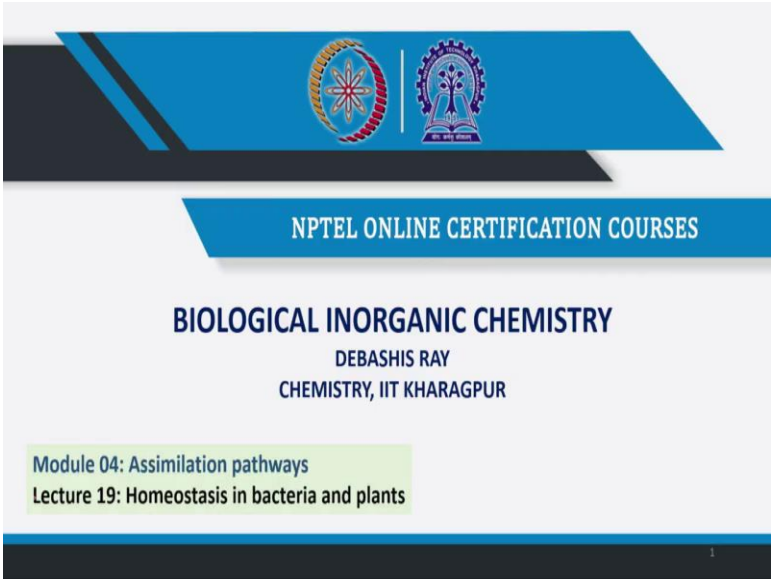
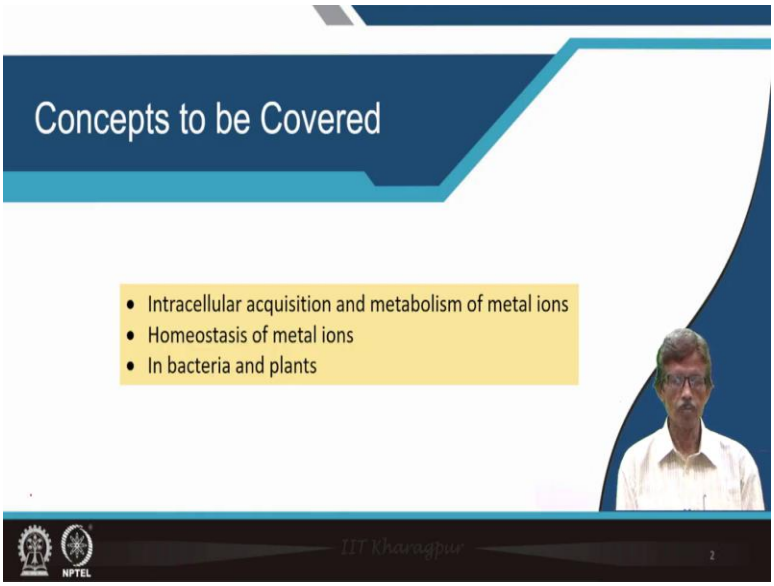
Biological Inorganic Chemistry
Professor Debashis Ray
Department of Chemistry
Indian Institute of Technology Kharagpur
Lecture 19
Homeostasis in bacteria and plants

(Refer Slide Time: 00:31)



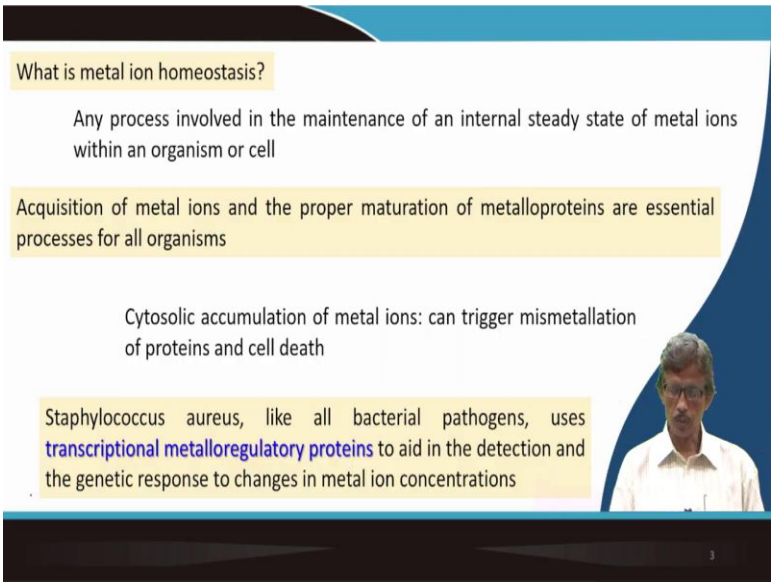
Hello. Good morning, everybody. So, where we stopped last time with that of your module 4 where we are considering the different pathways for metal ion assimilation. So, today in this lecture 19 will talk about what can happen for the homeostasis in bacteria and plants.

