Functional Genomics

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Lecture No 05
Genome Editing Approaches Part 1

So welcome to this new lecture on the course functional genomics. The previous lecture we have looked into how genes can be knockout meaning mutated in animal models like mouse.

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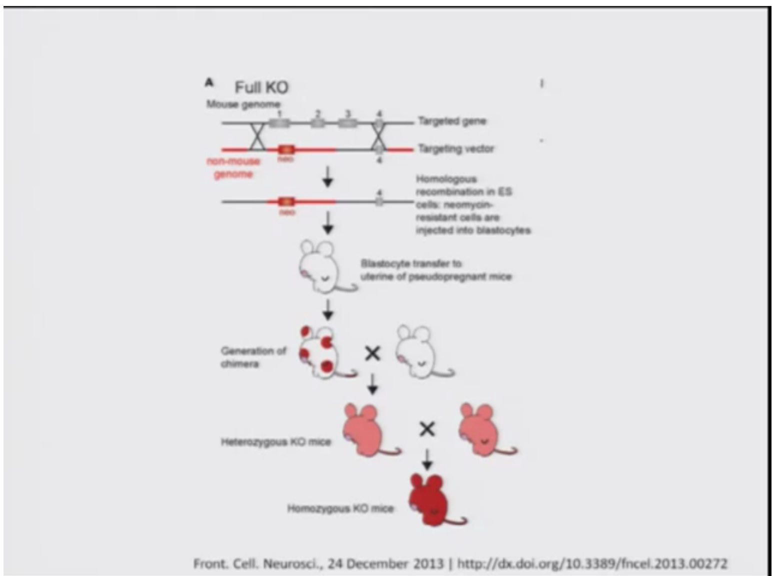

Wherein we discussed how a specific region of the gene can be deleted therefore you can understand the function of the gene in the organism (())(0:36). But you know although this is very powerful technique to understand the gene function this approach has its own limitation and that's true for any approach. It has its own merits and some undesirable characteristics for which we have to overcome with some other approach. For example when you go for a global knockout of a gene wherein you wish to delete the gene and all the cells of an organism. If the organism can survive and you will be able to see the phenotype but that at times you know there are challenges.

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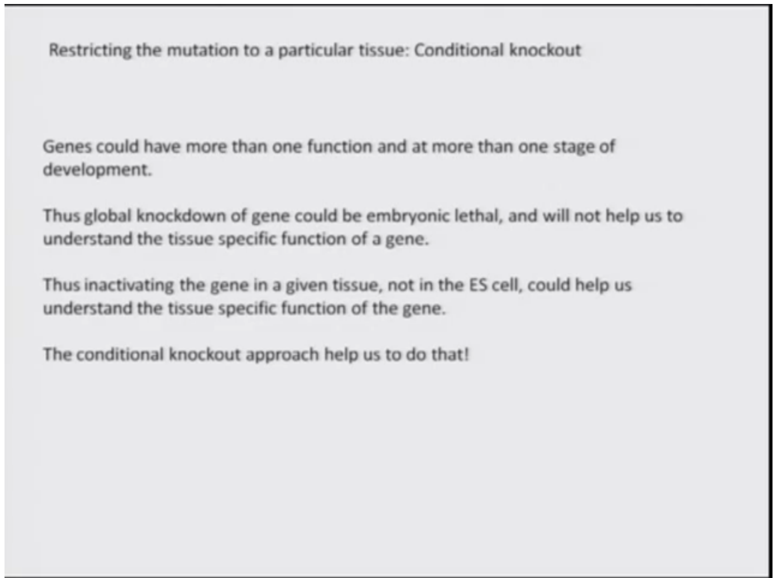

For example you know when you knockout a gene that embryonic development (())(1:23) this compromise therefore you will never see an animal coming out, out of birth. Therefore people have developed a new approach called conditional knockout, meaning you delete the gene only in a group of cell or tissue or when you want the gene to be deleted not right from the word go and that is what highlighted in this slide here that restricting the mutation of a particular to your particular tissue. If you can restrict that then probably you know you will be able to decipher the function of a given gene in a given tissue, so that really helps because it is not that all the genes are you know the function is restricted to given tissue.

So it may be working in several tissue, so when you knockout the phenotype that you see n phenotype. You really do not know you know is resulting from a defect in a particular system or tissue or is it you know an effect of compromise in multiple organs and tissue systems and so on. So genes that have more than one function and more than one stage for example they could have functional development and after that ones the animal becomes adult, they may have some specialized functions so if you really want understand how the gene contribute to this two distinct for example phase of development then you want to restrict the mutation to your certain developmental stage or to a certain tissue.

So that’s the global knockdown that we discussed in the last class. You know if it becomes you know a gene is so critical that their embryo dies so that will not really help us to understand the function of the gene in the adult because you are last embryo because it only tells you that it is critical for the development but it does not tell you ask to what is a function of the gene in the adult or in given tissue and so on. So the approach is to inactivate the gene in a given tissue not in the embryonic stem cells like we discuss, if you a recall the discussion, he said that we will introduce the cassette knockdown cassette into the embryonic stem cells and the gene is deleted and you are going use this embryonic stem cells to create the embryo chimera and then and then eventually you will get the global knockdown.

So that that you know if it is embryonic lethal the embryo does not survive or if it has a problem with reproduction then you are not going see that phenotype in successive generation so (()) (3:46) loose the line that you have created. So if we can restrict you know the mutation to a specific tissue or mutation happening only at later stage of development or when you want that then it helps you to dissect the gene function in all the tissue that gene express in different development stage and so on. So that's where you have the you know next generation of knockout approach which is called this conditional knockout that really help us to dissect the function of the gene.

