therapeutic targets. Further it can be used for experimental validation.

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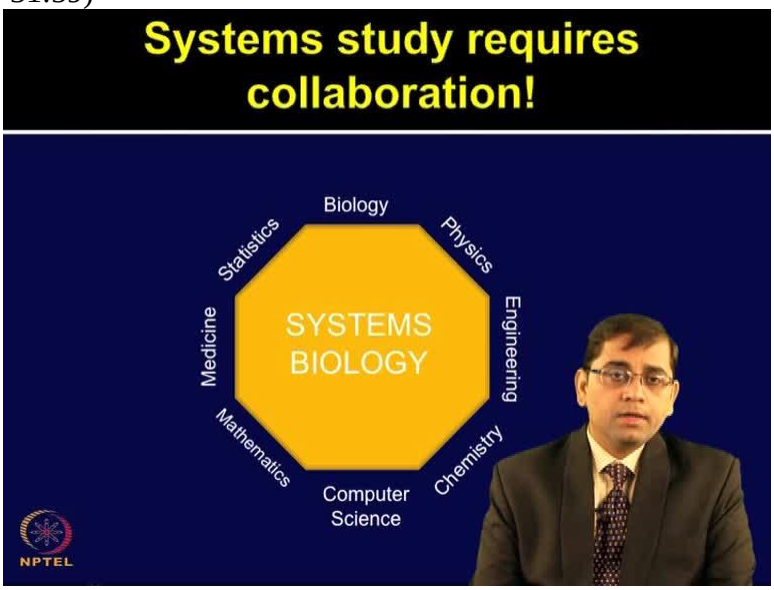

Studying systems control cannot be done in isolation in an individual lab. It requires different expertise and collaboration from scientist working in different disciplines of biology, physics, engineering, chemistry, computer science, mathematics, medicine, statistics and many other fields.

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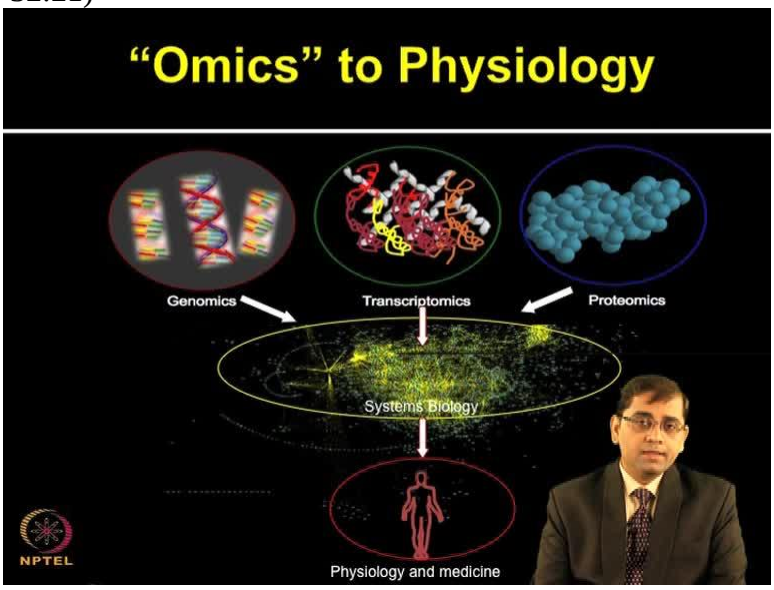

And eventual aim of systems biology is to employ the omics level information obtained from differ levels such as genome, transcriptome and proteome and derive that information at the systems level, integrate that information and quantitate some models and then propose to use it for understanding the physiology and apply it in medicine. This omics and physiology flow can be well maintained by employing systems biology tools.

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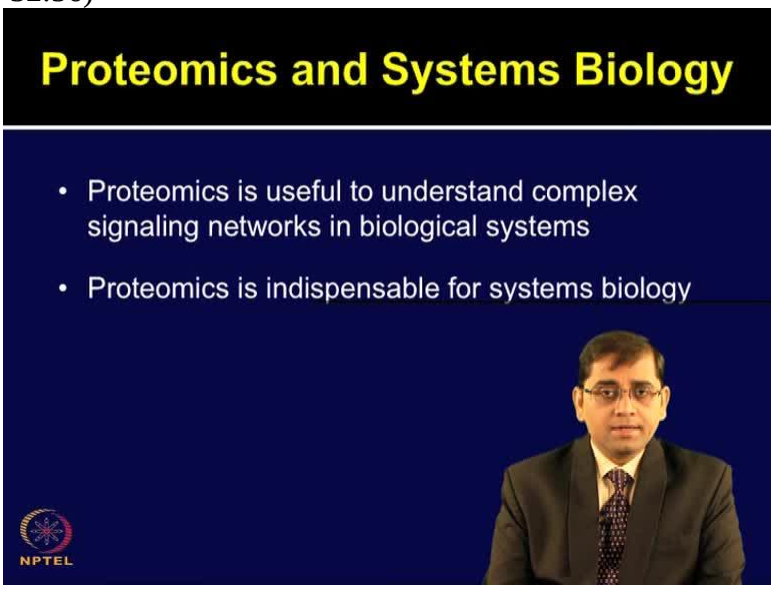

So how can proteomics and systems biology be integrated? Proteomics as we have studied it provides useful information to understand the complex signaling networks in a biological system. It is very indispensible tool for systems biology. The global analysis of proteome is important however there are many limitations in each experiment and thousands of proteins can be studied. Therefore new approaches and systems level investigation and predictions are required.