(Refer Slide Time: 00:50)



What we see there that we can have the cells, the biological cells of plant origin to the human being. So, we have seen also that the different cells are needed for the acquisition of the different amount of metal ions. So, we can consider that that intracellular acquisition as well as the metabolism of the all these metal ions which are available in their surroundings and definitely from the definition of homeostasis of metal ions and in this class we will be considering only on bacteria and plants.

(Refer Slide Time: 01:32)



So, let us start with that question. Basically, what do we know about the homeostasis, because it is directly related to any other species not only for the metal ion, but in this particular class since we are talking all the time with the metal ions, we are not going away from the metal ions. So, the role of the metal ions, their functions and their all other activities with the biological molecules we can see.

So, the typical textbook definition, there are different sorts of definitions and you try to understand all these definitions clearly, because when a biochemist or a life scientist can talk in terms of the homeostasis, they talk in some other way, but it is the corresponding maintenance of internal steady state.

That means, we must have some huge amount of metal ions in our pool, in our body pool and how we maintain that particular metal ion because they are bound to the different proteins, they are bound to the different metalloenzymes. Apart from that, they are also the degraded thing that means the metal ions are coming out from the all these metalloproteins and metal ion, enzymes. So, how to maintain the internal steady state of these metal ions within any organism or cell that is the terminology what people can think about the homeostasis.

So, we have seen earlier that how acquisition is taking place of the metal ions and their proper maturation. What does it mean? The proper maturation is nothing but their incorporation, the corresponding metalation, the metalation of the different organic huge molecule organic macromolecules which are your apoproteins. So, apoproteins through the insertion of the metal ion giving you the corresponding metalloproteins and they are essential for all the different processes of the organisms.

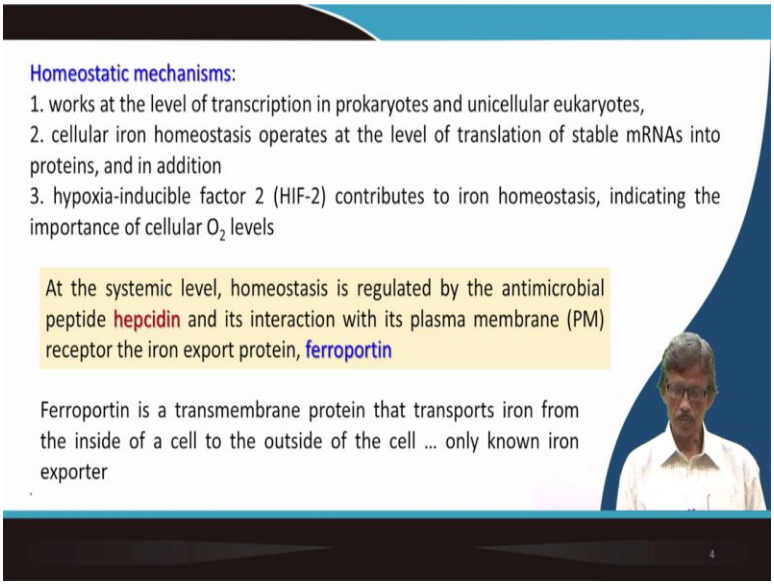
So, cytosol we know the corresponding medium which is present within the cell. So, it can go for accumulating many number of metal ions if it is available from the environments, a biological environment, not our global environment is the host whether you have a fungus or a bacteria or a plant or the human being. So, from the host, how the cytosol can take up those metal ions? And sometime it can so happen that if there is any mismatch during that metalation process we all know as I give you the example, that EDTA4 minus, the ethylenediaminetetraacetate anions when it is tracking or trapping the metal ions, it gives you the corresponding metal ion complexes of say calcium or magnesium or any other metal ion.

But in the protein thing, what you can see that instead of going inside the protein cell, you can have a different type of metal ions. So, mismatch of the metalation reaction is also deadly can lead to the protein or the cell death. One example is staphylococcus aureus, all other bacterial pathogens, because why we are studying all these things, because we are also very much dependent on the metal ions and bacteria is also depending on these metal ions, but they are the pathogenic bacteria. They do harm to us. They go for the different disease conditions and all this.

So, all these bacterial thing, if they are typically pathogens, they can go for transcriptional metalloregulatory proteins. So, this is a big name. But do not worry about the name. So, it can have the different parts, you know the protein part, you know the metalloregulatory protein, the protein which is involved in the regulation of the metal ion concentration in the environment and we know the transcription factors, the translation, transcription in the DNA, RNA knowledge, we all know this. So, they help in detecting the genetic response to changes in the metal ion concentration.