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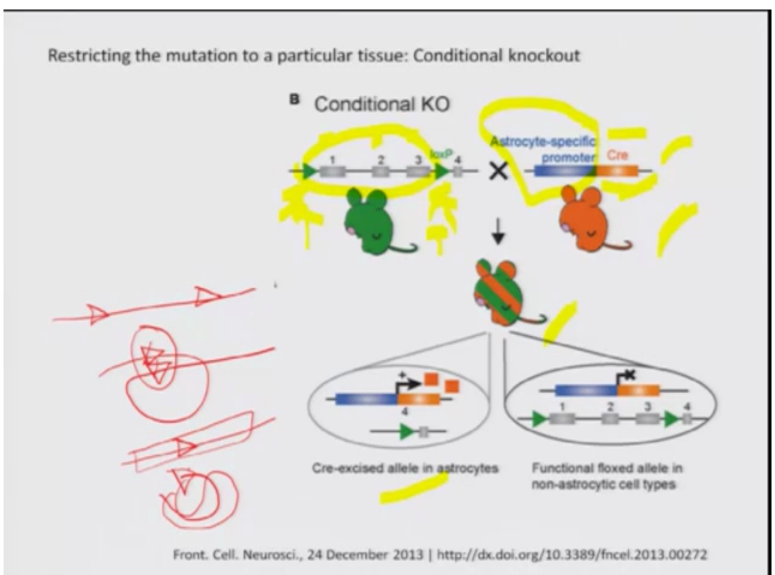

Let's see what is the difference between the global knockout that we discussed as compare to the restricted what you call as condition knock knockout approach. The difference comes in the way you create the cassette the DNA vector their use to knockout the particular gene. So the difference here you as you can see here. So you can see there in global knockout what you do is that you take the flanking region of a gene or a segment of the gene which you want to disrupt, you want to knockout. So here what we do in this kind of conditional knockout, you really do not delete that you know the segment that you want be deleted in the cassette itself in the targeting cassette itself but rather you introduce some new sites. So that's what shown here in the green you can see here on either side of it.

So these are exon one, two and three and then you want to delete if you ever a miss to remove all these three exons of gene therefore you know you sort of understand the function of the gene because it has lost its function what you do is whichever region that you wish to delete on either side of it you add your small sequence which is called as loxp sites. So you can go to the literature and found what it is a small sequences and these are the sequence that are identified by a recombinase which is bacterial (())(5:43) and these recombinase what it does is it promotes what is called is sites-specific recombination meaning when there are sites you know adjacent segment of you know insist meaning in the same DNA the recombinase will identify these sites and whatever is in between these two sites it will be deleted and the DNA fragment you know that is removed now that region gets filled in therefore the genome is restored but your last tail segment.

For example between this site and this site you know whatever is in between that will be last because a cre recombinase. So you have to have it to generate the conditional knockout first you create your targeting vector in which you are able to you know put or introduced the loxP sites on the region with in which you want delete the gene. So for example here you have added loxP sites in the cassette and then the procedure is same so you introduced this inside embryonic stem cells you go for a positive selection or negative selection and make the animal and so on. But as strong as the loxp sites are there in intronic region for example in this (())(6:52) shown these are very small sequence that are normally do not affect the function of the gene.

So in other words even if you have introduce loxP sites and the the gene is functional because it may be the sides there in the intron so when they RNA is being made its splice or it does not really affect the function. So by creating an animal which has these loxp sites on either side of a gene segment which you want to delete, this animal is normal because it is normally it is functional so when would you delete the gene because that's the beauty of the system. So you can condition it meaning you can restrict the deletion happening only inside in tissue or a particular development will stage as you wish okay.

So for that to happen you have to have the protein what you called as cre recombinase okay this is as I told you these are bacterial origin so it is not normally expressed in in mouse and rat and whichever organism at least you are trying to create conditional mutated. Therefore what you need is you need to have a transgenic animal meaning an animal which is expressing your foreign gene therefore it's protein that is required in this case it is a transgenic animal which expresses the cre recombinase okay. So that is what shown here, so now what transgene you would use or which transgenic line you would use? It all depends on your your plan like where do you want to delete the gene, for example what you shown here is a cre recombinase this is coding sequence of the cre recombinase and you have you know linked that coding sequence to promote of a gene which is normally expressed in astrocytes.

These are a specialised group of cell that are present only in the central nervous system. Say in the brain these are not neurons these are non-neuronal tissues but restricted to nervous system. Now what is unique about this is that since we have you know driving these cre recombinase coding sequence and their a promoter of a gene that is normally expressed in astrocytes so if you know the cre recombinase is express in the cell only in those cells your knock down or knock out will take place. For example, when you cross this these two animals the one that has the loxP sites with the animal that carrying the recombinase, when you when you get these hybrid which has got both now in this animal you know in astrocytes this differentiated cells.

You would have cre recombinase therefore the knockout or the gene will be deleted only in this cells. In other tissue for example, digest system or even the neuron or during development this gene is for absolutely for normal therefore you do not see any you know defects. So whatever affect that you are going to see now is because of the last of the gene in astrocytes that with with confidence you can say that. Now say suppose this gene is also express in intestine for example the whatever gene that you want to delete so how would you test the function of the gene in intestine, so again you to have a transgenic animal in which the cre recombinase is expressing in the you know cells that are therein the intestine.

So if you do that you are going delete the gene intestine or in testis or in ovary or in lung. So it all depends now once you have created a conditional knockout animal wherein in the loxP sites or inserted and either side of the gene now it all depends on you with which you know the transgenic animal that express cre recombinase your crossing. So you can choose the you know transgenic animal that express cre recombinase and then whichever disease cell you will be able to delete, so that is called as you know a conditional knockout. So for example here what happens is when you have cre recombinase is going to go and identify those to LoxP sites and then introduce what is called as site specific recombination.

So what happens in these cases the recombinase you know you have this is your DNA and you have LoxP sites here the cre recombinase actually helps in bringing this two sites together there it can do by something like this. So you this site this site came together and the recombinase sits here and basically cuts the DNA here and here. Therefore you have linear DNA in which the recombinase cut exactly at the LoxP sites and introduced then you would properly have a circular DNA which has a recombinase site these LoxP sites but this will not survive because is not going to have application or gene is not going to have telomere, it's not going to have centromere so within few you know deviation it would be last say it is not functional but this is part of your chromosomal DNA.

