**RNA Biology**
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**Lecture - 40**
**Dosage Compensation and X-Inactivation: SRP and Different Modes of Compensation**

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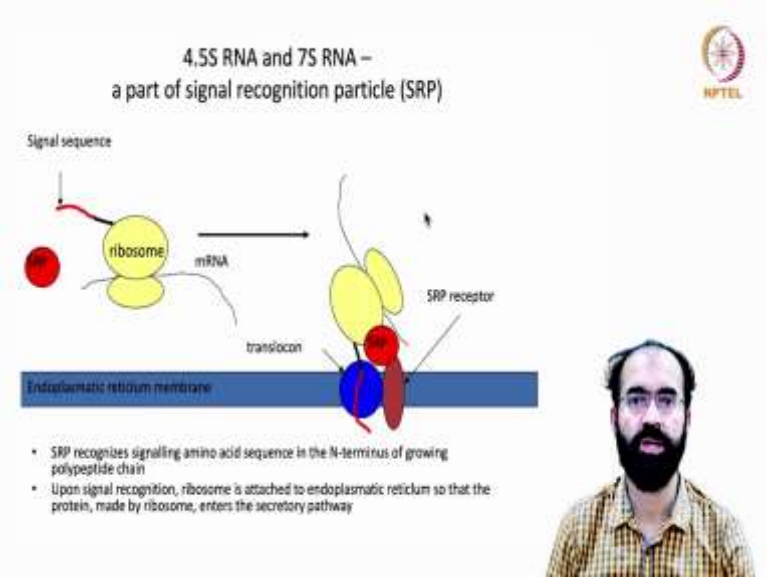


Hello everyone, welcome back to another session of RNA Biology. So, we were here in the previous class that we were trying to understand the signal recognition particle how it is governing the movement of proteins that are supposed to be pushed out of the cell or secretary molecule say growth hormone or many hormone, many enzymes or several. There are plenty of such protein molecules are there that is pushed out of the cell and the function is outside the cell not inside the cell.

So, they always will have a signal peptide sequence which has to be read by someone or recognized by someone that is signal recognition particle and that is pushed into the lumen of the endoplasmic reticulum.

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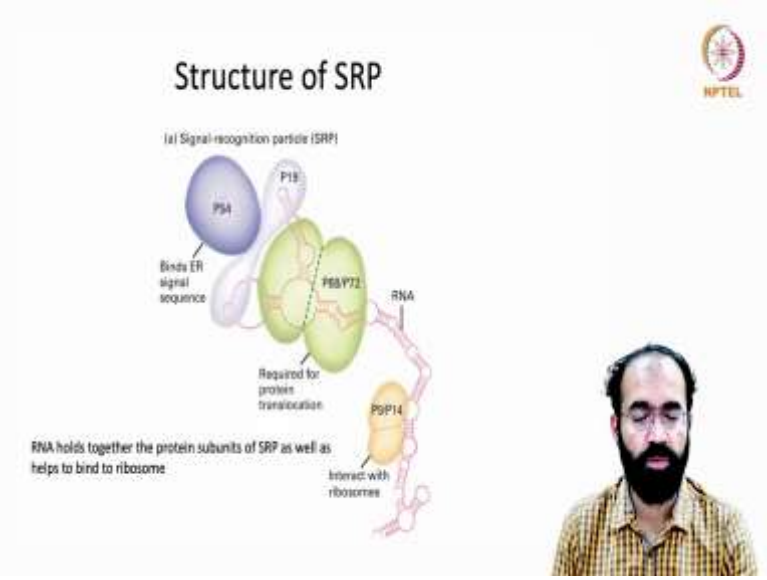


So, if you look into the structure of SRP Signal Recognition Particle it is a group of a protein as well as RNA complex. You can see a bunch of protein molecule such as P54 and it binds to the ER signal sequence, P19 and P normally stands for the P stands for Protein and the 54, 19 etcetera all meant for kilo dalton their molecular weight.

And P68, P72 it is a dimer and then comes the RNA part, then P9, P14 another two protein they interact with the ribosome and this P68 and P72 is required for protein translocation, that is they bind on to the signal recognizing sequence on the protein. That is we usually refer to as signal peptide that is recognized by the signal recognition particle and RNA holds together the protein subunits of SRP as well as it helps in bind to the ribosome.

So, this non-coding RNA is responsible for gluing together just like think about a garland where you have a thread and you just sue the beads on to it with a through a needle or something and you end up getting a garland like a pearl garland or some plastic beads or glass beads you just sue through a needle.

