Applied Environmental Microbiology
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Lecture – 24
Environmental Genomics IV

Dear students, in today's lecture we are going to talk about how the genes of a bacteria or any other microbe for that matter evolve over time as do did through replication and through succession in environment. So, one of the major source of changes genetic changes for microbes are mutation and the other are the drifts are genetic drifts that happen within microbial communities.

So today what we are going to do is we are going to go through the reasons why genes evolve over time and remember genes are very important because genes are the reason or let us say genes are the information code of any microbe and they decide what proteins can be expressed what activities the microbe can do or not do and thus it is very important for us to characterize the genes and know.

When genes change through succession or through a genetic drift, then the characteristics of the microbe also changes and today we are going to explore the genetic evolution of microbes. Alright, so previously I have told you about the basic paradigm of microbiology where the D N A stores the information and then the R N A is transcribed from D N A.

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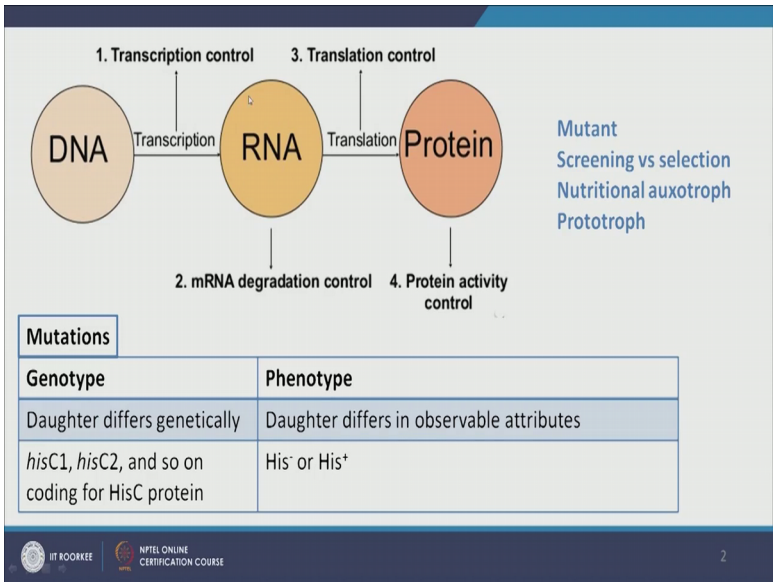

So, remember if D N A is let us say; 5 mega base pair big, R N A does not have to be 5 mega base pair big, in fact it could be only let us say 600 base pairs and thus in this case R N A would be a transcribed copy of 1 gene, it in bacteria and prokaryotes many at times and messenger R N A can have multiple genes can be transcription copy of multiple genes, the difference between D N A and R N A for revision is that; here in D N A behave A T G C, here we have A U G C since in thymine nucleotide we have uracil nucleotide.

Another thing you need to remember this that R N A are usually short lived and as the name suggests that we are talking about m R N A, messenger R N A their main job is to serve as a template from which the protein can be translated. Now in when the cell carries out this process of transcription and translation there are various steps through which the cell can control how much protein is produced and when? Now this is very important to understand that if a D N A is present; let us say, D N A is present for methanogenesis it is not necessary that the proteins required for methanogenesis would be expressed, it is quite possible that there is some control in transcription process and the gene that codes for methanogenesis or the proteins required for methanogenesis will never be transcribed to R N A.

Now, this is very important because this forms as one of the first control for a cell transcription control, using different enzymes or other signals the cell can force a part of

D N A not to be transcribed, the second step of control for the cell would be degradation of R N A as I mentioned R N A in general are short lived compared to D N A. Now the interesting thing here is that; different kinds of R N A have different life spans, some messenger R N A are short lived and some of them are long lived now this is another step of control for microbes, because the short lived m R N A will have less time to express protein.

So, if there is a particular protein that is required in less quantity all the cell needs to do is ensure that the messenger R N A which is a transcribed copy for the which will act as a template for that short lived for the protein required and short amount this m R N A should have shorter life. The protein that is required in larger quantity; obviously, it is m R N A should have much longer life, so this is the second step of regulation.

The third step of regulation of protein formation is in the translation step; even if I have made m R N A let us say, transcription is successful the m R N A has not destroyed yet even in the translation step when a copy is made from m R N A there is quite possibility for the cell to control how much protein is expressed, for example; let us say, initially there was a need for a particular protein let us say history protein which helps in degrading histamine. Now the history protein but the moment history protein is enough the cell can stop transfer the translation by controlling this translation step, again here it can use different enzymes signals to in to inactivate the m R N A so that further protein cannot be made.

