Functional Genomics
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Lecture No 06
Introduction to Functional Genomics

Welcome back to this course functional genomics, you know in the previous lecture we have discussing about how the genome editing tools have really helped us to engineer the genome you know in wide variety of species. So we are going to continue the discussion and see what are the other ways by which you are able to engineer the genomes, right.

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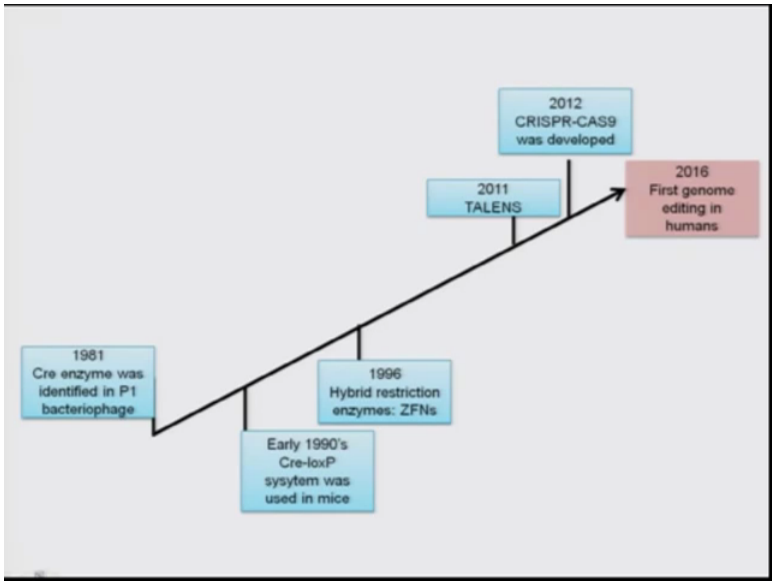

So what is shown here in this slide is some of the approaches people have used to create you know or engineer the genome. We discussed the cranes and which what is it leave us described in in the bacteria of bacteria forge or bacterium system and how that can be used for you know deleting the genes in the mouse and then we came and looked at the hybrid restrictions enzyme is called as a reference and then we looked at the recent development that is CRISPR - CAS9 and then we also discussed how this CRISPR-CAS9 has helped in editing the genome at least as a clinical trial in humans right. There are many other such advancements some of them we are going to discuss today or the following.

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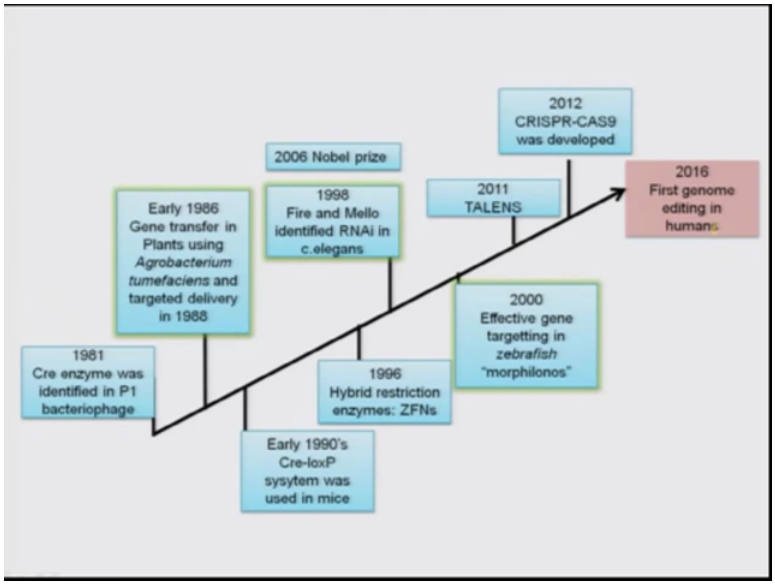

One is in early 1986 people have you know started engineering the plant genome and that is done probably you know it is covered widely in your text book called the you know the TI Vector and so on. So it is kind of a delivery vehicle like the way we have used plasmid and these are agro bacterium which are able to infect the introduce the DNA into the plant cells at least in the culture then we can you know grow a plant out of it.

That are the very first step in introducing the DNA into the plants but now we have a variety of you know approved by which you are able to you know kind of engineer the genome or affect the way the genome functions. One such very tall discovery that led to the noble price was called as RNAi or RNA interference which was first introduced in studying the gene function in a very popular model system called C.elegans it is a worm is a nematode caenorhabditis elegans.

And people have used this model to understand how the development takes place and how the genes regulate development. So this is called as RNAi because you create a kind of a short RNA that that can affect the function of a given gene right and then of course very similar powerful tool has come especially in again understanding the development of another model system zebra-fish which is called as the morpholinos and both these systems have not restricted its applicability only to this systems. But you know you can go and use it in wide variety of systems and that is something that we are going to discuss.

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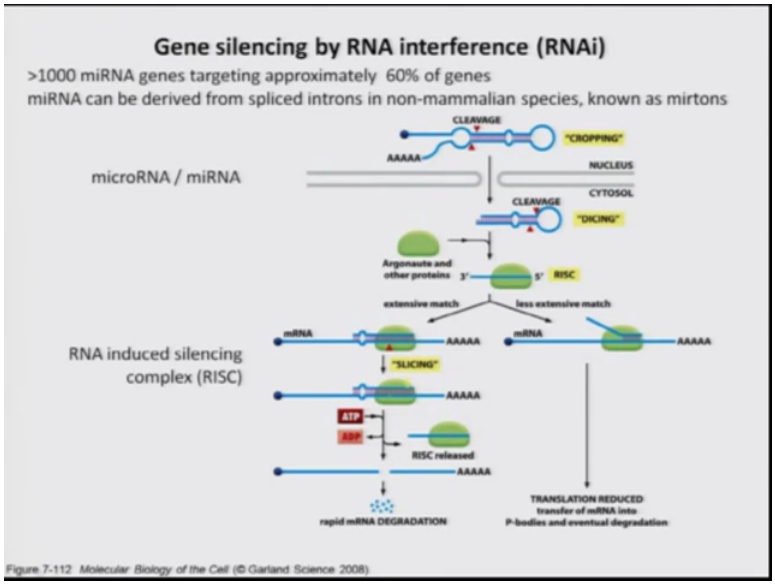

Let us first look into how this RNAi system functions. So the RNAi is you know a way by which you are able to silence a gene when I say silence it is not that you are preventing the expression of the gene but you are doing something that equivalent to almost the gene is not being expressed. So the way we do is we use RNA to interfere in the function of the gene and and its you know as it is known as RNA interference so in short people call as RNAi, so what is that so this is one of the mechanism by which our own system is able to regulate the half -life of a variety of mRNA.