But whatever DNA element that was part of the gene that was present in between is deleted so in this way you are able to delete the gene you know by driving the recombinase in whichever tissue you want to do this. In fact the technique is so robust that you can now a days we can pretty much you know decide when do you want to delete the gene. For example this there are drugs that are available when you feed the animal with the drug a given gene may be turn on. So assume that you know the cre recombinase is under a promoter which is you know whose expression you can sort of tune from you know what you are feeding to the animal. So in this way you can desire us to when you can induce a cre recombinase and and you can shut down the gene.

So this is a powerful technique and people are now trying to understand for example during ageing process or a good disease model you know whether you know you will be able to bring in some therapeutic approach all these are being tested by creating such kind of animals. So that is a very very powerful tool. So I will you one such example where you know there are advancements that really helped us to understand the human disease using this kind of conditional knockout models.

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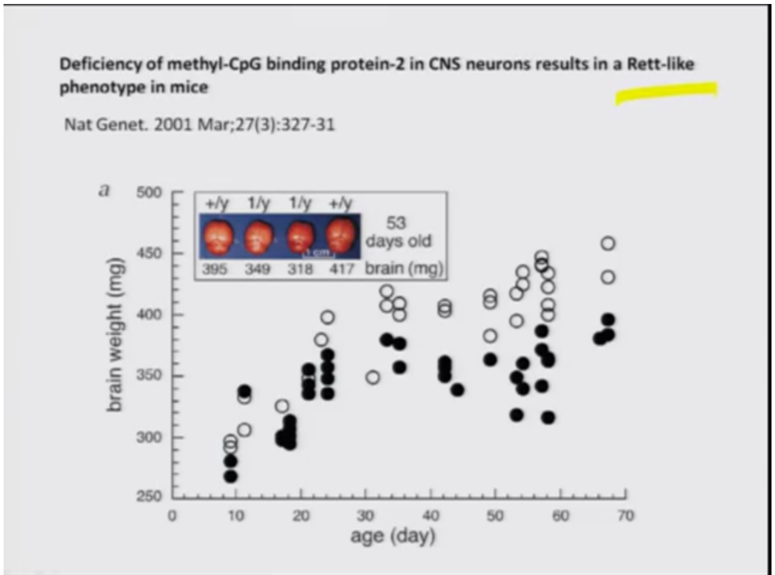

For example one of the very very common neuro neurological disorders is known as Rett syndrome. The Rett syndrome is a you know in human it is mental retardation and its known that it is you know (())(13:19) so on but what is amazing is that there are not many male patient that were known to have Rett syndrome. So that where the theory is either it is they do not survive or it does not affect the male its very milder phenotype and so on and what is when they identified the gene for these Rett syndrome then what they found was very surprising because the gene that is mutated and leading to the Rett syndrome phenotype it is protein that is expressed in wide variety of cells in fact it is incubate is meaning it is expressed in every cell type that you can think of and second it has a very very generic function at least from functional point of view because this is a protein that goes and binds to regions in the genome.

Which are methylated cytosine bases these are called as a CpG I lands which are normally present in the promote original many genes when the cytosine in such CpG phos that is P stand for the phosphate. When you have such methylation then more often these genes are mocked for a you know kind of silencing meaning it should not express and so on. So this is consulate to be transcriptional factor which identify subsequence and modulate the gene expression and is expressed in variety of tissues and CpG methylation is known in all the tissue tissue types. So they never it was surprise when they found that this is gene that is causing the Rett syndrome because it is purely a neurological phenotype. I mean you otherwise you do not have any problem except that these kids are mentally retarded, their IQ level is very low and so on.

So this was surprise so then they would like to really dis dissect how this gene may cause a neurological phenotype though the gene is expressed in wide variety of tissues. It is expressed in neuron in the nervous system. It is also express in the non-neuronal cells in the nervous system and there was one study before that were they have knockout the gene in you know in mouse and then the embryo did not survive even you know a for embryonic growth this particular gene was you know consulate to be very very essential. So they went (())(15:29) induce this particular model conditional model wherein they have driven the cre recombinase under a promoter of a gene which is normally expressed in a differentiated neuron okay.

So therefore once the you know the animal is you know sort of grown and have a differentiated neurons and you will not have the cre recombinase but the moment a neuron differentiate the cre recombinase would express because it is under a control of a gene which is normally expressed in the differentiated neuron. So now the recombinase would go and introduce the deletion because otherwise a loxP sites are normally it allows the gene to perform function but once you have the cre recombinase its sort of results in the deletion of the segment of the DNA that are between the two loxP sites as a result these neurons now lost this particular protein and then they looked at the phenotype of the gene is the mouse.

They have understood as to what goes wrong in such condition therefore you have a mental retardation phenotype and now in fact this animal model is being used for you know screening drug and there are very beautiful you know studies which we have identified drug molecule that are in clinical trial so that really talks about how powerful this kind of approaches in understanding the disease process and that can be translated for the human. So you can see here now what we are talking about is the brain weight because you know often you must have seen when the baby is born one of the physical examination that is done normally every ones in six months to look into the size of the head.

So heads are confirms they normally measure because that is an indicator as to whether the baby is growing normally or not? Normally the patient that their kids that have suffer from neurological phenotype the circumference could be you know somewhat smaller or lower than what you see in the normal per patient. So this is a study which really dissected and showed that you know that when when you lose this protein only in the neuron you now you have a phenotype the symptoms that are very similar to what you see in the in the human condition. So that you know otherwise you know you are unable to study this because we are knockout in every cell they embryo dies than your not having a model to study in the adults so this really helps us. So likewise there are number of studies people have used this model to understand the function of the protein.