So, some genes will be available there, we will find that the genes are there to encode for the corresponding protein synthesis and that protein synthesis is also important because the apoprotein what is formed over there can take up that metal ion, a particular metal ion to give you the corresponding metalloprotein. So, the synthesis of those proteins are important and the genetic coding is also important. That is why the genetic response is also important to have that.

(Refer Slide Time: 06:17)



Then this mechanism of homeostasis that means how we can have a constant level of metal ions in our system, say in our body also, so all these cases they works at the level of transcription in prokaryotes and the unicellular eukaryotes also. So, during the transcription process, the homeostatic is started operating for these two types of cells.

In case of iron homeostasis,, when it is taking place for the cell, because the cell is taking up that iron. So, it is taking part in the level of translation of stable mRNAs into proteins. In some cases, it is in the transcription level and some other cases it is in the translation level. So, these things only we have to compare when it is happening in the transcription stage and when it is happening in the translation stage.

Then hypoxia inducible factor 2 HIF-2 which is also can contribute which can also, can control or sometime it can disturb also the iron metal ion homeostasis indicating, therefore, the importance of cellular O2 level. So, hypoxia we all know that is related to the corresponding oxygen saturation. If there is shortage of oxygen, we do not get oxygen, right amount of oxygen for our regular cell operation or cell functions. Since that particular iron homeostasis is related to the different oxidation states of iron 2 and iron 3 so it will be dependent on the O2 level also.

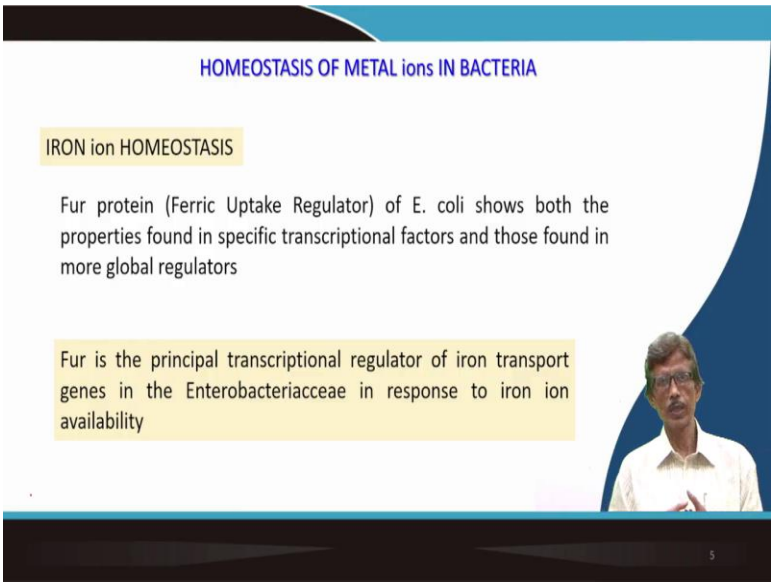
So, small peptide molecule like hepcidin, sorry, hepcidin, H-E-P-C-I-D-I-N, hepcidin, so it can have the microbial thing or the microbial environment can produce or synthesize that hepcidin molecules these are small peptide molecules during that homeostasis process and it can interact with the plasma membrane receptor, because all we now know that like the ligand, the ligand is there, ligand is available to trap the metal ion to give you the corresponding metal ion complexes.

Similarly, the receptor molecules which are sitting on some membrane or the cell wall is responsible for trapping some proteins. So, if we have some iron export protein like ferroportin, is doing the export that is why portin, is the work is export work is moving iron from one side to the other, so the export business. So, iron export business is taken care of by ferroportin and this is again their corresponding receptor. So, the interrelation between these hepcidin and the ferroportin we can see.

So, this ferroportin is also a transmembrane protein that transports iron from inside of a cell to the outside of the cell that is why it is the export protein. So, what is the definition of ferroportin,

where we can find that ferroportin, so these are the very basic understandings only and we are talking all these very basic things only. That is why they are only known iron exporter. So, if we want to move the iron that means the iron from inside of the cell to the outside of the cell because the reverse we are talking about because we are much more concerned about the capturing of iron such that it reaches the cell inside. Similarly, sometimes also if these are very much active we can also lose iron from within the cell.

(Refer Slide Time: 10:05)



So, these metal ions and the bacteria and how the homeostasis is operating. So, take one example because always I say that we can have iron first, then copper, then zinc and if possible the manganese. So, these four metal ions we will be talking about and during the last 10 or 15 years many information have been gathered. The most one or the highest level of knowledge is only with the iron.