For example you know for everything you know if you look into the system, if you have driven a two wheeler or a four wheeler which are automobiles you have, you know what is called as a gear box you know you change the gear then you accelerated you are able to go fast. So whenever you need you can go fast or you need more power then you go to you know shift to gear one or two and then you are able to give more power at the same time you have another system to break to stop the vehicle.

So you have to have all these controls, exactly the same way the cell is able to regulate the gene when you have more amount of protein for a given gene how do you do it. There are various ways one you can make the gene to make more copies of the RNA, so in a given say one minute you make hundred copies of messenger RNA right this is one way. The other way is let the mRNA stay for longer therefore they can make more amount of protein because everything has a

half-life. The RNA has a half-life the protein has a half-like, so you make the RNA to live longer therefore even with hundred you know RNA you are able to make more proteins because this is another way of doing.

So exactly the similar mechanism can be used to regulate it. You have made RNAs and RNA are being converted into proteins but now we have to shut it down. We do not want protein to be there so what we can do we can degrade the protein or we can make it inactive but if even if you do that if RNA is there it is going to continue to make the protein right. So what way you can do that even if you shut down the gene the RNA is going to be there so what way we can do we have to degrade the RNA.

So how will you make it specific only certain RNA are degraded and so on. Our system has you know small RNAs which go and target a given set of messenger RNAs and that targeting meaning it identifies sequence it goes on binds and help the system to degrade those RNA that have the complimentary sequence, so that is what is called as microRNAs or miRNAs that are present in our system that is show in the schematic.

So you have these RNAs called as the miRNA or microRNA which are you know single stranded RNA that are obviously which are made but they have sequence that are complimentary so therefore they can fold back from such kind of base pairing like a loop and these come out of the nucleus to the side of the side of the plasmid where there are enzymes like dicer and risk complex and many other which we can look into the details when we share the you know links for you which you know sort of cleaves them and make small segment of RNA like what you see here.

So these 20-25 base sequences you know go and bind to most often the un-translated region of the you know RNAs messengers the 3prime un- translated so when they go and bind they have two distinct function. One for example now when they bind like this something like what is shown in here now this can result in you know the translation arrest now the RNA is not being translated. The RNA is there but they are not allowed to translate the cell now gets the messenger kind of message that when this microRNA comes when they are cleaved and small RNAs are being made.

And they go and bind and then prevent the translation of the protein so we are able to block the translation, the other you know function of these such RNAs whenever there is the complementarity between the short RNA and the target mRNAs large like what is shown here extensive then what happens that it goes and forms this kind of a duplex with the RNA and then you have of course enzymes which go and recognise this as duplex and then degrade the RNA.

So mRNA is being degraded so this is the way you can do that so what people have done is that you look for you know RNAs that you can make something like this for a given gene and introduce inside the cell or animal and then using our own machinery to degrade the RNA you can degrade the RNA that you want to degrade therefore the cell is almost like not having the protein, so the only difference in here is there is not that the knock down what do you call here that is by degrading the messenger. It is not 100 percent right it can be 50 percent, 60 percent, 80 percent, 90 percent but never be 100 percent but still it is good enough because even if you have depleted a cell of a given protein up to 90 percent that is almost not having that protein.

So we can see a given phenotype so that is the advantage of the system so what is interesting that you may find is that there are about you know more than you know thousand genes that make this mRNA right and they target about 60 percent of our genes that are there, so it says that the majority of the genes that we have owns you know RNA. Their stability their half-life whether it is translated or not translated is regulated by such short RNA called as microRNA right.

So and where are these microRNAs comes from miRNA comes from. They come from a variety of source it could be simply a small segment of intron of any other genes which is spliced out. Now we believe normally the textbook says the intronic region is spliced out but we never bother about what possible functions it has. It looks like that spliced out RNA is called also function as a regulatory RNA and you know its its there are its present in a wide variety of systems not restrict to only two humans right.

So that is one information so let us see how we can do that so we can use the same machinery you have a machinery in a cell which identifies such RNA duplex you know short RNA bound to miRNA therefore it can degrade so what you need to do is if you can provide the shot you know RNA inside you are able to you know regulate the level of that particular messenger thats what people have done.

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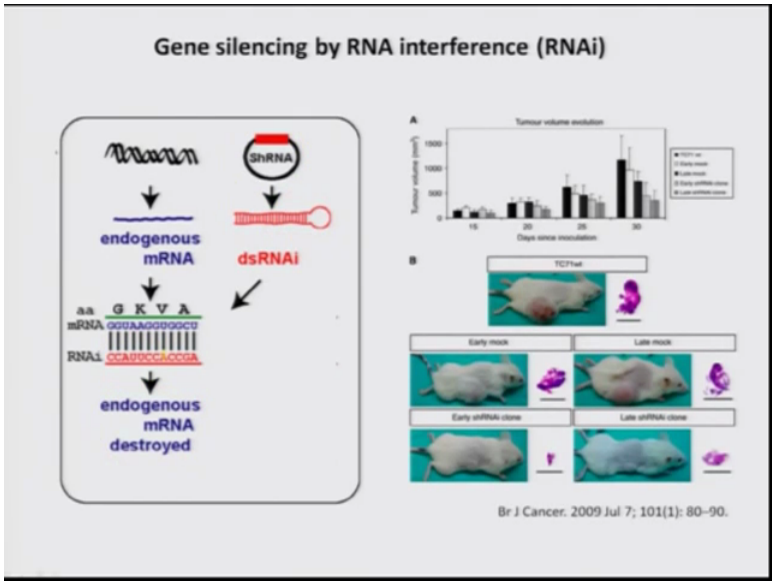

So one way to do is we can for example make a plasmid which expresses you know like a harping loop like in a sequence which are specific to for example a given gene transcript right. So when you make this and introduce inside the cell or you make the cells to make this then the one of his strands that is how the system functions goes and forms you know complimentary base pairing with a target RNA and then off course because its such kind of duplex are recognised where the machinery and they are being removed.

So we are using the same machinery that otherwise regulates the normal function of the gene. So in this way we can target any of this you can make any of the duplex of the RNA which is you know RNA at duplex and introduce inside the organism or the cell. The RNAs is targeted or you can make this kind of construct which keeps on making such duplex and then you know the cell is able to degrade the target sequence right. So this again has a wider applicability because you know for example what is show here is one of the papers that that as shown that you know in a given tumor.