There is a fourth step of control where let us say; initially, there was lot of lactose and the cell expressed proteins to digest the lactose to break it down and use it as energy source and carbon source, but all of sudden the media has changed and now there is no lactose the lactose has disappeared. So, these lactose proteins that help degrade lactose have to be inactivated or rendered useless, in this case the cell there is no point for the cell to stop transcription or to destroy the m R N A or to stop a translation because the protein has already been expressed, in this case what the microbe can do is render the protein ineffective.

Now, there are many steps of doing this there is in this course we will not be going through the regulation regulatory details, but this is a very important information to understand to understand the limitations of studies that are based only on D N A or only

on R N A or only on proteins. In fact, as I mentioned in the last lecture we need to account for all of them D N A, R N A and protein to get a realistic picture. So, to summarize presence of protein gives me an idea of activity in recent past or in present, presence of R N A, m R N A gives me an idea of activity in now because m R N Aare usually short lived and protein is longer lived in m R N A, so m R N A is a snapshot in time protein also captures some of the past because some proteins most proteins live substantially long.

D N A lives the longest, D N A gives me the potential which may or may not be translated into an active protein alright. So, now, let us come to cells now as microbes evolve and as I succeed from parents to daughters and again daughters and so on and so forth they undergo changes they can be because of natural reasons the natural error that happened when cells replicate.

So, when the D N A replicate and the parent cell has grown twice the size to not split into two daughter cells, it is quite possible that there is an error and in making the copy of the D N A and thus some mutation has been introduced.

Now, most of the mutation that are introduced in such way are not meaningful they do not really harm the cell. So, these not harmful mutations over time can collect and eventually we might have speciation where the daughter cells are very much different from the original ancestor. So, we are going to look into the mutations first understand how genetic evolution occurs how even anthropogenic xenobiotic such as; heavy metals and at times radiation can actually increase the rate at which mutations happen, how we study them and how and why mutations are so important for understanding the microbiology?

So, let us define the terms wild type wild type is a micro which implies that it has been isolated from the environment and it has not been mutated, so it is the original or the wild type strain. When one of the gene or but if there is any geno typical difference in the wild type daughters then they are known as mutant. So, let us say I have wild type strain 1 o 0 1 o o then the mutant has some differences and then it could be a strain 1 o 2 or 1 o 3 or something like that, so that is wild type verses mutant.

Then we have screening verses selection. So, screening versus selection it is quite possible that even when the mutant is geno typically or in terms of genes different from

the parent cell or the wild type it is quite possible that it has similar behavior, because this mutation does not cause out not render any important protein useless or does not create the cause damage to the cell, in this case we both the mutant and the wild type will behave very similarly so we cannot screen them, but over time we can grow enough colonies and we can notice that even though they digest the same food, even though they can they are resistant or susceptible to same antibiotics, but there are slight differences in the shape of the colonies they make.

So they do everything as it like each other the wild type in mutant, but there are certain surface differences morphological differences so if you grow enough colony you can pick out the mutant from the wild type this kind of process is called a selection.

At times when a mutant is made the difference in the qualities are so clear and clear cut that we can screen out the mutants from the wild type; one example I want to give is cloning process, so the routine cloning that we do in our laboratory for amplifying a particular gene and sequencing it or for expressing a particular protein that I am interested in the routine cloning process usually the genes that the cells that the competent cells that successfully pick up the gene that I am interested in cloning they become resistant to an antibiotic typically ampicillin or kanamycin. So, if I take a let us I will show you by diagram.