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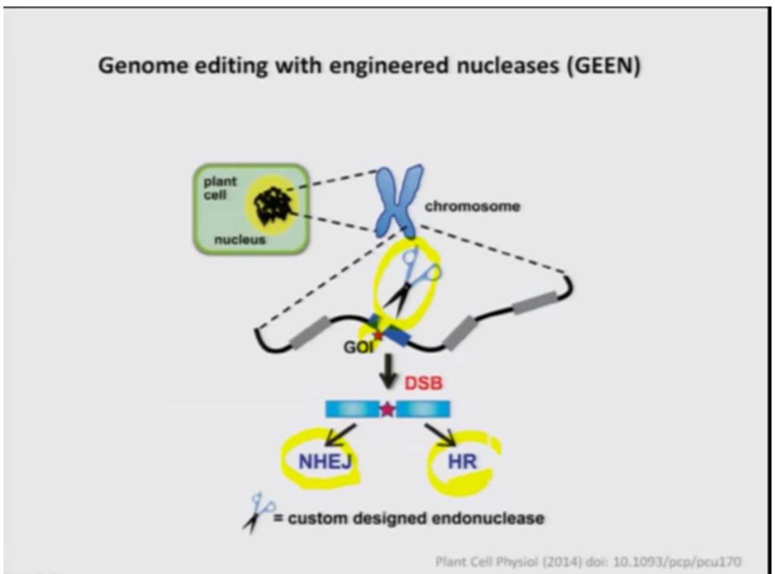

Now that is one model but what you need to understand is that creating a transient you know knockout construct is a very very tedious process because you know it requires manipulating the genome and creating a construct and things like that. So it is really you know time consuming and and a probably it is quite good in case of model where you are able to have the genome sequence then you know genome what you call as structural wall genes are known and so on but it is not kind of universal you can applied to everything. It has its own limitations. So therefore in parallel there are many other approaches that are being tried out. Now one of the approaches that is getting much more attention this this because of its more precise control as to what can be done.

In fact what we are talking about in gene targeting or conditional knockout is that you are able to create mutation but you know the technology that is coming now is can you sort of correct an error that is there that is resulting in a disease. Right so if we can fix that probably that individual can become normal so therefore, there are many other approaches in the meantime people are trying to a engineer the genome to either understand that disease process or understand that gene function and one of the promising approaches that is being considered to be much more powerful than the loxP system or global knockout is called as genome editing all right using certain engineered nucleases right. So this is just this cartoon gives you an overall view as to what it tip.

It's it has a wide applicability. People used return plant cell in fact it is started to begin with for the plants now it is applying even to the human I will talk about little latter. So it is basically what you have is you have engineered nuclease that can identify a given segment a small region of the genome and then it can cut there and then the around cellular machinery has a repair mechanism. Right if we have damage in DNA or repair mechanism tries to you know fix that and it is disease in this it is expected that our repair mechanism would you know try to fix that damage cost by the engineer nuclease in this process there may be some changes that is happening.

So there are two different process one called as NHEJ meaning non homologous end joining and homologous recombination right. So this homologous recombination normally happens in the you know in the mitotic cells whereas the this process we believe it happens in almost all the cells because even there there could be damage and you know self-rise to repair itself. So what it does is that this engineered nuclease will come to that little later is what the nuclease is? It is not something which can go on cut anywhere it is like more like the restriction enzyme that discuss about almost this is the same there it recognize certain sequences right and then it goes and makes double sided cut there.

Just like restriction enzyme and so once you made cut then our system in the it is whatever we are talking about designing the live cell so you are not extracting the DNA outs. So you are using a cell and you are introducing the nuclease and this will go and cut somewhere and as a result you have DNA damage and our system tries to repair in that process you may delete some segment few bases that is good enough for gene to be in activated. So if you have an enzyme which recognize certain sequence and that such kind of sequence that present over a gene or close to a gene which is likely to disrupt the function you can use this and it it makes the changes. So this is the the principle are the basic concept behind this nuclease let see what they are.

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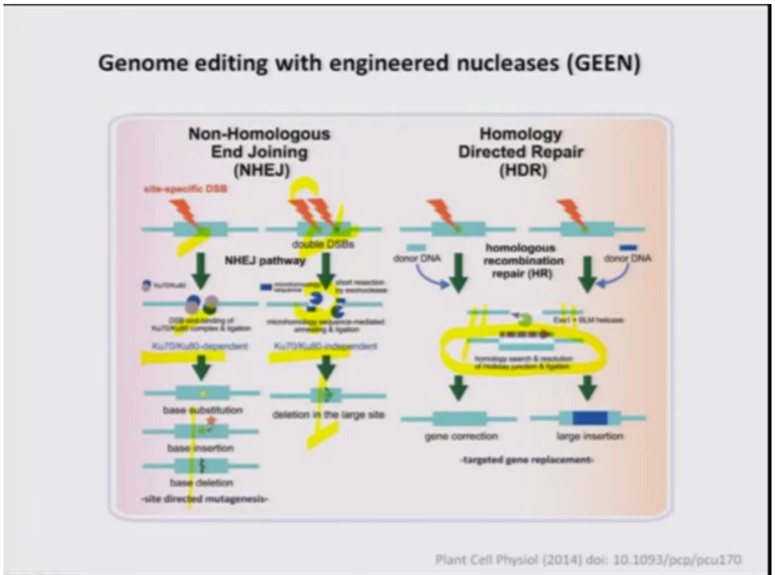

So before we get into let us look into this two process one is called to non-homologous end joining process and homology directed repair or recombination what we discussed. Basically this is what you are talking about say suppose you have an enzyme which goes and makes cut in your genome it could be a single cut or it could be double cut depending on how many sites are there in given segment of the genome and then what happens in this processes once the cut is made our cell including human cells have the machinery to fix the damage because you know our cells that expose to various stress and some of them can caused DNA damage but immediately our system make sure that you know damages are fixed and and whatever you are talking about this proteins are nothing but the proteins that are involved in repair process.