The system investigation is required to study the complex dynamic structure integration with the biological system whether it is at cellular level or at the organism level, ultimately it is responsible for their function and behavior.

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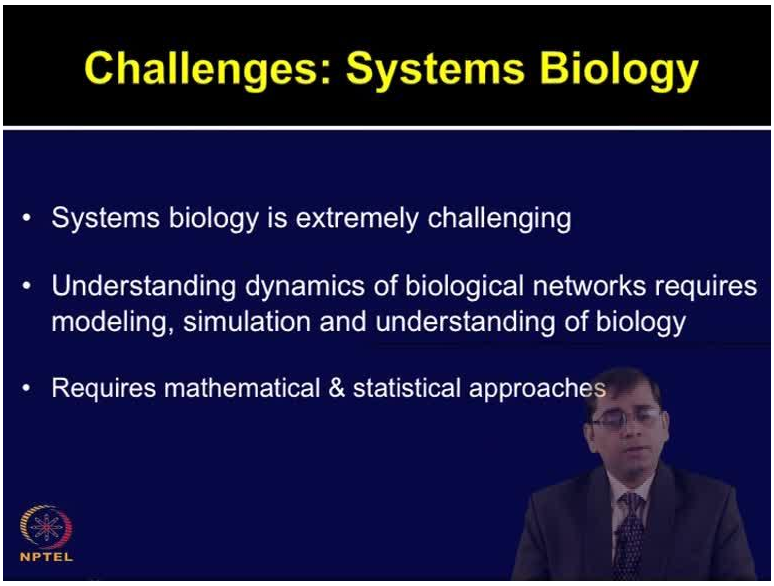
Points to Ponder:

- Systems biology cannot be studied in isolation
- It requires expertise and collaborations from scientists from different disciplines of biology, physics, engineering, chemistry, computer science, mathematics, medicine, statistics etc..
- They help in integration and interpretation of data across the central dogma and help in building prediction models.

Section III

Challenges in Systems Biology



What are the challenges of systems biology? Systems biology is extremely challenging, the emphasis to understand a given system understanding the dynamics of even simplest biological networks not only requires understanding of biology but also its modeling and simulations. The disintegration study can be used for studying from cells to proteins to gene or integrative study could be used for putting these pieces back together and then understand and make prediction and control of functional biological processes.

All of these are very challenging but currently being addressed by applying various systems level tools.

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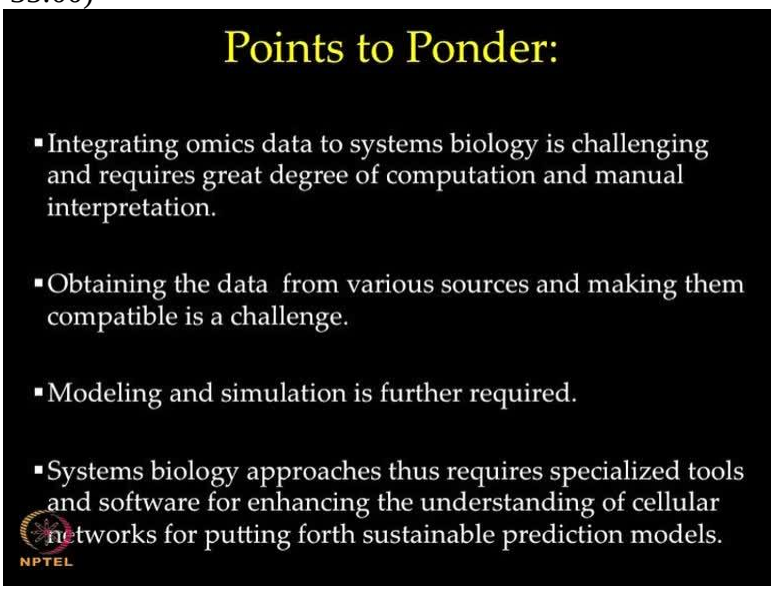

In summary, today we discussed some advance principles of systems biology, we saw how in the omics era the technological advancements in genomics, proteomics and metabolomics have generated large scale datasets in all the aspects of biology.

These large datasets have motivated the computational biologist and systems approaches with objective of understanding the biological system as a whole. While proteomics continues to generate the quality data at the proteome level systems biology approach would help characterize and predict these dynamics abrasions in biological networks. Thus such interactomics based approaches would impact basic as well as applied sciences alike, thank you.