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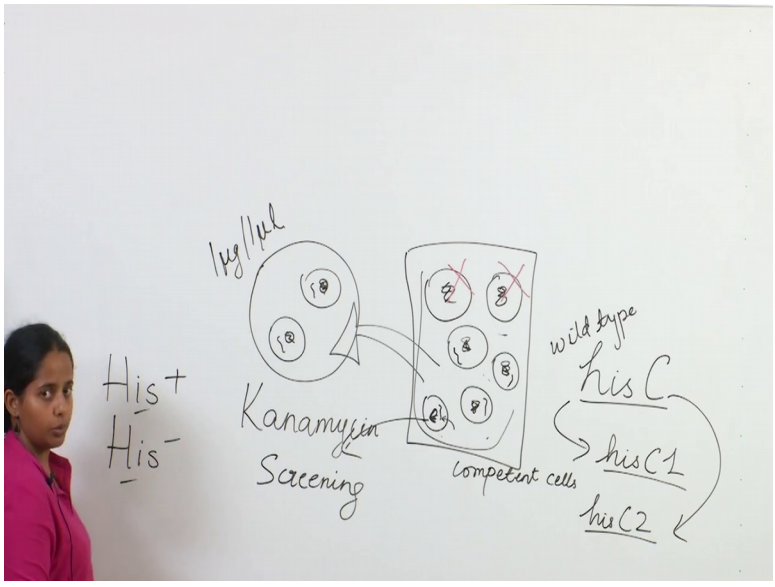

So, let us say this is my box of bacterial cell is some e coli harmless e coli, now all of these e coli have certain genes in them their chromosome all super coiled chromosome now all of them are competent, so when I talk about competent cells we will talk about it in more detail either in this lecture or in next lecture, but all of them are competent cells which means that they are ready to pick extra D N A lying around them. So, there is any D N A fragment lying here there they can pick it in and bring it inside their cell these cells are competent, so let us say their competence is lying here.

Now, in the typical routine cloning process thus we introduce the genes the genetic element we want to clone we put it around competent cells we create the right chemistry, the right temperature, the right conditions for these competent cells to pick the D N A fragment up and when they have picked up the D N A fragment it so happens I mean the way these e coli have been designed is that they become resistant to an antibiotic, let us say now after they have picked up these e coli are resistant to kanamycin, now it will so happen in my experiment that not e coli will pick up the D N A I wanted them to pick up.

So, some of them have not picked the D N A I wanted them to pick up. So, they are just sitting here, now if I have a plate and in this plate I have added kanamycin; I have added kanamycin at the right concentration, so only the cells that are resistant to kanamycin will grow in this plate. So, if I take the f I scoop this out and I pour them here and I pour them here only those cells will be able to grow, who have picked up the right genetic fragment and the ones that had not they will die out.

So, the ones that have not picked the genetic fragment they will die out and the ones that have picked will successfully grow on the plate, such a process of removing now remember because the ones that have picked up the gene their genetic composition has changed some new addition has been made so they are mutant, whereas the earlier one was in the way wild type even though in case of routine cloning it is not wild type because it is not picked from environment, but this is the original one. So, when we isolate the mutant from the wild type on basis of their quality such as; resistant to antibiotics or being able to degrade some sugar or being able to synthesize some protein or not synthesize some protein this process is called as screening.

So, screening when we do the different main difference between screening and selection is in selection both the wild type and mutant can grow, but in screening only the mutant

can grow or only the wild type can grow. So, one of them is removed and thus it is easy to isolate them. Alright the next term we need to understand is nutritional auxotroph's, sometimes when a mutation happens let us say there is a gene his C 1 his C 2 histamine according genes, so these genes if they have been knocked out then the cell can no longer synthesize histamine and thus it requires histamine to grow successfully such mutants are known as nutritional auxotroph's.

So, nutritional auxotroph's are those microbes that are mutants from the wild type and they require a supplement to survive and the initial wild type is called as prototroph, because prototroph is what we are comparing the nutritional auxotrophic.

Now, that we are talking about mutation one of the word that have been thrown around a lot is genotype geno typical differences. So, as the name suggests genotype is referring to genes, so any genetic differences we are talking about gene you typical differences phenotype is your morphology and observable attributes things that we can observe such as; morphology, motility, color now both of them are very helpful when we do screening or selection of microbes, but let us try to understand mutation in terms of genotype and phenotype. So, if the daughter cells differ genetically from the parent cells then this is genotypical difference, now geno typical difference as we have mentioned may or may not have an observable attribute difference.

So, we might have knocked out few genes, but there are essential genes and we really cannot observe the difference between wild type in mutant, it is quite possible that it is only single nucleotide difference and it does not make much difference in the behavior and observable attributes of the cell. So, we might have a geno typical difference, but without any pheno typical difference; however, in general it is nearly impossible to have phenotypical difference without any geno typical background, microbes usually they behave as their genes ask them to. So, if there is a pheno typical difference for example, there are two pseudomonas aeruginosa one makes really good biofilms the other does not so we might know that the mutant which does not make biofilm, so that is a pheno typical difference that perhaps has geno typical difference compared to the wild type that makes beautiful biofilms.