So, one such regulator which will regulate the corresponding homeostatic state of the iron that means how much iron you will have inside the cell that can be taken care of if we only study with E. coli, so the small things, small model sample, bacterial sample you can have the E. coli. We know the E. coli is also responsible for many diseases and many infections. So, people can study it for several years on E. coli.

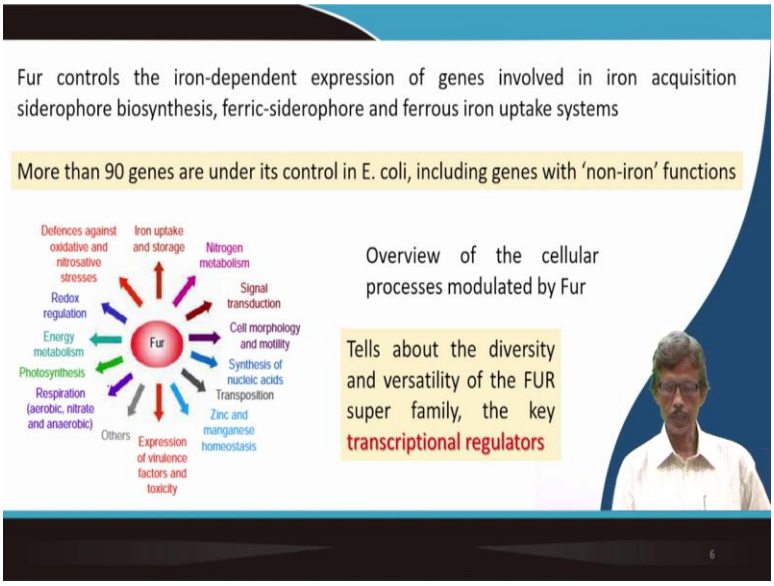
So, on this E. coli also you can find out Fur protein, F-U-R, which is ferric uptake regulator. Uptake, so remember the name will also tell you that it is taking up iron. So, whatever property it

is showing is found in both specific transcriptional factors and those found in more global regulators. So, during the transcription process, it can have the presence. But also in some other global regulators, where in some other functions it can also control.

So, this is the principal transcriptional regulator. So, during the transcription process fur proteins are available and those fur class of proteins, is a bigger class, many number of fur proteins are there and sometimes they are only leveled as ABC, ABC1, ABC2 or ABCD2 all these things. So, do not worry about all these nomenclatures. These are naming of your iron as Fe, naming of your nickel as Ni.

So, this transcriptional regulator of iron transport genes, so the genes are available, which are available for your protein synthesis. And then you find out that you are getting some regulator proteins and in response of iron availability only. So, if iron ion is there that means the presence of iron is functioning as a triggering mechanism or it is giving you the signal, the proper signal that okay you can go ahead many number of iron is there so the iron dependent processes we can see. So, for this particular case, we find that you have the fur synthesis.

(Refer Slide Time: 12:47)



And the function, so the fur controls, what it controls, the fur is there, before that you have the iron. So, iron is also present. So, many number of iron ions they are and those iron ions are there to control the function of fur and this iron independent expression of genes. So, iron presence should always be there. For that particular process, you must have the iron acquisition, how, the

iron acquisition through siderophore biosynthesis. All we know because we have studied some time back also. We are giving the examples. We have the drawings also that enterobactin also, the structure of enterobactin, the triester, the cyclic triester with some three pendant groups by dented part which is capturing this iron.

But now if we talk about the genes, so little bit of more biological part because these biological inorganic history classes obviously will go back to your mode of metal ion chemistry, mode of coordination chemistry and all these things. But to start with how the latest advances in the knowledge and research is going on to find out the corresponding typical thing that means how these genes are involved. We know siderophores per se around 50 years or more. Siderophore biosynthesis is also standardized, but which particular genes are involved.

So, triggering those genes, if we do not want to have this biosynthesis of siderophore in all these bacteria or if the biosynthesis is even there how to stop the function of this biosynthesis for that corresponding trapping of iron such that it can take up the iron from the host, the human being. So, it can control for the siderophore biosynthesis and irons, the ferric siderophore and ferrous iron uptake system. So, ferric siderophores are there, but also at some point it can also control the ferrous iron without that siderophore.

So, many numbers all these genes have found out and some of them are dependent on the presence of iron. So, more than about 90 genes are under control, under its control in E. coli. So, E. coli can control these particular genes, including the genes with some non-iron functions. So, when you have the iron function, what do you find? That you can have the fur protein molecule. So, fur can have some specific functions, but these genes, if you study in detail for these E. coli genes, they can tell you okay no in absence of iron there are some other functions are there. So, those are your non-iron functions in diseased conditions or in many other cases also.