And or you make some flower garland there also you say thread and RNA acts like a thread and these proteins are glued together like a bead. So, that is the importance of a non-coding RNA in maintaining the function of maintaining both structure and performing the function of signal recognition particle.

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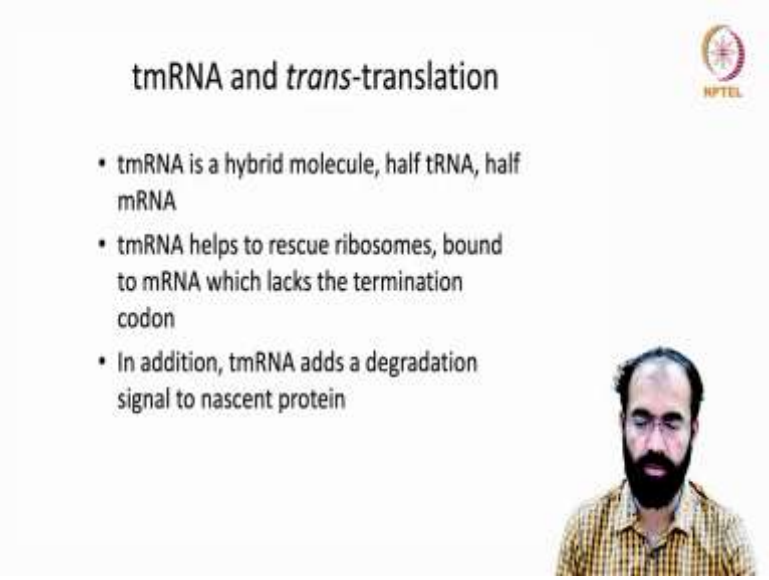


Now, let us see another group of RNA that is tmRNA and trans translation. Let us see what is this. tmRNA is a hybrid molecule, half tRNA and half mRNA. So, it is doing the job of both, it can do both the functions. So, we call it as a tmRNA.

tmRNA helps to rescue ribosomes bound to mRNA which lacks the termination codon. Remember we saw in the previous class some examples that how different RNA decay takes place like nonsense mediated decay, no go decay or like many examples were there where we talked about the RNA meant for degradation. But some cases it need not necessarily be degraded that is what we are going to see.

tmRNA helps to rescue the ribosomes bound to an mRNA which lacks the termination codon. Usually if termination codon is not there it will go all the way till the tip of the RNA, right. And we saw example where it is meant for degradation, but it need not happen so always there can be some exceptions. And if a tmRNA is available it can be rescued. In addition, the tmRNA adds a degradation signal to the nascent protein. If stop codon was not there, the product that has come may not be of any use.

So, the tmRNA will have few codons that will add few amino acids onto this unwanted or useless protein sequence that it made from this mRNA which lacked this protein. Because if this protein stays back if this protein start doing some function which a cell do not want or an undesirable function this will give to lot of trouble to the cell.

So, this tmRNA not only rescue the ribosome not only detach the ribosome from the mRNA, but it can also do something to cause the degradation of this protein which formed from that RNA which lacks this stop codon.

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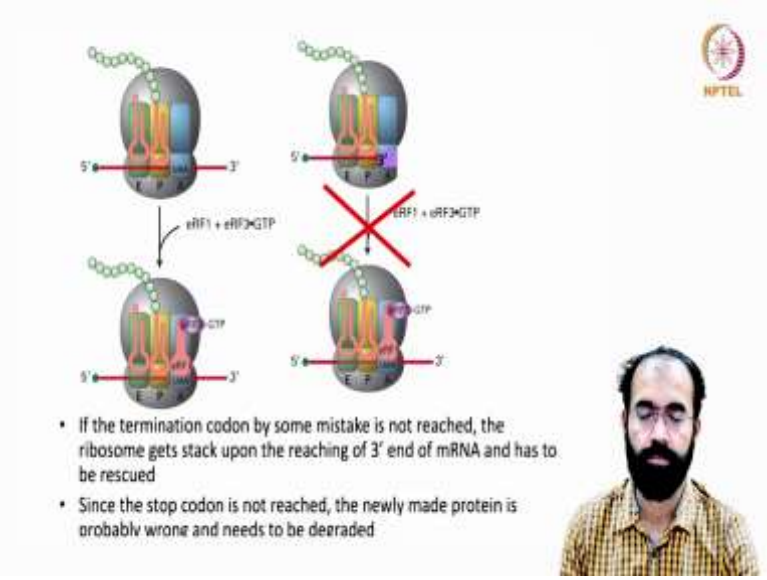


Let us see how is it doing. So, normally a RNA which you are seeing here and you have this black color 2 ribosomal subunits and you also have few sites. Acceptor site and peptide transferase or peptide bond forming site and escape or exit site.