Now, one thing I want to show you the geno typical differences are often referred written on named by the name of nomenclature is often done like this usually you will have a

three alphabet written in italics here it is; his, followed by a capital letter and maybe a number or not. So, his C 1 is the first mutant in his C gene, so this is one of the ways of writing genes will not let me tell you. So, when we are writing in computer or in the words or any electronic media the way we write gene names is be italicized the gene name now C is not italicized only the first three letters are and if you are writing it by hand then you need to underline it. So, if I were to write his C; which is a gene by the way, by hand in a hand written document I would write it like this underlining the name.

Now, if there is a change in a geno typical change in this particular gene I make this as wild type let us say and there is a geno typical difference then I can call the mutant as his C 1 and let us say I get another mutant I can call it his C 2 and so on and so forth, now if we have phenotypical differences then we do not reuse italics we write them normally and we often write plus or minus to show pheno typical difference, remember phenotypical differences are observable difference.

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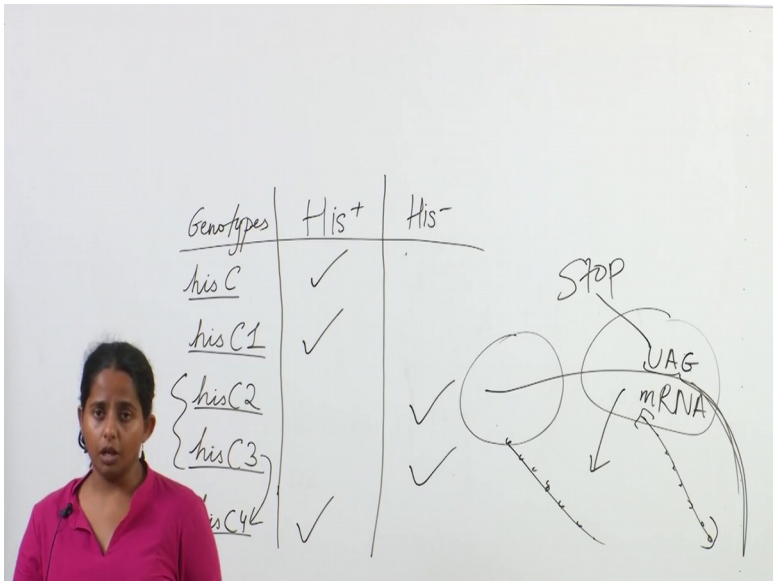

So, ability to synthesize histamine could be His plus yes it can do it or His minus no it cannot, so this is your wild type and this is your mutant. Now dear students take a careful look at this table, here I have listed five different genotypes five different four mutants and the wild type gene, so the wild type gene is his c and these are the four mutants and this is my convention of writing it by hand, now it is quite now the wild type is his plus

so it has a ability to synthesize histidine there we go perfect metabolize histidine very nice.

Now, after I have introduced one mutation it is possible that it can still metabolize histidine. So, let us say even despite a mutation in his C gene it can still metabolize histidine and then when I do another mutation his C 2; now, it can no longer metabolize histidine and thus it is His minus, clear now let us say another mutation his C 3 also cannot metabolize histidine here, now it is quite possible that I do mutation 2 his C 3 and I create a mutant his C 4 and this reverses the effect of the mutation that happened here and his C 4 can now metabolize histidine and again so I have his positive.

So note here; some mutants can metabolize histidine some mutants cannot, but all of them are geno typically different from the wild type. So, the geno typical differences may or may not translate into phenotypical difference, another thing I want you to notice that once a mutant has lost inability to metabolize something or to do some action it is quite possible to mutate it further so as to reverse the effect, so that it starts behaving phenotypically similar to the wild type even though geno typically it is still different. So, even this is a possibility for example; initially let us say, we have a susceptible pathogen and it undergoes mutations and now it is antibiotic resistant pathogen it is quite possible it will undergo mutations and then again become susceptible to antibiotics while still being you know typically very different from the original wild type.

Alright, so now let us look at mutation there two types of mutation you want to pay attention to; one is the induced mutation, the other is spontaneous mutation. So, spontaneous mutation has names such as these are they happen spontaneously without any anthropogenic or environmental influence.