So, many of these functions we can think of and we can see also for these fur proteins. So, these fur proteins, it gives us an overview of the cellular processes, the different cellular processes modulated by fur. We know that a ligand is there, the ligand anion is there. It is reacting with the metal ion. We can consider it as the corresponding complex in process. But within the cell what is happening? Within the cell what we can find that, it can also, the different cellular processes and those cellular processes can be modulated by fur.

So, from the top left the defenses against oxidative and nitrosative stresses are an uptake and storage, all we know, the nitrogen metabolism is also there. So, this fur is also interesting you see not only is the signal transduction, synthesis of nucleic acids, transposition, zinc and manganese homeostasis also. You see the right hand side bottom part the zinc and manganese homeostasis.

So, it is not that that fur protein depending upon is iron, so iron uptake so is going your iron uptake and this particular thing can go for many other reactions or the cellular processes, but also sometimes we will find that for the corresponding homeostasis of zinc and manganese it is also responsible.

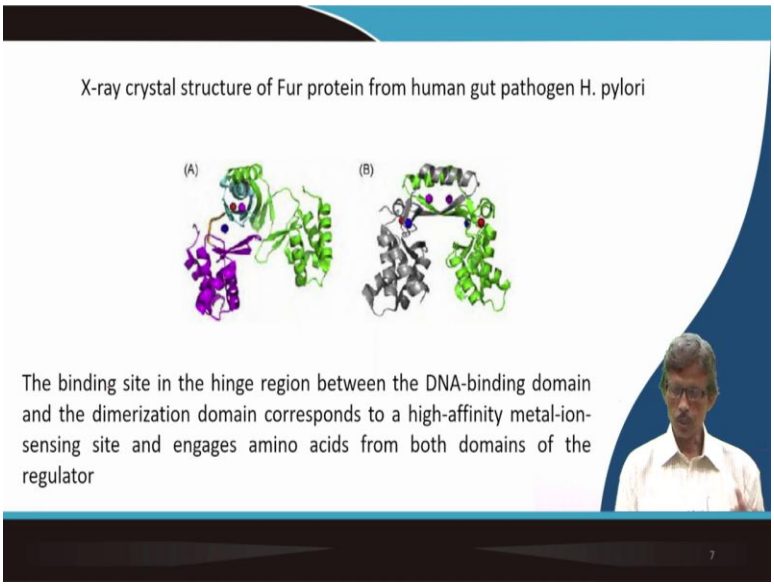
So, we can have the energy metabolism even we can have for the some function in the photosynthesis also. So, is a very big super-family of these molecules. And it also tells about, it states about the diversity therefore, starting from your defenses against oxidative and nitrosative stresses. We know that we can produce ROS, the reactive oxygen species, the superoxide, peroxides and all these.

So, it can go for the defense mechanism that means this fur even if it is you have the iron bearing fur, but it can destroy those ROS, the reactive oxygen species through electron transfer reactions. So, those are the oxidized thing. When you oxidize the O2 molecule, you get the superoxide, you get further oxidation, you get peroxide.

So, these oxidized form if you are able to take out this particular electron, the electron which is taken out for this particular one that means the reduced form of the O2 and the reduced form of superoxide, so then iron through its redox cycle can destroy all these ROS and RONs reactive RNS, the reactive nitrogen species also that is why you have the nitrosative stresses.

So, starting from there to up to the redox regulation, we can use these all these things. So, we can have the fur super-family which is controlling all these reactions and all these processes and it is also the key factor for transcriptional regulators also. So, if you had the transcription process and we can regulate that particular transcription process, we can have the fur protein.

(Refer Slide Time: 19:04)



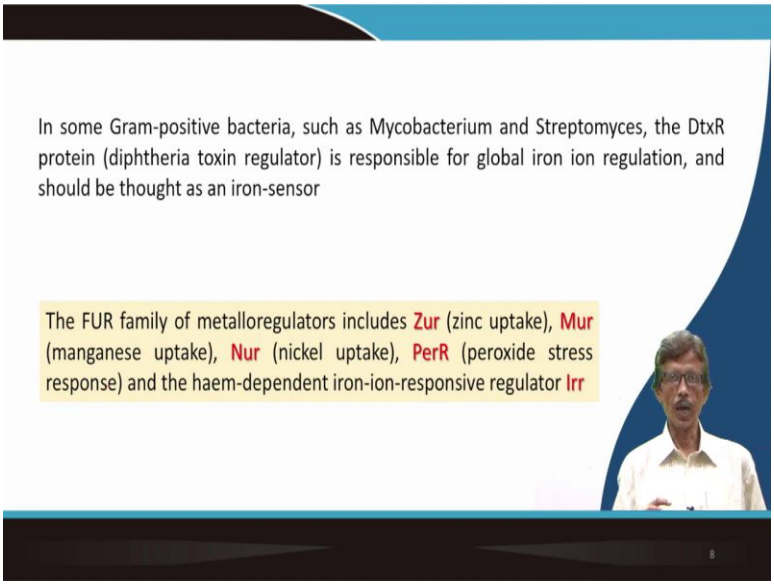
So, one such example people not only studying the processes, but also have studied the corresponding structure and how the metal ions are there, how metal ions are incorporated related to some very well known thing that helicobacter pyroli, H. pyroli we know it is there in our body also, in our large intestine to small intestine. So, in human gut it is present. So, this fur protein can be found out in that particular environment or from the human gut and (())(19:38) crystal structure, the single crystal excess structure gives us this particular structures of these two forms.