Let us not worry about what is name and what is the specific function but there are two ways by which it can function say suppose you have a a single cut then, what happen is? It tries to you know polish the DNA bit removes whatever it is and then tries to you know seal the DNA. In this process what happens is that there could be few additional bases added at the site of you know you are the cut where it is made or it there could be few bases that have deleted. So it is a kind of random process it can be substitution, it can be insertions, it can be deletions.

So as a result you know you are going to have some change and some of them could affect the way of the gene functions because it can change the coding region so therefore the protein is not made. Your last (())(23:34) so it is basically altering the function of the gene but what kind of changes will happen you do not have any control. You expect that you know that would have some (())(23:44) function of the gene which is more likely to be. The other process is that you have selected the enzyme such that are you have identified a gene with certain you know sequence such that they you know you have made two cuts so if you have two adjacent cuts close nearby then whatever the fragment that is lost in between that is sort of degraded.

So what the cell tries to do is the chromosomal DNA which is much larger now because small part is last. This two are joined together now. Interrupt in between whatever fragment that was that is last. So as result you are going to have a mutation which is more like deletion. Say suppose a gene given exon say its 2 kb and you have two sites you know which is separated by 2kb fragment. If you cut then allow the system to repair. You have lost the two gap in between and you have sealed it therefore the genes you know two exons or one exons whatever is last.

So that's the way to do it. The other is more in mitotic cell is this homology directed repair where basically this cell looks at region that are sort of complementary you know and then you are able to go on fix it so for example you can you can have a construct kind of where you have region that are homologous then it can go and try to stich identify that stich and so on. So this a kind of a you can create insertions using this method but this is you know you cannot use it in all the cell type so depending on what you want do. So that's that is the concept behind this whole thing.

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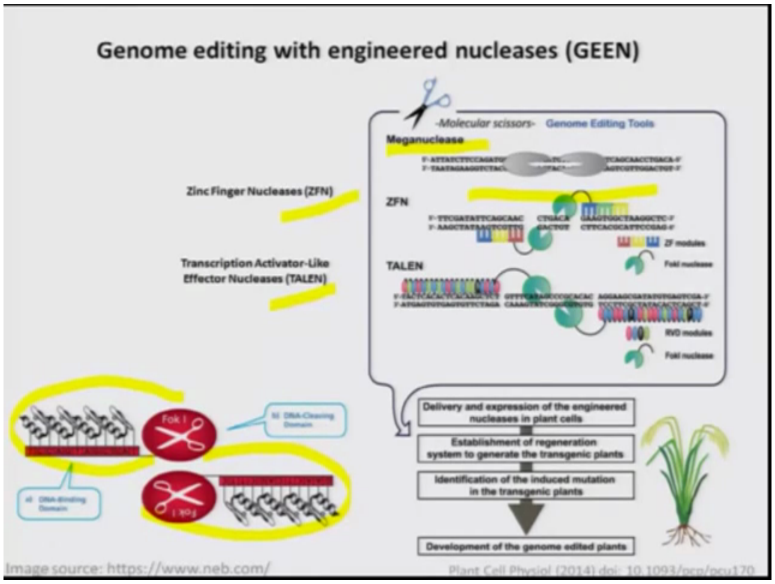

Let us look into what is this nuclease. What are this nuclease is people use for genome editing. First nuclease that that people start using was like restriction enzyme as we said so we have you know a domain which is basically domain that is able to cleave the DNA just like a restriction enzyme. But restriction enzyme are two domains one is the nuclease domain which helps the DNA to if the enzyme to the cut DNA then you have the other domain which give the specificity as to where the enzyme should go on cut like as you have see restriction enzyme. They have a unique you know recognition sites the given sites sequence are identified by enzyme and it goes and cuts there.

So here to it's a same principle so you have DNA binding which is the only difference here is that the recognition sites are very large like to be 14 basese,16 bases therefore the frequency of such sites in your genome is very few right you know the probability that that you would find that kind of combination is very low and therefore it is not going to if you have used smaller you know enzyme that identify four base then it is going to chop off your genome that not desirable because it it does not allow the cell to cell bind.

That here it is very few sites therefore you are going to make a cuts in only a few sites so that really helps right. So where are the enzyme the earliest enzyme people have used is this enzyme called Fok1 and then of course what they have done is? They have deleted the DNA binding domain of that particular protein and then try to fuse it with some other you know DNA binding protein therefore you made an engineered enzyme. So by doing this you can make this nuclease Fok1 to identify whichever sequence that you want right or you know you much more you know control over which segment it can go cut. So let us look into two such examples one is what is called as a zinc finger nuclease right the other one is called as transcription activator like effector nuclease okay.

ZFN is the abbreviation and tell this one. So let us see what it is both use the same concept that you have a DNA binding domain which gives a spite sites specificity and then you have this nuclease domain which cuts very close to the domain. So often this enzyme function is dimer because you have one right and then you have the other one therefore you know you can make two cuts and so on. So that is what shown here in figure for example it all started with the enzyme called Meganucleases now that had limitation because it can cut only such sequence wherever it is there right. Then they have engineered such in nuclease with what is called zinc finger nuclease. Here the zinc finger is nothing but these are the structural domains that are present in many transcription factors.

So you must have studied about transcription factor. These are set of proteins that goes and identify certain sequence that are located on the promote original of the gene. So therefore you can restrict which gene is turn on or turn off. Most often these are activator so these proteins go on bind to such sequences and then activate and more often such sequences are present in repairing room to domain in a two segments and they go as function as a dimer and so on. So what they have done is they have used this DNA binding domain and the fused to the nuclease domain such that you know a theoretically you have these segment identifying these sequences and then you have this nuclease domain likewise you have another molecule of the same protein bind here and the nuclease domain it can make a cut wherever you want.

So it is not as simple what they have done is they have created large in number of mutants for these zinc finger binding domain and such that they looked at what are the sequence on which it can binding. In fact they can you know if you have a disease sequence then they can engineer the enzymes such that you will identify that sequence. So what you have done is? You decide where do you want create the mutation and then create DNA binding domain which can bind to that sequence you know thats the way now we can tell your enzyme to cut wherever you want.