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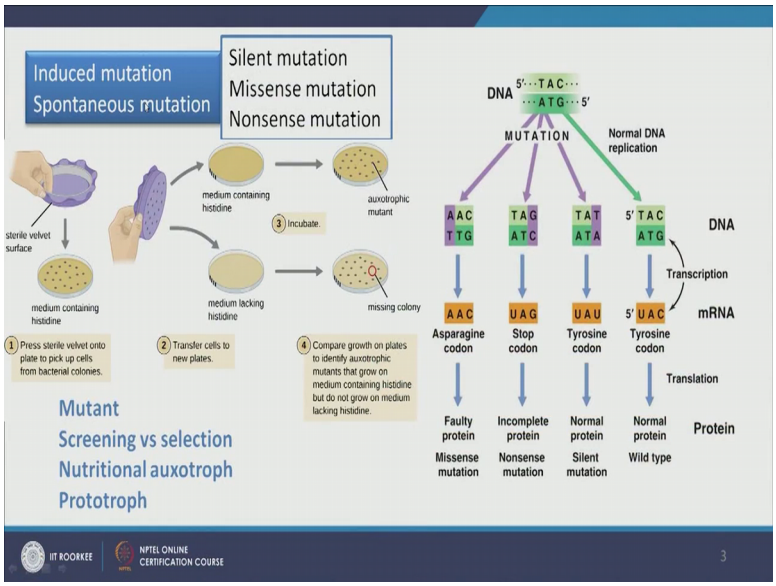

So, as I mentioned before during replication when D N A is replicating it is possible that there is some error or let us say there was an error and now the microbe is trying to repair the genes yes my dear students; note this very carefully that, microbes have the ability to repair genes. So, when microbes are trying to repair the genes it is quite possible that in the repair mechanism they introduce some errors.

Alright and then induce mutations we have here, in induced mutation we actually cause mutation by changing the chemistry around them or by exposing them to mutants by exposing them to chemicals or radiation environmental factors that encourage mutation, now in we can broadly classify mutations into three different forms; silent mutation, missense mutation and nonsense mutation. So, when a mutation happens, but it does not change phenotypical characteristics the cell can do the same thing it was doing earlier there is no phenotypical difference or activity based difference between the wild type in mutant then these mutations are called silent mutations.

Thankfully most of the mutations in on earth in our life are silent mutation; then we have missense mutation. So, in missense mutation what happens is that? We get the protein but the protein is faulty protein, so protein can still somewhat do the job but it is not as good as the wild type and this is missense mutation. Now in nonsense mutation what happens? In the m R N A messenger R N A the pump the because of mutation a stop codon is introduced so a stop codon would be U A G, so in m R N A wear something else

should have been for example; let us say, we have a long m R N A chain this is your messenger R N A and here you were supposed to have you U A A alright, but we have a mutation and now instead of U A A you have U A G correct.

So, when your ribosome starting at the beginning of the m R N A it is making protein as it moves so this is the protein chain which it is making by the time it reaches here and it has made a some level of protein it will read this as U A G and U A G happens to be a stop codon. So, ribosome says the gene stops here I should fall off, so it drops here and we are left with this size protein and all the rest of the m R N A that was supposed to be translated is not translated and thus the protein fragment that we have is incomplete and it cannot do it is work in this case we call such protein mutation has nonsense mutation, because the ribosome drops prematurely because it has read a stop codon. So, whenever a mutation results in ribosome reading a stop codon it is a nonsense mutation and these are very dangerous.

So, let us look at this picture here; this is your original D N A it underwent mutation, so originally it was T A C from 5 prime to 3 prime end and A T G from 3 prime to 5 prime perfect complement to each other they all underwent mutations or different types. So, when T A C mutated into A A C and A T G became T T G it coded for this particular codon coded for asparagines and thus you have a faulty protein this is missense mutation right, because actually the original was not was supposed to code for tyrosine but now it is coding for asparagine so this is missense, the faulty protein might do a good job might do decent job might be fairly good or might not be effective in doing the job it is required to do, but because it is just one amino acid in a long chain of proteins it is quite likely that it will be a faulty protein but not deadly.

Now, if T A C undergoes mutation in the third nucleotide and T A C becomes T A G we get A T C and this T A G is read as U A G, now U A G as I mentioned is a stop codon. So, the ribosome drops off and the protein is incomplete this is nonsense mutation and it is very dangerous for the cell. On the third level; the T A C becomes T A T it becomes A T A, now A T A, T A T is a codon this is the m R N A, U A U and it so happens that U A U also codes for tyrosine which the original U A C was doing and thus there is no difference, that if I still make the normal protein that all of the wild type would make even though there is a genotypical difference such mutation is called a silent mutation, now if you look at the left panel here we have a very beautiful image of how we select

different microbes that are mutated? So, here we have auxotrophic mutant, so nutritional auxotroph.