We can see this is a mouse model with the tumor that is growing because we can you know place few cells that have the tumor of genecity and the tumor grows in the animal, so if you want to really test any other drug that can regulate or reverse the growth of the tumor than you can use this model and that is what they have shown for example you know there are two different RNAi construct meaning the different RNA target they have looked at and they have delivered this

RNAi duplex into the tumors and they found that if you inactivate a given gene you know in the system.

And the animal is not developing such big tumor as you have seen otherwise right in other words if you can target a specific gene and block its expression using this method RNAi we are able to control the growth of the tumor right. So it has a tremendous potential, now what it says now you can pack this RNA duplex into certain chemical capsule like balls and you can target it to whereever you have the tumor and if they are delivered there then that is going to inactivate the genes by degrading the RNA that are being made as a result the cell will not grow into tumor or we can regress.

So in this along with other combination of therapy you will have a better control over treatment right so that has the potential so this is one way we can do. The advantage is that it is very simple we can we can make we can target any genes that you want because all you need to make is a small segment of the RNA whether the duplex or the shRNA that you make so much easier and it also allowed people to look at knock down a large number of genes in fact we have now libraries for humans all the genes for which you know you want to study you have libraries of the RNAi.

So simply select what you want and you can knock down and then study. This also has application in other field for example you can engineer plants to make you know double stranded RNA for a gene that is not present in the plant but in the parasites or nematode that infect plants right.

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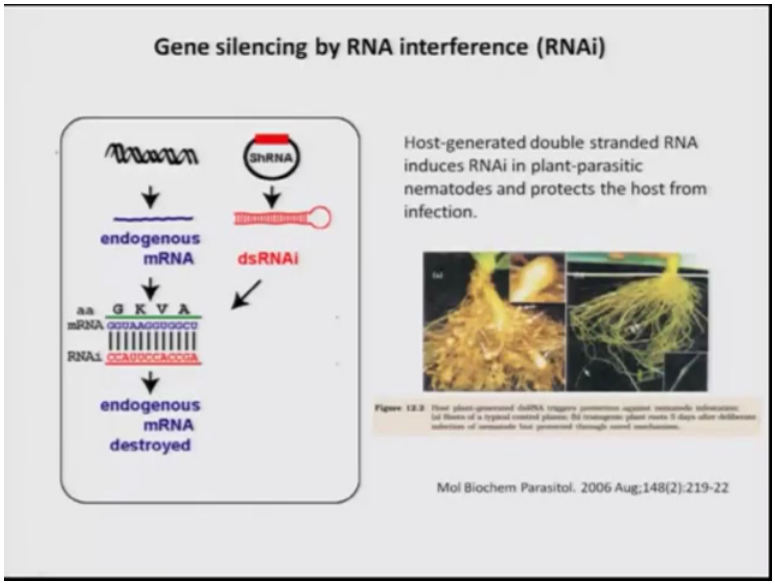

One of the example that is shown here that is come from our own institute from IIT Kanpur wherein they have engineered the plants such that it makes a double stranded RNA for a gene that is normally critical for the development of parasitic you know young ones the larwa forms. So they are the one that infect the plant and what is shown here you can see in this left side is that this is the root of a plant which is a heavily infested with the parasites, so the parasites gets in and they form this no dues and as a result the plant do not grow well it is the infection.

But if you can make the plant to make a double stranded RNA for you know some of the genes that are critical for the development of the worm the nematode the parasites then what happens even if you now infect the plant with these nematodes because the nematodes eat the plant cells for their survival as a result the double stranded RNA gets into their guts and that can sort of you know trigger a systemic you know kind of a silencing of the gene as a result the nematodes do not grow and therefore the infection is minimized.

So these are some of the applications really powerful applications using the RNAi. This techniques that you spoke about the RNAi and others these are you known some of them that are very specific to the genes because you want to delete the genes that you want or mutate a gene that you want or alter the gene that you want but there are also other methods people have used which are not target specific but phenotype specific meaning I am looking into a function say for example vision using Drosophila model.

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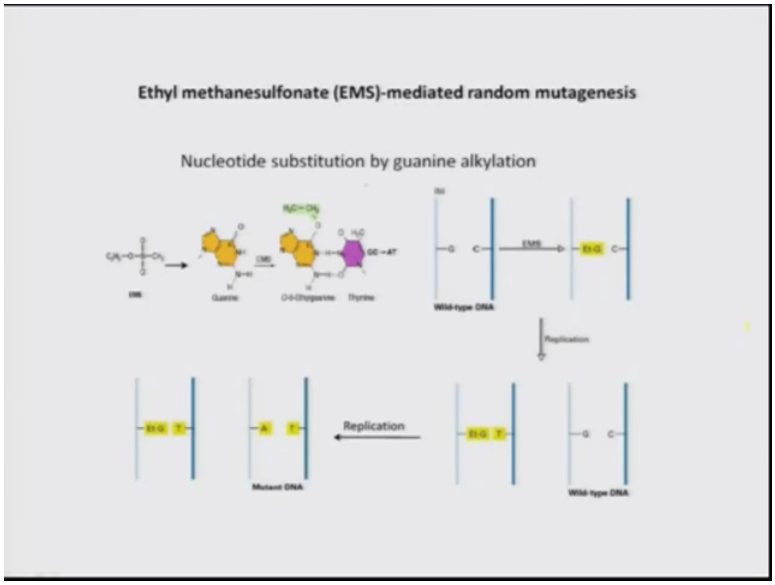

So what I do I expose the fly to a chemical what is shown here is Ethyl methanesulfonate EMS they call it and if you expose the animal to this chemical and this chemical is known to cause mutation but normally these are base changes. What happens is chemical especially with guanine it interacts with and then it makes what is called as co-ethyl guanine that the way it is changed, as the result what happens as this gets changed now this G no longer pairs with G but it can pair with T.

So if you have a cell in which such kind of modification took place and that cell is dividing then what happens now this ethyl guanine now sort of mimic the base pairing as if it is A and the new strand that is being made will have T in place of you know C, so in that way you are able to change the base and if it happens to B a region where there is a codon and the codon is altered then you are going to have a different amino acid.

If this is the reason where you have some transcription factors you are going to bind so it may not bind and things like that so you are creating mutation randomly and you are going to screen a large number of (())(16:52) the that come out and then look at the phenotype and if they have a desirable phenotype like for example loss of vision then you are going to look at where this mutation took place and then you can go back and map you know the genome right so this is this is other much simpler method people have used right.