So that kind of you know system is award there are you know now there commercial companies which have specialized in making these enzymes one of the Signal Reach, so what they do is that you give the sequence or they predict what sequence in the region that you want to delete and then they engineer the protein such that it will identify the sequence. There is a you know laborious process involved in it. The other one is very similar to zinc finger domain but they have used in enzyme again not enzyme sorry its transcription factor which are normally made by the pathogen that are infecting plants. So these are the pathogen that you like our virus and others they come and get into your system and then hijack your system. For example virus can get into your cell and then hijack use the same machinery transcription translation to make their own protein.

The same way these are the protein that that all made by the pathogen but now hijack the system so they have used these you know the gene that code for these proteins. That are transcription activators to engineer genome mainly the plant genome for example what is shown here is the plant genome. So again the concept is very similar you have your DNA binding domain fused to the nuclease domain and you can engineer such that whichever sequence that you want now we will able to cleave over then.

So the basically you make this protein and then deliver so you make the protein to get in the cell and express there right and then once the protein is there is going to cleave that a region of the DNA and you know if such kind of cuts are made then cell tries to repair in this process as we discussed little earlier. There may be lost. There may be gain. There can be alteration as result the function of the gene is lost right. And then he allow the plants to grow and look at decide phenotype in case of plants in case of animals are cell lines likewise we can do that. You know you can go for, for example the embryonic stem cells you can introduce these nuclease there something is happening you, you know look at the cell whether desired change is happen. If it is happen then go on create the animal, so it is going to have such kind of changes.

So this is another robust method because you are not really looking at all those construct that we discussed. So the time spending making constructs you know you no longer we have to do. You can engineer the nuclease then introduce into the cell it will do rest of the things. So it becomes much more easier and more efficient as compare to the knockout that we have discussed but again this is global thing so it is not a conditional right so that’s let us see another process but the more recent development in genome editing is this what is called is CRISPR cas9 mediator genome modifications and these is come up in huge way because it uses very different kind of things.

So in fact you can now you know the previous the tools that ZFN and the other one that we spoke about so there you are depend on a protein whose binding domain detects as to where the cut can happen so you know you have to think of certain sequence on to which you know you know your protein goes and binds and there could be complication that time because the sequence could be such that you know you are unable to get the DNA binding domain for that particular sequence. The complexity makes it of the genome where do you want to mutate cannot times limit your efficiency with which you are able to generate such mutants of DNA binding domain. So you may not be able to get them so that is the limitation because you have to screen large number mutant that times it does not work out.

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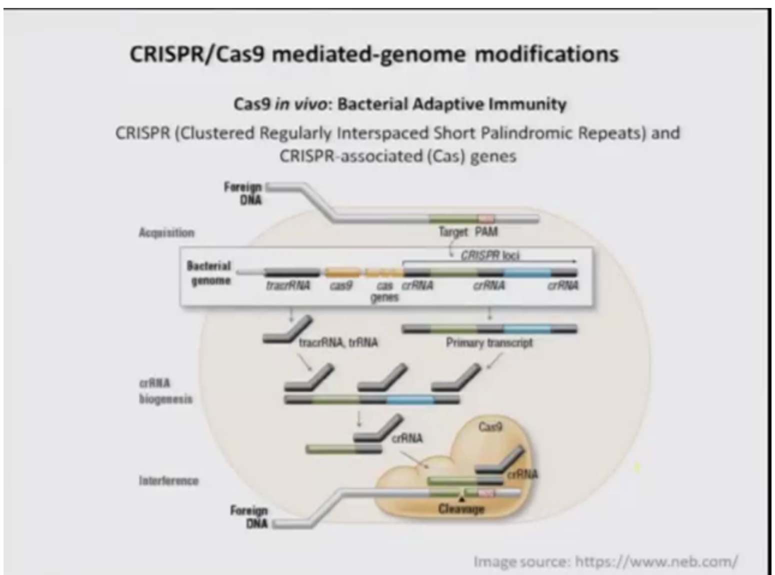

So this CRISPR cas9 really does not bother about such DNA binding domain right so before you get into let us see what is these system is and from where we evolved such kind engineered nucleases. So basically just like we have immune system in our body where we have you know cells that fight against any foreign you know bacterial my virus that gets in the bacteria also has a immune system and these cas9 is one such system which prevents the entry of for example any foreign DNA bacterial fights for example because this is they infect that bacterium and and they or some other viruses can introduced the DNA.

So they want to be immune and identify such DNA and remove them right. So this is pretty much that's that's the mechanism that it uses this called as clustered regularly interspaced short palindromic repeat. Pretty long sequence but what it says is this you have segments in the genome are the bacterium in which there are palindromic sequences. The palindromic that you have for example abcd this is the sequence and then it would be reversed for example cba right that's the parameter right now such kind of short sequence palindromic sequence are clustered meaning present close to each other but these are enter space meaning they are not continuous but there are other sequence in between.

Such sequence really help in you know fight against the (())(35:09). So therefore it is called as you know abbreviation of this cluster regularly interspaced short palindromic repeats called CRISPR and then you have you know genes that are associated with these cluster right CRISPR associated therefore they called as cas9 right. So whatever cas genes that's what it is. So how does it work so you have what is shown here is the bacterial genome and then you have these cas9 gene and then close to that you have this you know the clusters right and and the idea is that in the wherever you have this the clusters then RNA is produced and then RNA really helps as a surveillance goes and looks for any foreign DNA if it is then it it gets degrade. How does it do that?

So as in when your foreign DNA comes in because of an infection what happens is that these system really takes part of the DNA and and sort of integrates as spot another bacterial genome around these clusters. Okay it puts that just like your immune cells if there are any foreign body get in it you know you your immunes cells attacks them digest the protein and presence part of this protein your image complex right therefore the system is alerted as to these are the peptide that possibly are present as foreign invader, exactly the same and then this cluster is activated therefore the transcripts are made the transcripts are such that you have this you know fragments along with the foreign DNA sequence.