So, we have a velvet surface and this is a medium that contains histidine and which is with the ability to metabolize histidine is in the hist C protein, now the both medium one of the medium has histidine the other does not. So, the microbes that need histidine to grow will grow only on this one and not this one, so auxotrophic mutants will only grow on this plate and not on this plate whereas, the wild type will grow on both. So, what I can do this is my medium that is containing histidine.

It has both of auxotrophic mutant and the wild type I can use sterile velvet surface to pick these colonies together and then I can plate them on both, so I can just do a like a stamp I can stamp them on both, now the medium that contains histidine everything will grow, but the those auxotrophic mutants that require a histidine to grow will be missing here.

So, this way I can pinpoint oh this is a missing spot, so this must be my auxotrophic mutant, so this way we can screen out our mutants alright. Now let us look at different kinds of mutations we have so far talked about missense nonsense and silent note that these are all single nucleotide mutations so they are all point mutations happening at particular point, very dangerous mutations that happen are frame shift mutations.

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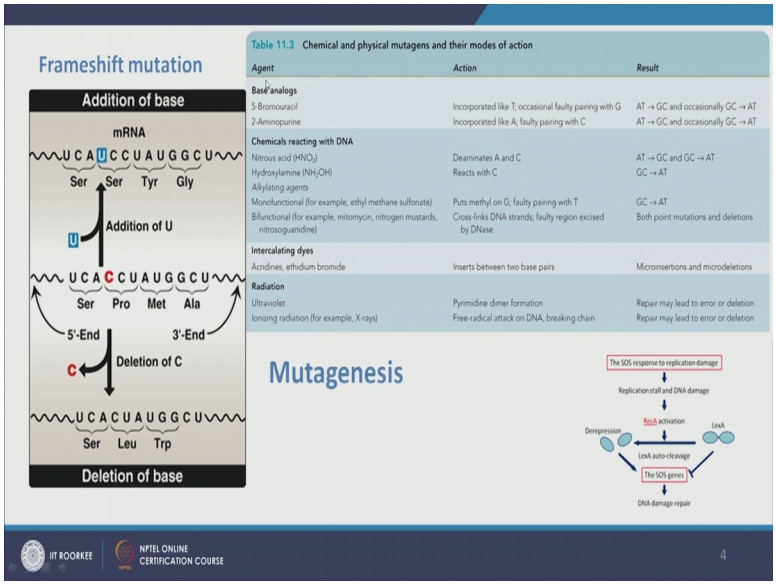

So, in frame shift now remember that D N A is converted into m R N A and then the m R N A is read by ribosome in group of three so they are called codons, in frame shift mutation let us say it starts with U C A but now it has skipped one step forward or one skipped non step backward and when this happens then the problem begins a real serious problem and the entire protein would be faulty this is a very dangerous mutations.

So for example: let us say, if we had U C A, U C C, U A C, G G C, you and so on and so forth we should have had different amino acids as I mentioned here, but now what happened is? This U has been removed. So, if we let us start from here this is my original wild type, so we have; U C A, C C U, A U G, G C U so the ribosomal ribosome binding here at ribosomal binding site.

And now it is reading in three and it is it will read you C A, C C U, A U G, G C U and add the amino acids as required. Now let us say we add a uracil here. So, this will create a frame shift mutation how? So, when we add a uracil here it is still reading the first three as U C A, but now instead of reading C C U it will read U C C which will call for a different amino acid and then all of them will shift and we will have very different sequence then what she was supposed to be. So, this is a frame shift mutation though the frame of reading has shifted.

Now on the other hand we can delete the C, so this C highlighted in red can be deleted and when it is deleted; now, the ribosome will still write U C A, but now instead of reading C C U it will read C U A, U G G, C U A and so forth again a very different protein will be made, now these frame shift mutations are very deadly because by deleting or adding a single nucleotide we can have very different protein almost always a very different which does not do the job at times it might be even dangerous for microbes, now this is when we are adding or deleting one nucleotide what if we are adding or deleting two nucleotides? If you are adding or deleting two nucleotides we still run into the same problem.