So, 3 sites are there. So, normally a tRNA with amino acid enters through A site peptide bond is formed here and then it will move to the E site the previous one and it will come out of the cell. So, any given time the peptide site the P site will have the entire stretch of amino acid that is grown. Because earlier this peptide was with this RNA this tRNA, but this tRNA jumped to this position while throwing the already existing until here only this tRNA had.

So, this amino acid was carried by this tRNA when it entered through the A site. So, this tRNA this tRNA had single amino acid here. So, remaining all amino acids were attached in this same manner onto this tRNA. So, it gave the whole amino acids number sequential number to this tRNA and it moved to this place. And now when a new tRNA comes in this P site tRNA will give the whole amino acid to that tRNA.

But here it cannot come, why? Because there is a termination codon UAA, release factors can come depends on what condition you are talking. But here it is not going to be recognized in that way. So, eRF1, eRF3 and a GTP joins because tRNA cannot come, it can come, it stays and it is situated here and this can be the normal way of disassembly.

However, if this does not happen because it is a premature stop codon or an unwanted stop codon on or it is in the end of the RNA there is it is in a place then it is in a place where it is not welcome. So, what happen? eRF1 and eRF3 and GTP do not come here and that mechanism is not going to act. That situation what happen, why it is happening?

If the termination codon by some mistake is not reached the ribosome gets stuck upon the reaching the 3 prime end. Because it is not reached because this RNA do not have any codon as you see here. Because it reached all the way till the tip of the RNA because there is no stop codon, stuck on reaching the 3 prime end of the RNA and there is no place for this eRF1 and eRF3 to come and occupy because there is no codon available, stop codon should be there.

So, they are stuck like this it is stuck with a peptide attached on to it. Since the stop codon is not reached because it reached till the tip, but not stop codon is reached the newly made protein is probably wrong and needs to be degraded. So, two things has to be done now. One is rescue the ribosome so that it can be used for another round of protein expression from some other RNA and this protein has to be marked for degradation.

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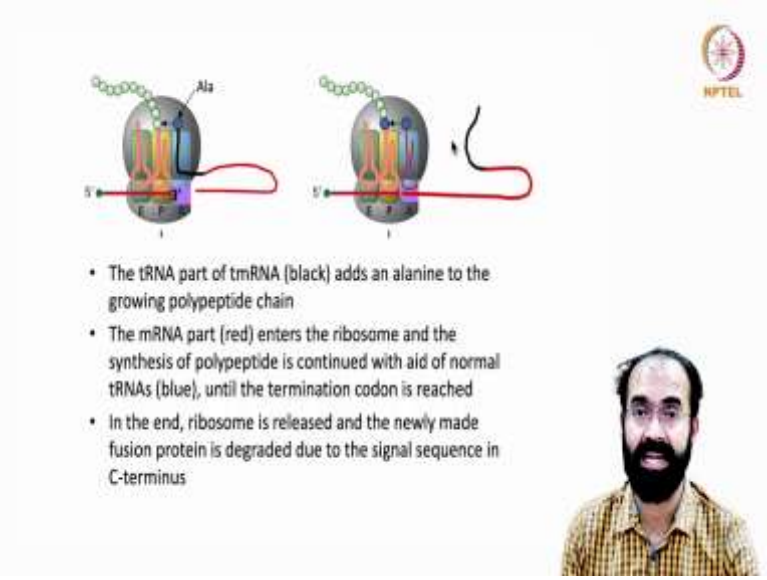


So, here comes the importance of the tmRNA. What it will do? It is having a alanine and a small stretch it can code for you will see that in detail. But most importantly it can have a stretch of sequence that can act like a mRNA part. So, this tmRNA what it did? It carried a amino acid in the tRNA part just like any other tRNA will do and it came and entered through the A site and it could accept the alanine from this tmRNA or tRNA part of the tmRNA and attach onto this polypeptide which was stuck.

Actually, this mRNA is only up to here, no stop codon came in and the ribosome do not know what to do, it is stuck there. So, this tmRNAs, tRNA part brought in and the alanine whereas, the mRNA part have a bunch of codons. So, what it will do? Once this codon attached in place then it can routinely bring in another normal tRNAS with a codon specific amino acids that can be brought in. So, another normal tRNA came in which has an amino acid.