So what happens is this RNA goes and looks for homology and wherever you have a DNA which is homologous to just that has got incorporated now it is go into you know degrade that you know cut that DNA and and that try to degrade this is what this how it is done. So in in other words you are going to cut a DNA whose sequence matches the RNA that cas9 gene regulates right. This is this is basically or this this CRISPR locker regu regulates so like what you shown here so we have a primary transcripts right which are made and then they have this region and this is what called as crRNA and if wherever it finds homology and its going to cut okay. So this have it is. So this is to protect against any invader that comes into the bacteria.

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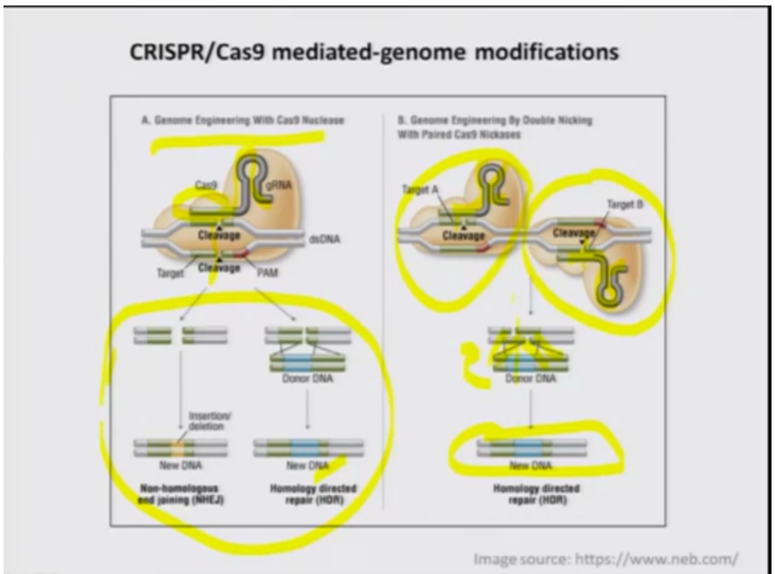

So let us see how we have engineered that you know complex a mechanism to our own advantage therefore we can edit our genome the way we wanted. So there are two ways by which we can do this is the first one that have shown on the left side is genome engineering it cas9 nuclease again it is a you know a nuclease because it cuts the DNA what’s the different between this and the ZFN nuclease that we have discussed earlier. So over there the nuclease is dependent on a DNA binding domain to identify a sequence where it has to cut here the nuclease is dependent on the RNA that comes along is the system therefore the RNA would look for homology for that particular RNA sequence wherever in the genome you have its going to cut it.

This is the RNA that is shown here and what you can do is we can engineer the RNA such that you put a small segment of the sequence which is complement it to the genome where do you want make them cut okay. So if you make the cas9 complex along with RNA which is called is that guide RNA because it really helps the nuclease to go on home where it should cut so now you if you wants for example gene X I want cut so I take part of that sequence that is in a critical region of gene X and make that RNA then is going to go and position the nuclease around gene X right. So then it can of course the nuclease it makes the double strand cut exactly the same way that you made and then allow the same mechanism so your cell is able to repair either by you know the non-homologous end joining or the other homology directed repair so in this process either you can delete a region or you can put in a new sequence like you know insertion so the same approach that we discussed earlier.

This is just to either delete or create mutation or to introduce a small segment into the genome but you can also the just like the way we discussed in this other two nucleases which has got DNA binding domain you can also engineer such that you have two complex that of the cas9 which sit close to each other you know segment of the genome because we can design the guide RNA such that it falls very close to each other of the genome and then what happens it cuts these are mutants of these cas9 which are called as (())(40:17) it does not make a double stranded cut but it just you cut one strand of the DNA right these are mutants that are derived.

As a result what happens now, again your system price to you know because of the nics that are created intervening sequence will not last then you can again put a new homology director repair mechanism it can it can help you to integrate your foreign DNA because it pretty much looks for the homology so it would only put the DNA where such nics are made. So it gets in and you can create the kind of sequence so for example you want to fix a repair you know fix a error in DNA there was mutation and you want to rectify the mutation put back into the while type so you can use these approach and therefore you know these new DNA can go on for fix this. So this is doable in this process you can correct the error right.

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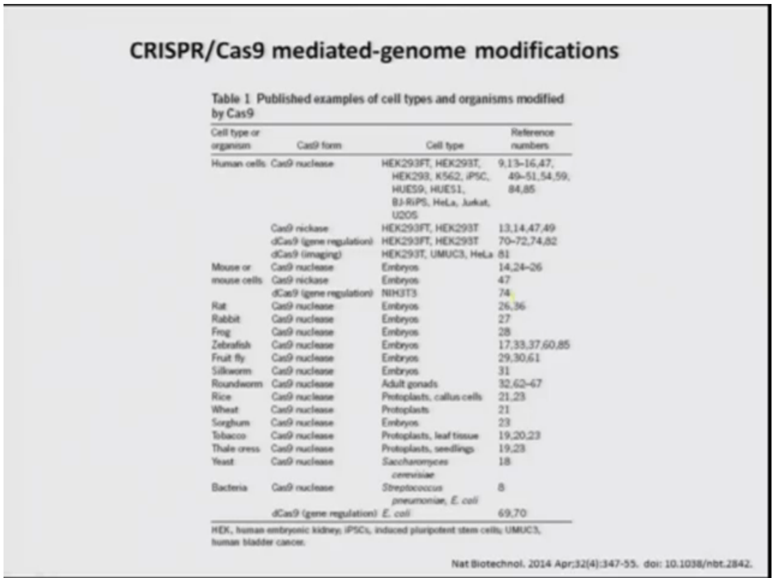