However if we I had or delete three nucleotide let us say that ribosome jumps a codon then one amino acid might be extra of one amino acid might be missing depending on whether three were added or three were deleted three nucleotides are added or deleted, but it is quite possible that the overall protein despite being faulty will do the basic job and thus it would not be deadly, but frame shift mutation if it is an essential protein it

will be deadly. So, now let us look at some of the mutants some mutagens and how they act? So we have base analogs so base analogs are very similar to the base nucleotide base, so for example; we have brome uracil it acts like T like U, but it has a bromine with normal uracil does not and it incorporates like T N A it causes faulty pairing with G when it should have actually paired with A, but now it will pair with G.

As a result then as a result we have a very different translation and sorry transcription amino purine it acts like a and it faulty pairs with C, then we have chemicals that react with D N A for example; nitrous acid H N O 2 it will delaminate A and C, hydrolase amine N H 2 O H it reacts with reacts with cytosine, then we have alkylating agents such as; ethyl methane sulphonate they put methyl on G and they fault and of courses faulty pairing with T and then we have bi functional such as; mitomycin, nitrogen mustard, (Refer Time: 35:55) they cross linked the D N A strands and they completely destroy your D N A structure and the faulty region is then excised by the D N A's which causes lot of genetic damage.

Then we have intercalating dies the intercalating dies there they have a very interesting way of destroying D N A or causing mutation in D N A what they do is? So, what these intercalating agents do is let us say you have D N A.

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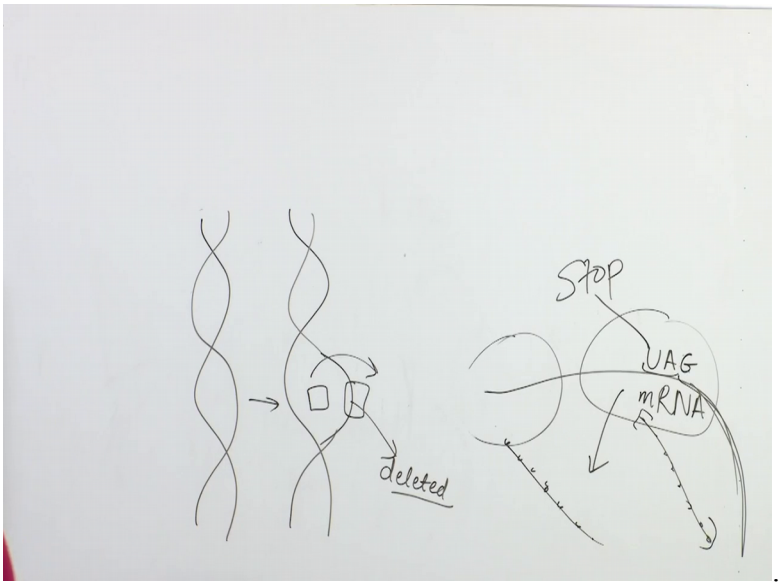

So, this is a schematic of double helical D N A and forgive my poor drawing, but D N A looks like a very beautiful oppositely paired double helix one helix running down to up

and another running up to down, but let us say you have this double standard double helical packed D N A now intercalating agents will come and intercalate here. So, what they do is? So, now you have an intercalating agent here and thus I am sorry your D N A becomes there is a gap in D N A, so when you start replicating it or when you start making transcribing it then often one nucleotide or some nucleotides are deleted.

So, this causes mutation 2, now ethereum bromide and other dyes that are used for looking at such as; cyber green, dyes that are used for looking at D N A all our intercalating agents and when transcription happens when translation happened they all add they all result in mutation. Now let us look at radiation ultraviolet rays are nasty and they are used for disinfection because they caused pyrimidine dimmers.

So pyrimidine dimers means they will form thymine dimers often sometimes they will also form cytosine dimers and when ribosome interacts with thymine dimer will not in thymine uracil dimer or time cytosine dimer then it drops and the replication process and some ribosome tag polymerase when it encounters thymine dimer so cytosine dimers it drops and replication stops and thus anything of a forward from the pyrimidine dimer will be deleted.

Then we have ionizing radiation. So, ultraviolet is non-ionizing radiation it is a free radical free radicals because they ionize which attack D N A it break they break the chain they destroy the structure. Now the cell is not they are not defenseless when it interacts with these mutagens, it has D N A repairing system as I mentioned earlier and usually it is controlled by these two genes; Rec A and nicks. So, these are the proteins Rec A and nicks and what this little schematic is showing you here is the S O S response to the replication damage. So, all of them were called replication damage all the mutagens.