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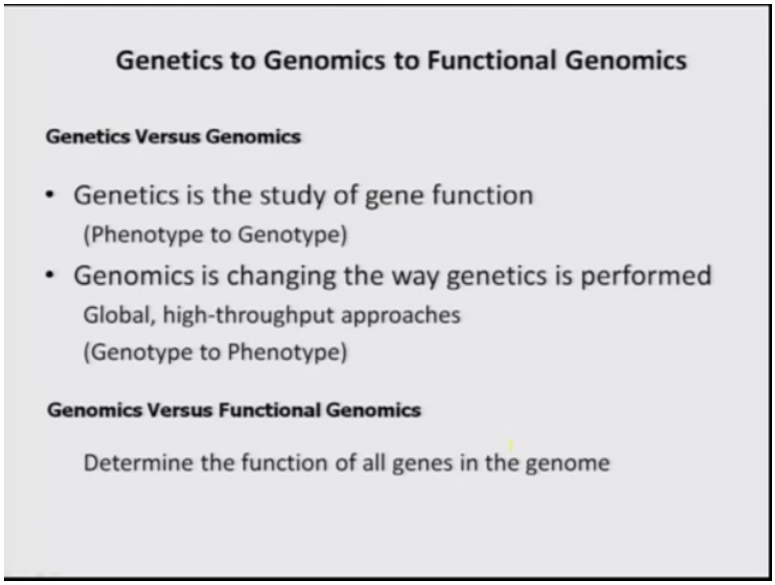

So there are ways what we discussed is how a genetics the forward genetics and then you have come to genomics we have understood large number of genes and that now called as the genomics wherein you know every gene you done not know what is the function you knock out and look at the function, now what you are going to do discuss is you know the topic that is functional genomics. How this has led to what is called as understanding the function the entire you know genes that are present in the genome as to what function they do.

So genetics just let us look into this genetics versus genomics, the genetics is the study of the gene functions so normally if you look into the classic Morgan model you have looked at the phenotype and try to map it to a region of the genome and then find the gene and then say this word is. Due to you know the way because now you have understood all the genes as to how many genes are there what kind of protein it can code for but what you do not know is what is the function.

A transcription factor may be helping you either to digest the product or it can be in your vision or it could be something else. So the protein per say does not say what is the final function in terms of phenotype. For that what you do you delete the gene and look at the phenotype right. So this is the genotype to phenotype that was possible you know from genomics point of view but still there are challenges so how are you going to assay like you know I do not know when I find

a new gene using a genomic approach. Now I delete it but I need to assay the function of it so I may not be able to see everything at the phenotype level.

For example a gene is involved in cell division and I knock out that gene so I will never see that phenotype because the every time you delete the gene the cell will not survive because it cannot divide. So in this approach given I have created a knockout or whatever it is, it is not going to help me in dissecting this. So you have to have a powerful assays to understand the variety of functions so here the assays is, the assays is dedicated to a particular function for example growth, cell division or the reverse of it.

What are the genes that help in the cell death right so all our cell have a finite division after that they cease to you know divide and they die, so there are now you know that there are genes that trigger cell death or pro apoptotic protein. So what are these proteins what are the genes, how do you screen for it, right. So it depends on what kind of assays so that is what called as functional genomics. Determine functions of all genes in the genome. So one assay is not going to help you screen the function of all the genes.

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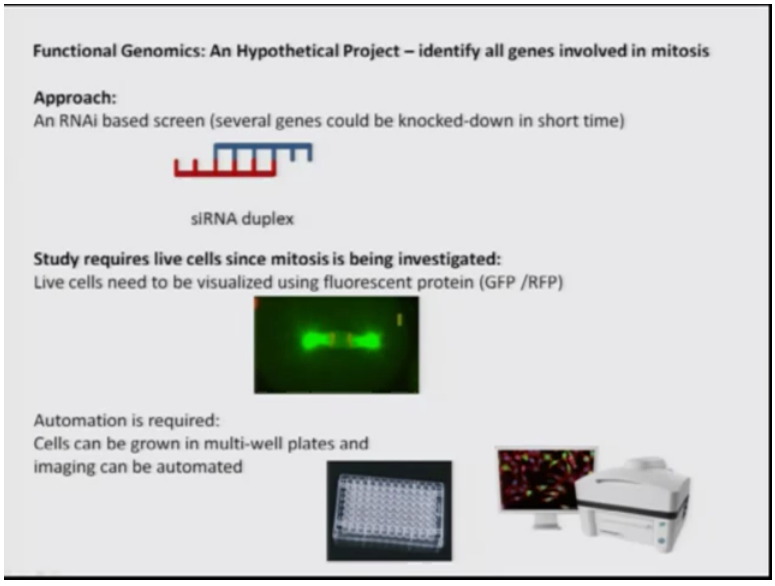

So we are going to look into some hypothetical projects I am going to show you then some examples from the literature how people have used this functional genomics approach to understand the function of a large number of genes that all of them function in one particular

pathway. Let us say our project is to identify all genes involved in mitosis cell division right we have all growing because of mitosis if you have a wound cut in your body, it gets you know filled up and healed is because of mitosis, so everything you know envy mitosis right.

So let us see so will you identify all the genes that are there in your genomes that help in mitosis right. The approach is you have now again you have to go for appropriate but there are we discussed a variety of approach we can use it for genomics applications. So let us look into a simple approach RNAi because human mitosis we are going to study and then we have the siRNA duplex for every gene that genome has, so I have the tools so I am going to introduce this double duplex into the cell as a result what would happen is that you know whatever RNA that carries this segment will be degraded.

So therefore the proteins in that cell would go down and if they are very critical for the function that function lasts so you can use a large number of genes for the screen because simply I have to you know put them into the cell. Second this requires live cells because I am studying a process which cannot be studied in a dead cell in the sense I am talking about cell division. So I have to look at cells that divide so that division is a process that I am going to score for. So I should be able to look at cell that are live so how will you do so normally when you talk about microscopy it is difficult to you know staying cells unless you have fixed them and you are using as antibody and so on.

So therefore you have to come up with ways so people have now we can use this called as fluorescent protein these are proteins that are normally present in marine animals which live in conditions where in it not that bright. They emit light kind of fluorescent protein these are called as GPF and people have engineered this GPF to make you know proteins to fluorescent different colours for example there could be RPF red fluorescent protein, yellow fluorescent protein and so on so what I can do is that I can use a protein which are normally present in your cell such that the protein in fused to, now GFP so now the fused protein would do the function of whatever the function the protein is be able to do but it also would flourish right so it will tell you where the protein is.