So, this alanine was from this tRNA part of this tmRNA and then this is from another normal tRNA which has an amino acid that is now attached onto it. And the tRNA part of the tmRNA which is shown in black color adds an alanine to the growing polypeptide chain and the mRNA part that is given in red color enters the ribosomes and the synthesis of the polypeptide is continued with the aid of normal tRNA as you see here normal tRNA present in the cell until the termination codon is reached. So, this tmRNAs mRNA part is having a stop codon right here.

So, that it will continue codon multiple codons until it reaches here. But during this process it is now adding a bunch of amino acids that is present in the mRNA part of this tmRNA. In the end the ribosome is released because you know eRF1 and eRF3 can happily come because it has a stop codon. And it is released and the newly made fusion protein is degraded due to the signal sequence in the C-terminus.

Because normally protein starts from the N-terminus. So, this is the N-terminus of the protein and this is the C-terminus. And C-terminus you have a bunch of amino acid added now onto this protein with respect to the codons present in this mRNA part of the tmRNA.

So, this addition of few amino acids will make sure that this protein should be marked for degradation. It is almost like a painting like some number is given to that protein that you know this fellow should be marked for degradation; this protein should not be here. So, that message the degradation machinery of the cell will get that message by looking at these amino acids.

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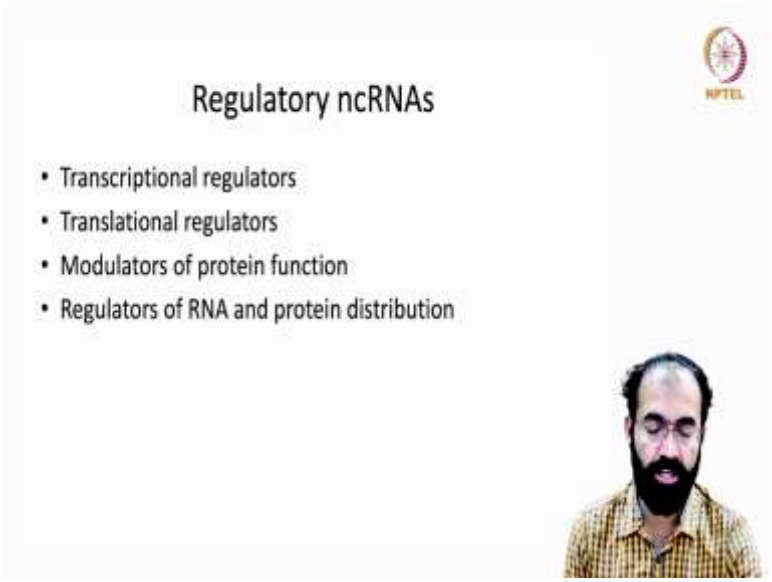


So, regulatory non-coding RNAs they can be transcriptional regulators. So, far we saw regulatory non-coding RNA that is meant for housekeeping function because tmRNA and various other non-coding RNAs are meant for housekeeping function. And now we are seeing the regulatory non-coding RNA. They are mainly transcriptional regulators

and they can also be translational regulators which act on to a mRNA post-factor or post-transcriptionally.

So, they are modulators of protein function, they can also influence the functionality of a protein. You may have heard how a phosphorylation affects the proteins folding structure etcetera like that association with a regulatory non-coding RNA also can influence.

We saw in signal recognition particle how an RNA is holding together a bunch of other proteins like that association of a regulatory non-coding RNA can influence or modulate the function of a protein itself. So, regulators of RNA and protein distribution so the regulatory non-coding RNA can influence, where a given protein should be located, where it should be pushed, where it should be placed etcetera.

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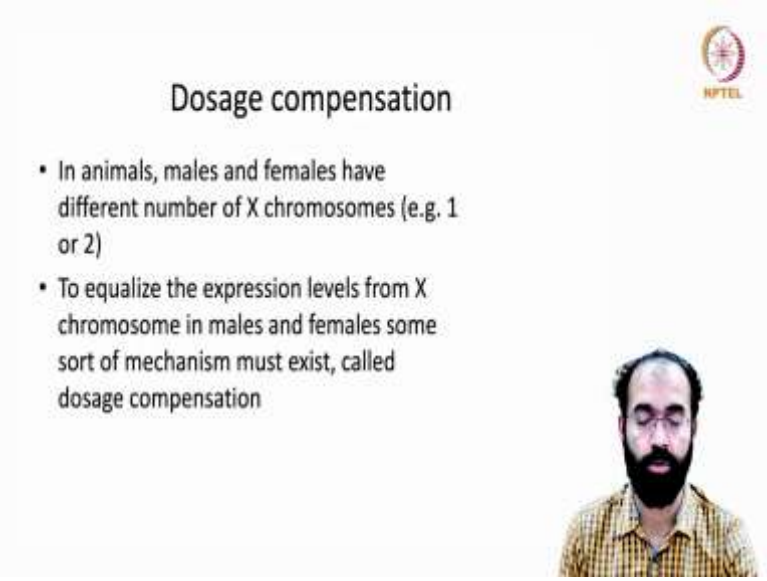