So, what will the cell do when it notices that there is replication damage? So, when replication damage is noticed it stops the replication process and it starts looking at D N A damage, it activates the protein rec A which deactivates nicks. So, nicks well nicks is a stopper on S O S response and it is important to have nicks here because if we do not have S O S response it is it S O S has the possibility to save the cell from the D N A damage, but also create D N A damage. So, S O S is all introduces lots of error in the genome, so the cell has nicks to stall it to stop it from happening, but when rec A is

activated nicks disintegrates it cleaves and it is no longer stopping S O S response and the S O S genes they get activated and the D N A damage repair starts.

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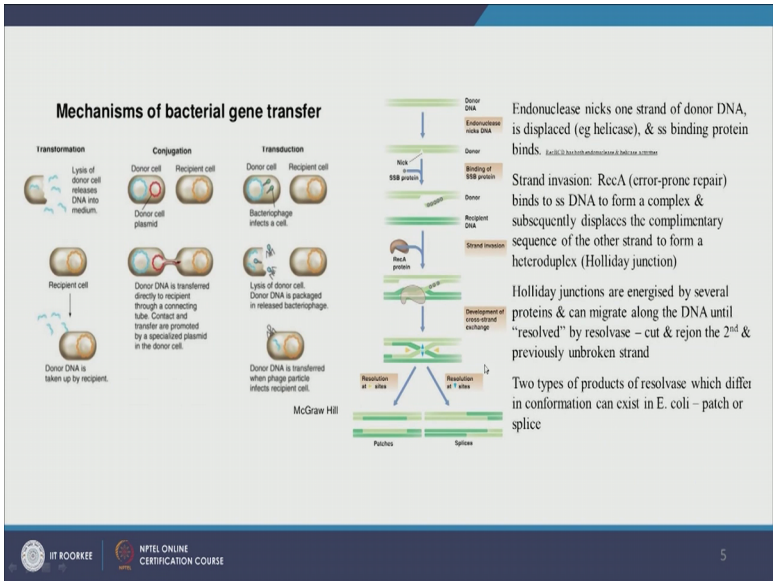

So, dear students we have already talked about these three different mechanisms of bacterial gene transfer. So, apart from mutation apart from selection the; another way in which microbes change the genetic qualities are by horizontal gene transfer.

So, we have talked about transformation just now where the bacteria they pick up the D N A fragments from the neighborhood. So, this is the blue fragment our line from a dead have been released from a dead cell and the recipient cell will pick them up this is transformation in conjugation to bacterial cell one which is the fertile will make a bridge and from the bridge the D N A will be shared, so both of them will have a copy now it is very beautiful process we will talk about it a little bit more in detail in the next lecture, in transduction virus are used. So, the virus here what they do is they pick up the genetic fragments some bacteriophage and when they go to the recipient cell they give away that genetic fragment to it and we will talk about transduction in detail also, but this is just a basic revision this is another way through which bacteria change their genes genetic sequence.

The next way in the bacteria undergo genetic differences is recombination. So, this is a very beautiful diagram of the recombination and I have some information here that you might read if you are interested, basically what we have is that endonuclease will create a

nick it will tear it will create a nick in the D N A and once this has been created it is quite possible it will overlap with the another D N A using Rec A protein. So, if you remember from here once Rec A has been activated S O S system is on S O S system is on in this microbe and it will cleave create gap in the and another double stranded D N A where this nick part can come here and they can exchange, so we can have different kind kinds of patches and splices this is a recombination.

So, this is all for today and just to revise in today's lecture we talked about how the genetic evolution takes place? Basically we talked about 2 directional movements in genetic evolution one is; vertical, when going from parent to daughter we have succession we have mutations and they cause different kinds of damage or do not cause damage to the cell that is one way of changing genes the other way is horizontal movement, so in horizontal movement we have a transformation, transduction and conjugation.

And the third way is when within the cell we have recombination, so in recombination some genes might be disrupted either by deletion or by addition or the operands might be shifted so the behavior of the proteins would change the behavior of the cell would change. So, my dear students this is all for today; in next class we will take this further and note how then we know how genes are changing, we can understand how close the microbes are genetically, how we can draw trees from it understand the similarity between bacterial, type the bacteria and will take it from there.

Thank you so much.