Say suppose if I am using a protein that binds to the spindle you know the fibres that bind to the chromosome and pull you must have studied in you text book. If that spindle forming protein as

GFP it would look something like this so the spindle would flourish then I can see that cell I say ok this is a particular cell division or not dividing at all, so I am able to analyse right. What is the other important element I should be able to automate so I am looking at some 40,000 genes to screen so anyone or hundreds of them could be involved in the process but I cannot you know study this manually.

If I have to study the function of every one gene out of that 40,000 from this particular process, I will be spending hours and hours with just for one gene to you know because its cells takes about 24 hours to divide. So at the maximum I can finish in 2 days or 3 days I can confirm that one gene whether it is involved or not involved. So it is not possible because you cannot spend without you know basically sleeping sitting at the microscope and observing its difficult tedious process so you have to have high through put meaning you should be able to look into a large number of samples at the same time it is automated meaning you do not sit there.

Machine does that right, so this this is doable because you know you can have plates that you know you have 96 wells for example what is shown here but you can go much closer to 400 or so and each one you have seeded some cells and then in the each one of the well you have given the siRNA for a given genes. So in one plate I am screening for 96 you know genes like ways I can you know scale up the reaction then you have a system in which you know you are able to analyse the cell but not by yourself the computer does.

So you have given the patterns and their algorithms that looks at the imaging pattern because you are going to look at cells from the fluorescence and the algorithm would look at the you know the kind of patterns that the fluorescent gives and it will call ok this cell did enter mitosis or it did not exit mitosis right. So this way you are able to analyse and tell what are the genes that are affecting the mitosis or not affecting the mitosis obviously the genes that are not affecting the mitosis are going to much larger because they have a variety of functions but then you are going to have a list of hundred genes or whatever that are involved in the mitotic function that is the screen that we use.

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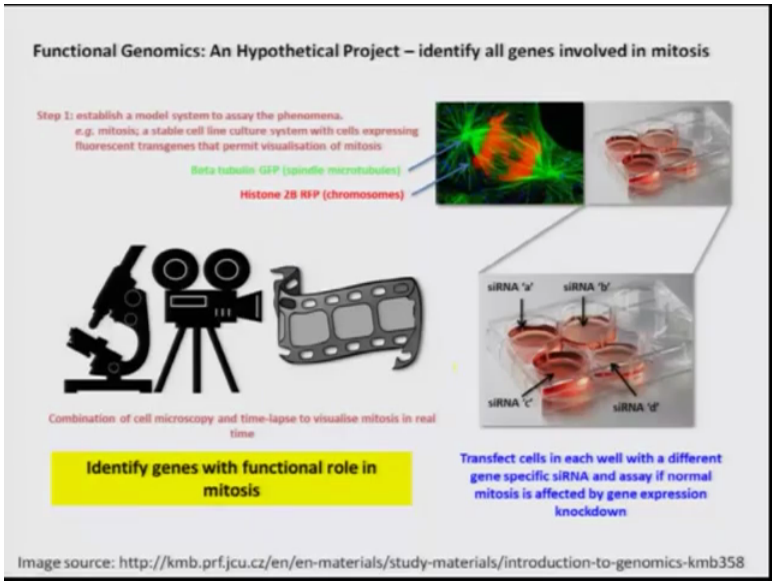

Let look into the flow chart how does it do so the step one establish a model system to assay the the process of cell division so what do you do, you know you have a cell in which you have engineered the cell line such that it is going to express a protein which binds to for example the tubulin the spindle micro tuble marker and for example histone which is a marker for thechromosome because the histones are the basic protein bound to the DNA, so what you are seen here beautifully is that these are the the red ones or the chromosomes and the green ones are the spindle that are pulling the chromosome to the two things so if I can make the computer to find this pattern in a if the computer was you know through microscope or looking at cells for 24 hours or 36 hours if that cell has shown this pattern that means that its able to you know divide that is what it is the system is able to score it right.

And and you are able to culture the cells in this kind of plate I describe and then you are going to what you do is that this is the process there is not a snap shot. You are going to look at the cell for a period of 36 hours because its division it is normally it is about 24 to 30 hours the cell might divide so you are going to look at that so the computer has to the camera microscope has to image it at the intervals for example every time minutes one it will take a snap. So when you have that kind of snap taken and then you know you run it in a continuous way then it would look like a movie and that image can be used for you know deciphering whether the entered

mitosis or existed mitosis or it is not at all entering or not at all existing. So that is easy to do in that way.

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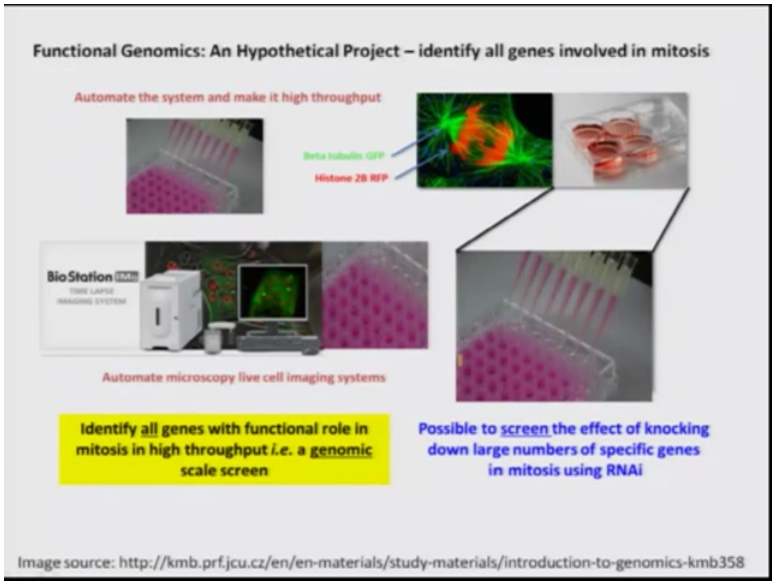

So then you have there are other challenges for example when you have 96 well pate then you want to add medium then you cannot take (())(27:09) keep on adding in one because then when will you finish dispensing all the liquid thats not you know, so you have to have you know either robotic work station or pipers that can dispense at the same time to either you know 9 well, 8 well or 16 wells whatever it is right so that that again helps and then you have for example a work station in which you know completely takes care of you know imaging at every interval and processing it and telling you all this things. So this is all doable and with this you will be able to at the end of 2 days - 3 days you will be able to call as to how many genes possibly affect mitosis.