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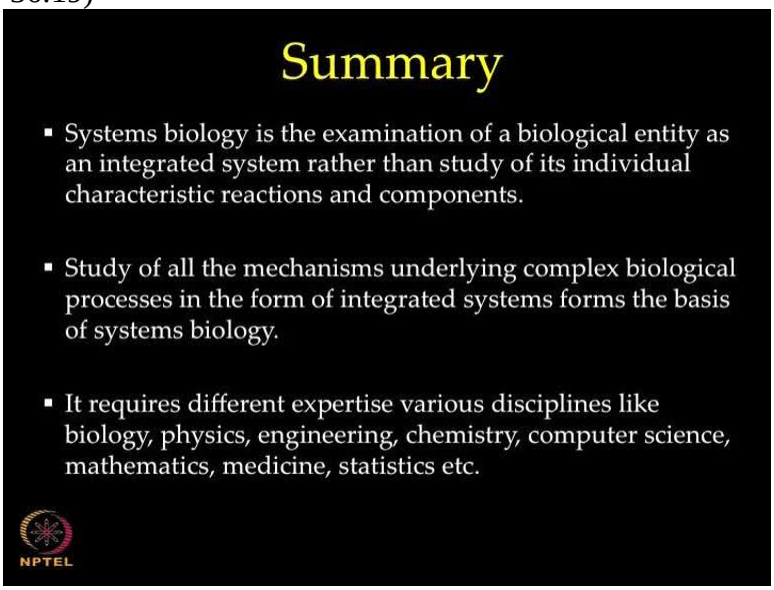

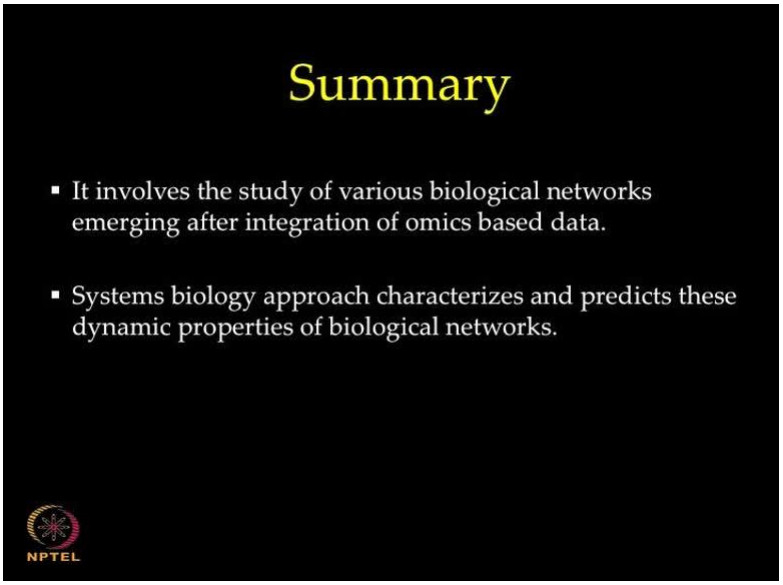

References

- Gupta, S., Venkatesh, A., Ray, S., and Srivastava, S. (2014). Challenges and prospects for biomarker research: a current perspective from the developing world. Biochim. Biophys. Acta 1844, 899–908.
- Ideker, T. (2004). Systems biology 101--what you need to know. Nat. Biotechnol. 22, 473–475.
- Ideker, T., and Krogan, N.J. (2012). Differential network biology. Mol. Syst. Biol. 8, 565.
- Ideker, T., and Sharan, R. (2008). Protein networks in disease. Genome Res. 18, 644–652.

References

- Ideker, T., Thorsson, V., Ranish, J.A., Christmas, R., Buhler, J., Eng, J.K., Bumgarner, R., Goodlett, D.R., Aebersold, R., and Hood, L. (2001). Integrated genomic and proteomic analyses of a systematically perturbed metabolic network. Science 292, 929–934.
- Ideker, T., Winslow, L.R., and Lauffenburger, A.D. (2006). Bioengineering and systems biology. Ann. Biomed. Eng. 34, 257–264.
- Kitano, H. (2002). Systems biology: a brief overview. Science 295, 1662–1664.
- Mann, M., and Jensen, O.N. (2003). Proteomic analysis of post-translational modifications. Nat. Biotechnol. 21, 255–261.

References

- Moxon, J.V., Padula, M.P., Herbert, B.R., and Golledge, J. (2009). Challenges, Current Status and Future Perspectives of Proteomics in Improving Understanding, Diagnosis and Treatment of Vascular Disease. Eur. J. Vasc. Endovasc. Surg. 38, 346–355.

- Patterson, S.D. (2003). Data analysis—the Achilles heel of proteomics. Nat. Biotechnol. 21, 221–222.

- Wingren, C., Ingvarsson, J., Lindstedt, M., and Borrebaeck, C.A.K. (2003). Recombinant antibody microarrays—a viable option? Nat. Biotechnol. 21, 223–223.