CRISPR/Cas9 mediated-genome modifications

Table 1 Published examples of cell types and organisms modified by Cas9

Cell type or organism	Cas9 form	Cell type	Reference numbers
Human cells	Cas9 nuclease	HEK293FT, HEK293T, HEK293, K562, iPSC, HUES9, HUES1, BJ-RiPS, HeLa, Jurkat, U2OS	9,13–16,47, 49–51,54,59, 84,85
	Cas9 nickase	HEK293FT, HEK293T	13,14,47,49
	dCas9 (gene regulation)	HEK293FT, HEK293T	70–72,74,82
	dCas9 (imaging)	HEK293T, UMUC3, HeLa	81
Mouse or mouse cells	Cas9 nuclease	Embryos	14,24–26
	Cas9 nickase	Embryos	47
	dCas9 (gene regulation)	NIH3T3	74
Rat	Cas9 nuclease	Embryos	26,36
Rabbit	Cas9 nuclease	Embryos	27
Frog	Cas9 nuclease	Embryos	28
Zebrafish	Cas9 nuclease	Embryos	17,33,37,60,85
Fruit fly	Cas9 nuclease	Embryos	29,30,61
Silkworm	Cas9 nuclease	Embryos	31
Roundworm	Cas9 nuclease	Adult gonads	32,62–67
Rice	Cas9 nuclease	Protoplasts, callus cells	21,23
Wheat	Cas9 nuclease	Protoplasts	21
Sorghum	Cas9 nuclease	Embryos	23
Tobacco	Cas9 nuclease	Protoplasts, leaf tissue	19,20,23
Thale cress	Cas9 nuclease	Protoplasts, seedlings	19,23
Yeast	Cas9 nuclease	*Saccharomyces cerevisiae*	18
Bacteria	Cas9 nuclease	*Streptococcus pneumoniae*, *E. coli*	8
	dCas9 (gene regulation)	*E. coli*	69,70

HEK, human embryonic kidney; iPSCs, induced pluripotent stem cells; UMUC3, human bladder cancer.

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So this CRISPR cas9 has become so powerful there ten within few years of you know introducing the system you will find that its application these one are the paper that came over two years back which talks about right from human source to we can see that there are few examples here human cells to mouse, rat, rabbit, frog, zebra, fish and what not from plants to bacteria to yeast you know you are able to edit the genome the way you want. So it is very very you know robust technique and wide applicability and and all you need is your sequence of the genome because you want to create guide RNA that is good enough. So in since now we have discussed there are so many species whose you know DNA has been sequence. It is much easier for us to really do such kind of editing so it really helps.

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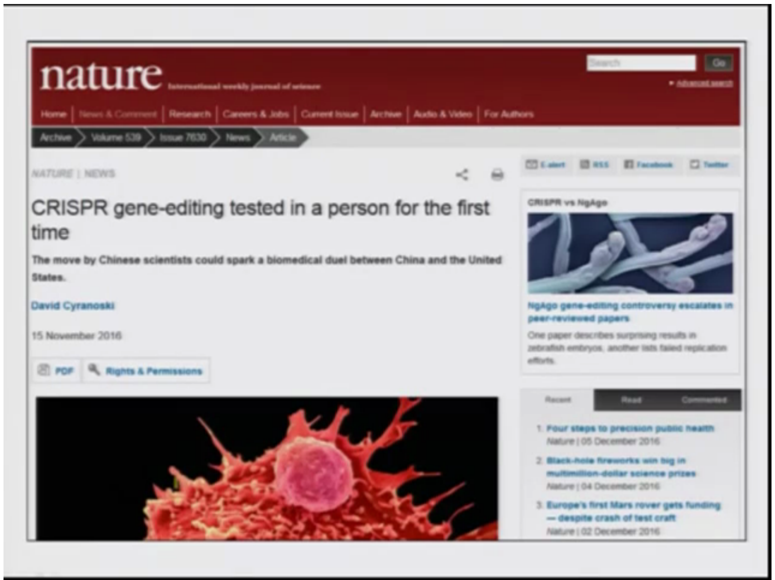

So it is not only restricted to the the you know the cell lines are animals in fact very recently last month in November 2016 the first attempt to fix mutation leading to your particular kind of cancer in this case it is lung cancer in a human case was sort of approved a Chinese group really used to this approach. The approach is to take the cells from the human body and fix the error and put back the cell therefore you know you are able to control this so we need to wait and see how good this approach is but it the promise is you know is enormous and people are thinking that if really works have and if it has the kind of specificity that we are talking about you should be able to fix the genome they may be want and it could be a powerful (())(42:57) potential this particular approach is called.

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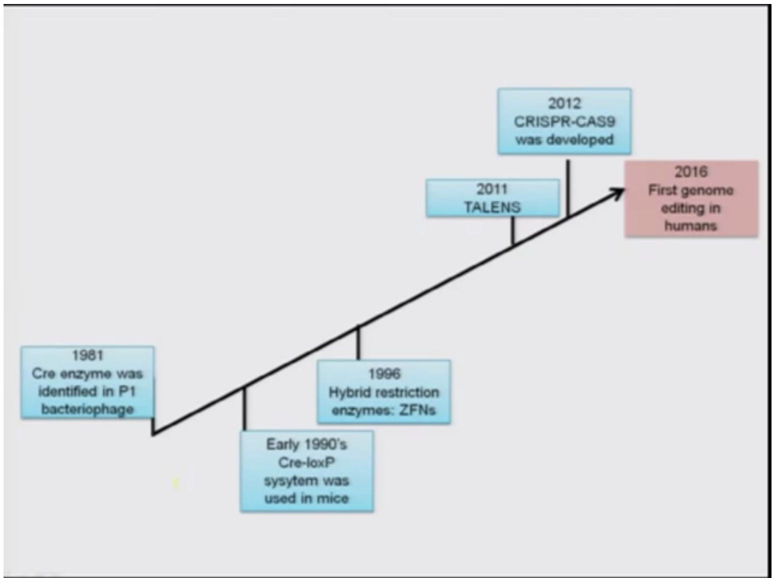

So that's a you know that's that is sort of brings an end to this lecture and then we will see the rest of the technology that help us in editing the genome in our next lecture.