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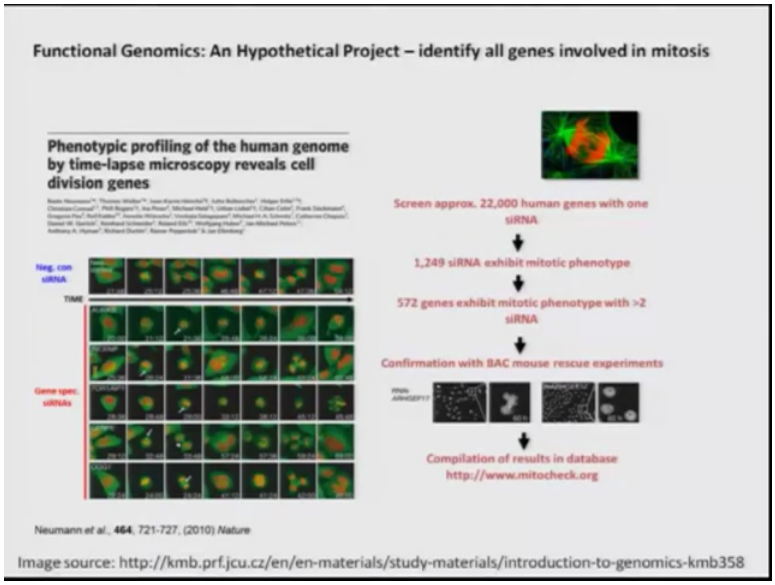

This is a land mark paper which used one such approach using RNAi screen to identify what you call as genes involved in mitosis cell divisions. So you can see that this paper came in nature and 2000 tonnes it is phenotypic profiling of human genome by time laps microscopy review cell division genes. What is called as human genome look at every gene almost and its function in cell division so now it is now what we have discussed earlier is that you are looking at the phenotype going to the gene through genomic approach you identify all the genes, now you want to delete the genes and look at functions now we are not looking at just one gene.

We are looking at every gene by using a high throughput screening you know automated process to identify set of genes involve in in a given function right. So you have to come up with assays and so on. So they have screened close to 22 thousand human genes and then then they found that 1249 genes siRNA exhibit mitotic phenotype and then 500-700 genes exhibited mitotic phenotype with you know meaning two different siRNA you can use and so on. Let us not get into that but these are validation right once you found some positive heats then you are going to validate and indeed that the case and so on.

So these are some of the images that are shown we can see that these are time lapse that is 21:48 hours and you can see that from you know 25 minutes, 46 minutes, 47, 47, 54 and so on and they are showing how beautifully this cell is dividing, you can see that if you look at very carefully you have the histone that is marking the chromosome, you have the spindle, you have the cell

divide so that is the normal process but in this conditions vary and knockdown certain genes that are like that shown here OGG or zenpep or whatever it is then the process is altered.

Either it is not entering mitosis or the cell division is not proper, you can see that the phenotype that varying the cells not properly dividing they are come together and it is not dividing that pretty much tells you that you know loss of this gene results in a phenotype that is not ideal for mitosis so here you went with an approach without really bothering about with genes and at the end of the screen you are able to identify hundreds of genes that are involved in the process. So that is you know high throughput process.

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Now I am going to talk about another such approach again its construct to be a land mark discovery wherein they identified human genes or the protein called it by the genes helping the influence of virus to enter our human cells. So the virus does not just enter on its own there are some it uses some of our own machinery to survive and infect ourselves. So if we can identify those proteins then we can come up with the better therapeutic or preventive measures that is the you know paper here. They identified genes that help in this process how did they do again they used automated process, let us not get into all the details but what is shown here is that a process that you have a viral particle you now that gets into the cell and then again it makes its own copies and again goes and infects and so on.

So what they have done is they have again used the same approach meaning you have a system wherein you are introducing the virus and looking it how it gets into the cell again makes copies of itself and then goes and does one more round of infection using time lapse videography and then on the top of it they have you know looked you know genes that affects this process. Now you go on knocking out every gene each one after the other or high throughput screen and see in which when you knock out a given gene or whichever genes when you knock down then this process of infection is altered right.

So the use of this very similar approach of image processing to identify genes and they are able to tell that it uses a machinery which is normally the cell uses to clear the abnormal proteins that are made in the in the in the cell. So it uses that machinery hijacks that for its own benefits and it is able to multiply and so on so that is you know the way they have done.

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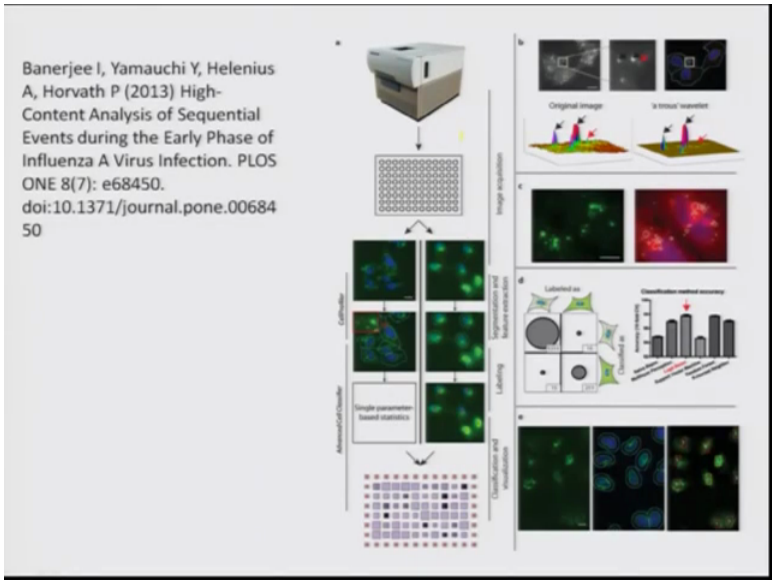

So again just to show a flow chart that you have had a system wherein you have a large number of you know what is called as a multi well plates you have seeded the cells, you have seeded the virus, you have ways to image certain events there and then the computer scores this images and tells you whether the infection is proper whether the virus is able to replicate and make more particle or not. With that you are able to target the genes that help in this process, so that is the way it is done.