So, the binding site in the hinge region, so you can have always we see the door or the window hinge, this is the fixed part. So, this is the hinge part. So, hinge part can open and close. So, this hinge region between the DNA binding domain and the dimerization domain corresponds to a high affinity metal ion sensing. So, you can have the metal ion or if you can have a different metal ion, it will be possible to sense that particular different metal ions.

So, instead of iron if you can have cobalt or if you can nickel, it can also sense, because in presence of iron only you can have the iron dependent processes. And this particular sensing site is important and engages amino acids from both the domains of the regulator. So, amino acids are there, their pendant groups are there and those groups are responsible for the metal ion binding, so same thing.

The metalloprotein formation and the metalloenzyme formation, only the structure is different, the genes are involved for the protein synthesis and when the protein is in your hand it can trap those metal ions. But sometimes before that also the expression of the gene is also required the presence of these metal ions.

(Refer Slide Time: 21:03)



In some other Gram-positive bacteria, so mycobacterium and Streptomyces, we all know these are well known for disease causing bacterium. So, these are pathogenic bacteria. So, one such protein is also there, which is known as DtxR, is diphtheria toxin regulator, so which is causing diphtheria in our body and that particular diphtheria causing species is also toxin in our body. And how we can regulate all these things, so that can also be studied with these Gram-positive bacterial stains and is responsible for global iron regulation.

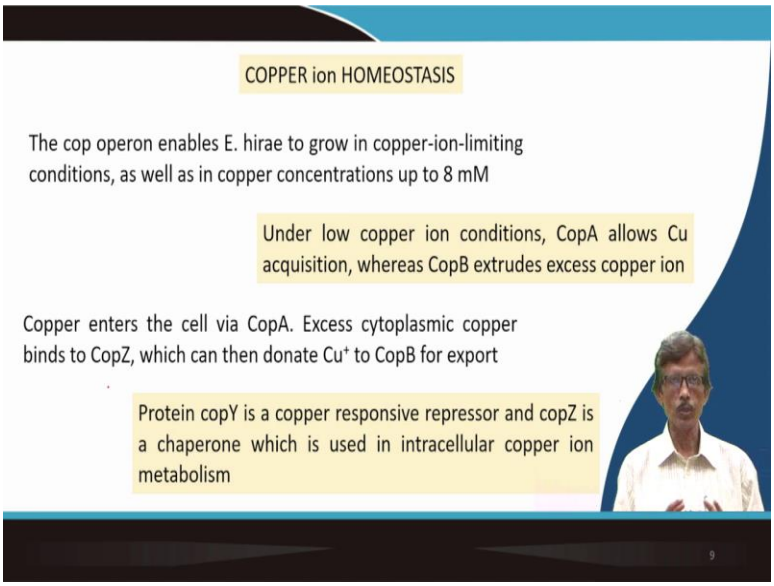
That means how the total picture of iron assimilation and the processing is dependent. And therefore, we can consider these as a good iron sensor also, because the presence of iron is important for all these biochemical reactions are corresponding biosynthetic pathways not only for your siderophore synthesis, but also for the protein synthesis. So, if you try to go for that particular protein synthesis, you must have the corresponding metal ion availability and that metal ion availability is important to carry farther for this particular type of family as your metalloregulators.

So, as I told you just now few time minutes back that your fur that means the iron uptake regulators, so the iron uptake regulators, so fur family of metalloregulators, so one we considered as one small is f, small u and small r, but when you the whole family we all capital FUR family we write. So, includes the zur which is zinc uptake regulator, the manganese uptake regulator, nickel uptake regulator and not only the metal ion uptake regulators, because it was first time it was identified, first time it was studied in, well, that it is iron. But later on it has found that not only iron, but some other metalloregulators are available which can take care of your zinc ion, nickel ion, manganese ions.

But not only that particular metal ion also you can have the P-e-r-R PerR, so the peroxide stress response, so oxidative stress we are talking about, such as you have the reduced form of O2 to giving a superoxide or peroxide and that responsible for your oxidation. So, you have the formation of these peroxides and these peroxides are can show you the corresponding oxidation reactions. We know that for destruction of peroxide from our body we require peroxidases or catalases.

So, peroxide stress response such that you are under stress of peroxide, the elevated amount of peroxide in your system, in your body. So, we can also sense that and also the haem dependent iron ion responsive regulator Irr. So, not the free iron, but also the macrocycle porphyrin bound iron which is your haem iron then that haem is also can control your regulation in terms of your iron ion responsive regulator which is needed for your presence of the haem ion.

(Refer Slide Time: 24:45)

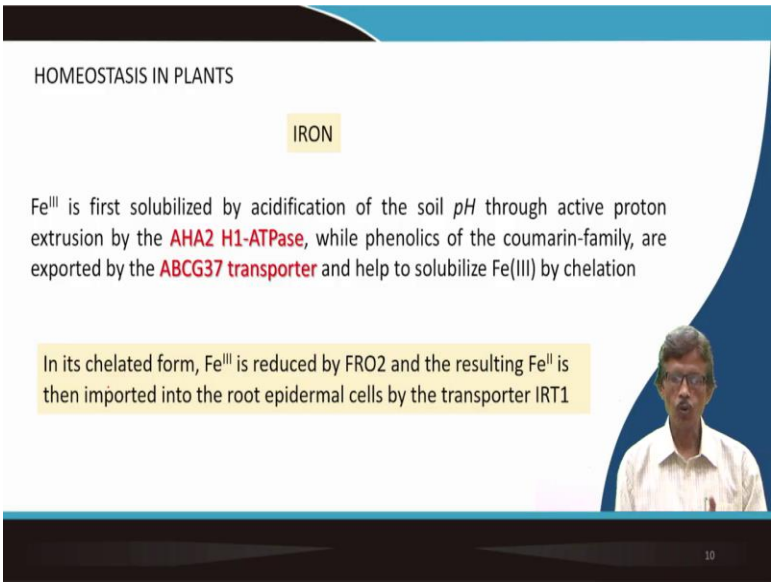


Then if we see that if we go further that for your copper ion homeostasis, what we can see now that what sort of copper ion it can have and how this fur is also useful for your copper iron homeostasis in this particular case. So, the copper operon, so is the copper operational protein which is required for one such bacterial cell is H. hirae to grow in copper ion limiting conditions that means there is a corresponding level of your copper which is not going down and which is not going up also. So, this copper operon is functioning within that particular limit of concentration.

But some time when the copper ion limiting condition means that it is the copper ion which can control, which can also think of the corresponding identification in terms of its what concentration it can have. But up to a level of 8 millimolar concentration it can work. So, these coppers operons are of different types like CopA and CopB, which can also control the function in terms of its copper assimilation as well as removal from the medium for this particular excess build up. So, copper enters through copper A, but it extrudes through copper B and it binds to a CopZ, which is then donates to copper plus to CopB for export.

So, these proteins sometime also the CopY is a copper responsive repressor and CopZ is a chaperone, so is a chaperone that is used in intracellular copper ion metabolism. So, all these terms what we are trying to see here is required for your copper ion metabolism, copper ion homeostasis in all these cases.

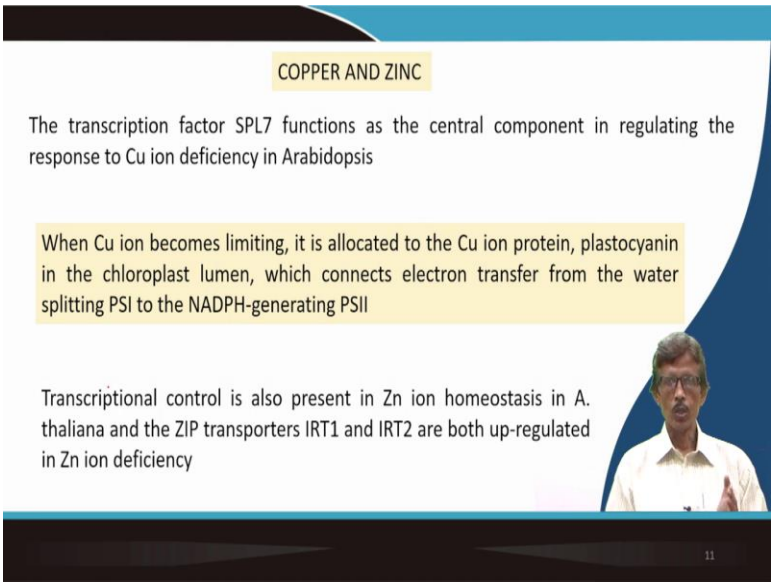
(Refer Slide Time: 26:47)



So, quickly we will see now how the plants are important and these, for these plans the iron is important and there are ATPases and ABC type transporters are there which can help in solubilizing the iron which is available to plant at a particular pH of the soil, acidic pH definitely we all know sometimes we add only the carbonates or the bicarbonates even the lime to the soil to adjust the pH of the soil. So, when you think or talk in terms of the corresponding assimilation of iron by the plant through their roots, we can think about the corresponding pH also.