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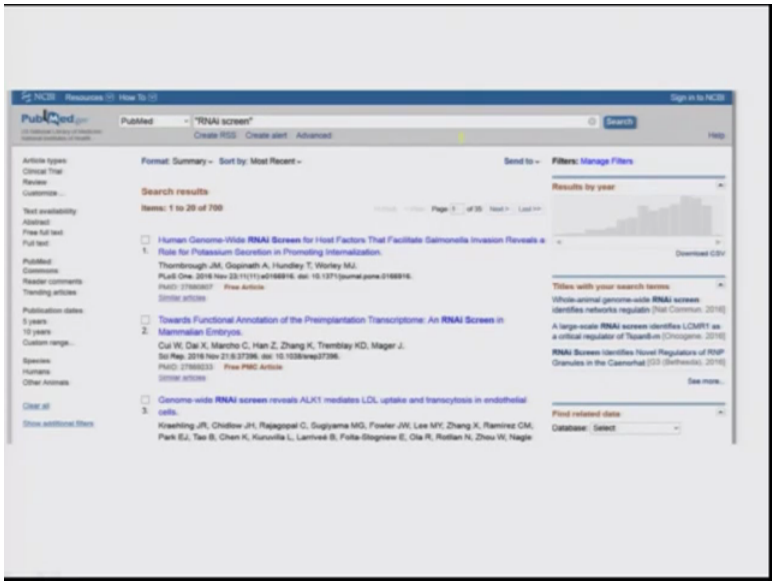

So again these are using the RNAi as I said the knockdown of the gene is done by duplex that people use and this RNA screen is something that now is routinely done and I just give this RNA screen. This is a pubmerge search and you will find that already when I say screen it is not individual RNA or individual gene. You are talking about you know screening for a particular process using thousands of constructs that knockout thousands of genes. So when you see that you are already 700 papers and you can see here on the right side this cystogram only talks about how you know a number of papers that used these RNAs screen as increasing by every year then you can see that this is you know probably 2017 now sorry 16 and this when probably this approach is came.

You find that the number of papers goes up that means that the technique is being refined and more and more groups are using it because it is very desirable to find the screen right. So that really helps us to identify that is what it is about RNAi.

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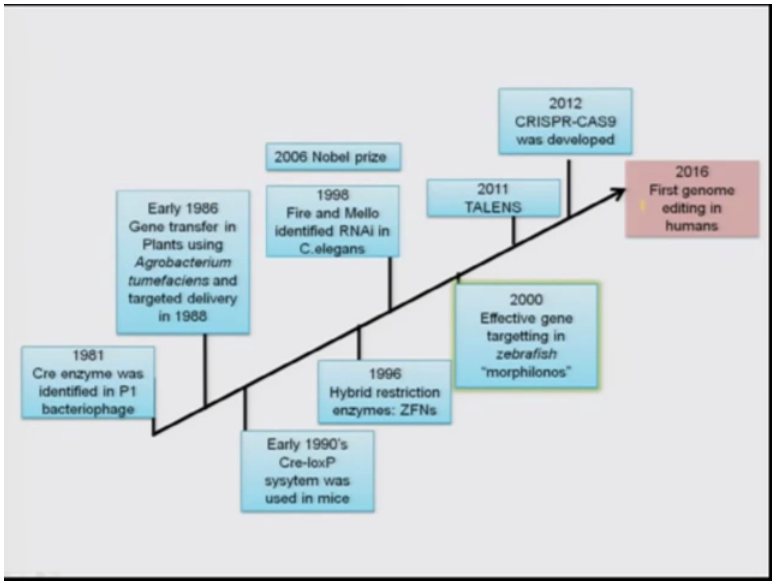

And because of its simplicity and the power you know to identify genes whose function is last you know this discovery led to the award of noble price to Mofire and Melo and that is really tremendous application in that. So we are going to go and look at another such approach which is called as morpholino again it is DNA sequence based knockout strategy originally you know described for zebra-fish.

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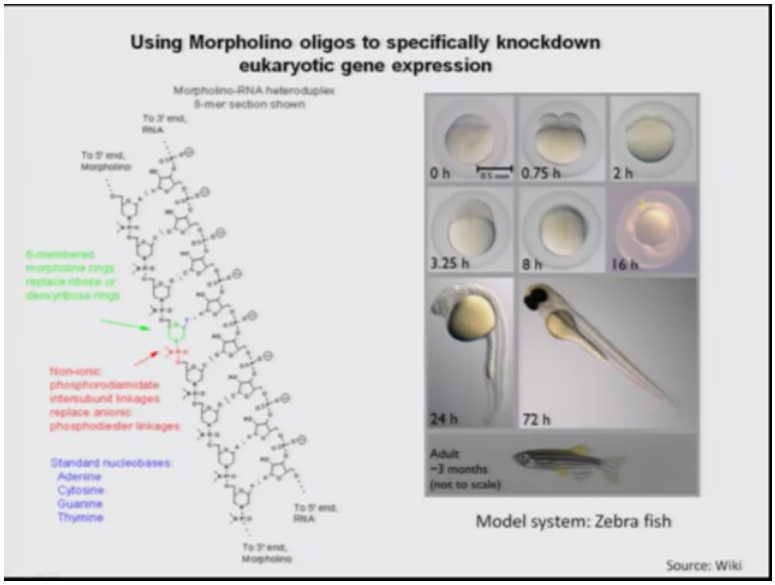

Let us see what it is, these morpholinos is nothing but oligos you know these are synthetic single stranded DNA but these you know the back bone of the the DNA that are made are modified such that you know it is a stable otherwise it may be degraded and these are antisense meaning they complimentary to given RNA and they have their efficiency to knock down the gene expression meaning it goes and forms a duplex like what is shown here and this is your RNA and this is your morpholino this is nothing but an oligo having a modified 5prime end and then you have this bases ok.

So they all form base pair just like that right and they have the same standard nuclear bases (()) (35:15) but there are changes like what is shown here these are non-ionic, this basically makes this nucleic acid to survive in the condition because your cell otherwise could you know degrade them right and this is the assay system with which people have developed this and validated it because these are zebra-fish. These are animals that lay eggs unfertilised eggs and sperm in the medium water and the fertilization takes place outside and you can see from zero meaning just fertilize to the formation of the young to adult happens outside therefore you can follow the development, you know under a microscope unlike for example mouse.

The development happens within the system, uterus then you cannot follow but here it is external therefore if you really want to study the genes that are involved in the early development. This is one of the best models because its vertebrate and external fertilization external development and the entire process is translucent. You do not need any colour any fluorescent protein and all so you can see that these are bright filled imaging just you are looking at an microscope you are able to see the all the process you can see that this is animal pole and you have the cells dividing and so on.

So then this embryo is coming out and you can see that. People have used this, so if you are looking at genes that are involved in developmental processes all you have to do is take these oligos and then inject into the egg and see whether the process is happening or hampered. If the gene is critical for the process then the RNA rather it is going to go and form a duplex that will not be translated so you will not have the protein made. So hampered the process will be hampered. On the other hand if there not involved the process the process will continue.

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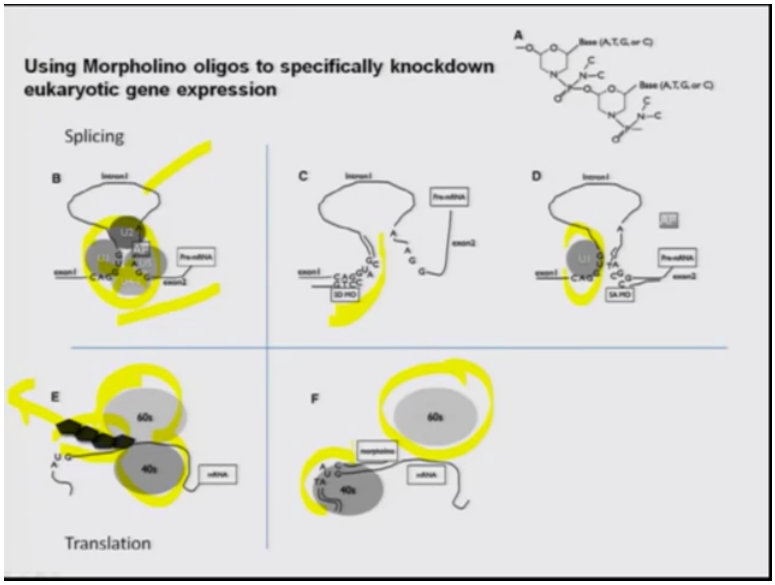

The question is how does this oligo or the morpholino really affect the gene or the RNA from splicing. There are two ways it can do for example it can even affect the splicing of the RNA, so what is known is that you must have studied in the textbook that when you have a gene which has multiple exons the introns sequence should be removed. What is shown here here that there are proteins that comes and binds and then cleave the RNA such that the intronic region is removed, so when you have this morpholino targeting to that kind of sequence where you have the intron-exon boundaries then what would happen is that it would prevent the formation of this (())(37:45) the complex that help in splicing of the mRNA.

So when that happens the RNA will not be cleaved and some of the factors is still bound to the RNA and the RNA will never come to the (())(37:57) as a result you know there is not going to be you know functional because it will not make any protein. The other process is that that you can make the morpholino such that you know you have this ribosome binding to the RNA and then reading and then you will have the peptide being made translation process so you can make morpholino such that the assembly of the large ribosome is hampered. You make it to the 5 prime end of the RNA and where the assembly takes place therefore you prevent the translation, so in both the approach basically you are you are the end result is that the protein is not made it is as good as not having the genes so that is how you do.

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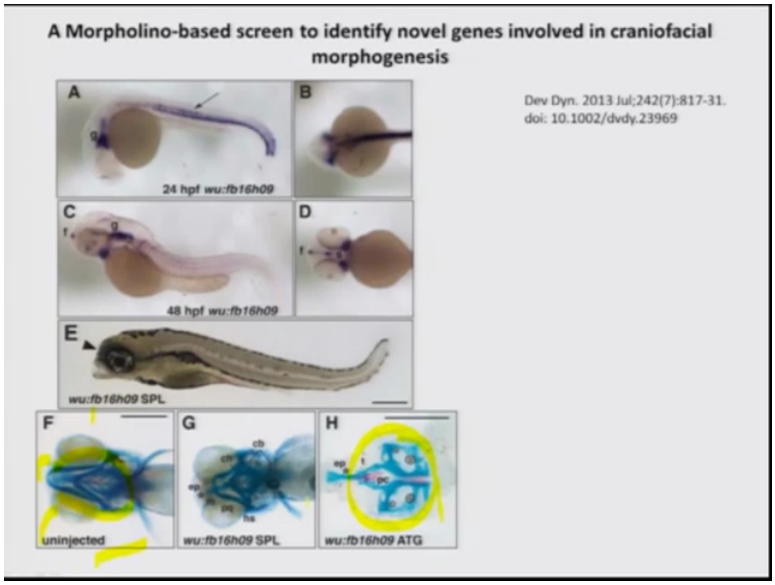

I am going to show you some examples again how people have used this approach to identify genes involved in certain developmental events. You must have seen that there are developmental email called as craniofacial morphogenesis meaning when you are the embryo is developing you have this colour which even forms the roof of your mouth and other part these are very tightly regulated development process there are people who have deformity in the development as a result they have a defect because it again because of some genetic defect that they have.

So what are the genes that are involved in such process right so this study here what is shown in this screen here they have looked at a large number of genes, they have used morpholinos to sort of affect the function of the gene and looked at how what are the genes that affect the skeletal the craniofacial development because these are all conserved genes because the same genes that functions the similar way in human as well. So you can see these are un-injected embryo they are normal you have the eye and then you have this this skull coming up and this is one such gene when you lose then the you know the skull is not being formed well. So in this way we are able to quickly come up with genes that are involved in this process you know.

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So that in fact you know there are several implications I will show you one more example in fact this paper used the zebra-fish model to understand you know a gene which is even involved in human development function. For example this particular gene called FAN1 mutation in the gene causes interstitial nefrtiti some defect in the kidney function and it is genetic mutation, it is a severe condition affecting the human. And and they found the mutation but they were not sure how does it really this gene or its or its mutation affect a kidney function. So they went to this in fact they stared with the zebra- fish model they used the morpholinos to knockout the genes and looked at how the kidney being developed their function and there are able to validate and show that the same gene is involved in human as well as in zebra-fish in the same function that is how the kidney functions right.

So that is the power of these approaches here your approach is unbiased you are not looking at any gene you are looking at every gene. Every end product is what is the functional out come in regard to kidney or regard to skeletal development regard to mitosis. You are looking at the process and you are looking at all the genes that are involved in the process so you are able to identify all the genes involved in that particular process. So that is pretty much the end of the functional genomics and we will end here and then we will again look into some other analysis especially on how you can look at the expression of the gene, because so far you are looking at

how you delete the genes and how you engineer the genome or destabilise the RNA and how the function is affected.

In the next lecture we will look into how we can look at the expression patterns, expression profile of various genes in a process