So, in the chelated form these Fe3 is reduced by FRO2 and the resulting Fe2 is then imported into the root epidermal cells by the transporter IRT1. So, like your bacteria, your plant is also having the same type of mechanism where you can have the transporter, you can have the epidermal cells and you can get these irons to the roots.

(Refer Slide Time: 27:51)



Then copper and zinc also the transcription factors only the names of these transcription factors SPL7 and the copper deficiency we can think of in Arabidopsis. So, these are some conditions where you can have for the plants the copper deficiency we can monitor and we can see.

So, for all these cases the copper is responsible for its two oxidation states copper 2 plus and copper 1 plus and as you all know the copper proteins we all try in detail the electron transport proteins plastocyanin which is there in the plants only.

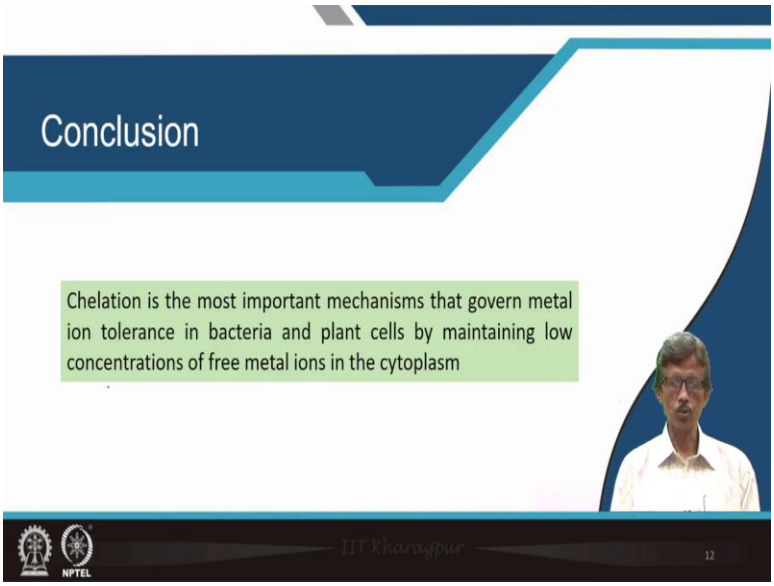
So, we, whenever we will in future we will talk about the plastocyanins. Do recall about these plastocyanin and their little bit of these functions and how they are related starting from all this NADPH generating photosystem 2 also. So, they are also there for the photosystem 2.

Then also the zinc, zinc homeostasis in A. thaliana, thaliana and ZIP transporters is also there and up-regulated the zinc iron deficiency. So, the homeostasis is telling us something that all these genes and all these things are there and which can control not only the typical abstraction, the assimilation process, but also their incorporation sometimes the corresponding function of these genes, which are very much important. So, not only your iron, it can also important for your copper, it can also important for your zinc.

So, in future when we will see about the corresponding proteins, the plant derived proteins, whether we can get some iron plant derived proteins or whether we can get some iron, copper plant derived proteins like plastocyanins. We will study in detail the corresponding blue copper

proteins. The color of all these proteins are blue in color. And we will study in detail about these copper proteins of the plant origin which are plastocyanin, which are azurin and all these things, because they are responsible for electron transfer and those electron transfer mechanisms are important for your, not only photosystem 1, but also for the photosystem 2 where we all know that you can have the oxygen involving center or water oxidation center.

(Refer Slide Time: 28:59)



So, what we have seen so far in this particular class is that the metal ion you have, the ligand, the big ligand, the huge ligand, the protein is there in your hand, so what we will be talking all these cases, so definitely some metal ion coordination is taking place. So, in all these cases, the chelation is the most important factor as well as is important mechanism such that you can have the corresponding tolerance of these metal ions in bacteria and plant cells.

So, if you just simply think the cells of bacteria and plant how they are taking up these metal ions and the chelation is important for the maintenance of the low concentration of free metal ions in the cytoplasm, because the cytoplasm should have its optimum metal ion concentration. If the concentration is more, it can be deadly. If iron is there, you can have the corresponding Fenton type of redox reactions, which can destroy the cell, which can destroy your life also within the cell.

(Refer Slide Time: 31:07)

So, references are from the Wikipedia page. You can go for many pages you can have, but you can start with the metal ion homeostasis page and you can read the corresponding cross references and the book of Biological Inorganic Chemistry. So, thank you very much.