Then comes the most important regulatory role of a non-coding RNA that is dosage compensation. So, it is a relatively new term or a new topic for some of you, but the word itself convey something dosage compensation. In nature you can see plenty of situations and circumstances there is a compensation of the dosage.

Like you can see in nature many strong, very huge animals they are all herbivores. Like you can see elephant, rhinoceros, hippopotamus like many by default or buffalo these are all herbivores. On the other hand, if you see carnivores there are big carnivores, but they are not huge in size.

In nature if you see what if a elephant size or a rhinoceros size animal was carnivore there would not be any vegetarian animal left on the planet because they can completely attack and eat every because they are big in size they need to eat more and they will completely wipe out the entire you know herbivore so-called herbivore species in no time.

So, what you should understand nature has made such a way that the herbivores are big in size, they can give a fight and they can also escape and they can also breed more, they can give rise to more number of you know they are they are breeding cap because plant is everywhere, they do not need to hunt and eat plant, they just need to go to a place where plant is available.

So, their life is better compared to that of a carnivore. So, carnivore need to be must most agile and they have to struggle to get their food. So, that is why the number of carnivores is never as many as number of herbivore. So, this is why I gave this example is some kind of dosage compensation, what you are seeing here is some sort of dosage compensation. You can see many animals like zebra or horse these are all quite strong and fast runners, but they do not have horns as a buffalo has or an elephant has.

So, since these animals are being very fast and very agile, if they have horns that is an extra weaponry for them. So, to compensate that they have teeth both in lower and upper jaws so they can like buffalos and all have mainly only on the lower jaw. So, horses and zebras they have both the jaw. So, they can bite nicely and you can think about giraffe and some antelope some deers. So, they have horns only during breeding season when they are breeding two males have to fight to win over the female.

So, they have horns only then as soon as the breeding season is over there, they can just simply shake off their horn and it will fall off, but during breeding season it is very strong. So, otherwise if they carry that throughout their lifespan it is. So, heavy and it can bring down their survival advantage when a lion is or some carnivore is chasing this horn can get hooked somewhere and it can reduce the speed and this deer male deer will be vulnerable.

So, females do not have it because they do not have to fight with another female. So, males have to fight with another male that is why it has got the horn. So, what you are seeing now is these are all different kinds of dosage compensation. In nature also there is

at molecular level you will see plenty dosage compensation takes place and we will see some examples what are they.

In animals males and females have different number of X chromosome either 1 or 2. We know human males have got only one X chromosome whereas, human females they have got 2 X chromosome. So, to equalize the expression levels from the X chromosome in males and females some sort of mechanism must exist called dosage compensation.

So, there are different ways of it just like if you have two kids at home if you are buying a pen for one the other will cry. So, you have to buy two pens and preferably of the same color or same type and shape. If one pen is blue color the other is green then the both of them will say I want blue, I want green.

So, if you are smart you should keep them of same size, same shape, same brand everything equal, then there is a chance that it is almost compensated. So, gene expression also need to be compensated similarly. So, if a female have got two X chromosome if both of them are expressed proteins then that will cause an imbalance.

Because every chromosome or every chromosome located genes when they produce protein they are not working alone, they always work in conjunction with proteins produced from other genes located in other chromosome. It is just like you think about anything like if one country is producing a lot of tire only, it is not producing vehicles then and no exporting also.

Then what you will do with this tire? All you know is a tire only. So, you need to have equivalent amount of cars also being produced so that this tires can be utilized. No point in keep on producing tires, same way with car no point in keep on producing car engines if you do not have tire or body options available. What will you do with this car engine?

So, cell also functions similarly. So, if you have a given set of proteins it has to be utilized or interacted with other proteins produced from elsewhere. So, more also problem less also problem. So, nature has made a strategy that one X chromosome is ok, and remember all our genes all our other chromosome they are in pairs chromosome number 1, 2, 3, 4 up to around 22 they are in pairs they are identical pairs.

When it comes to 23 all the problem starts. That is either you have an X and X or you have an X and Y. In males it is X and Y which is not pair you cannot call it as a pair. And in female it is X and X there you can call it as pair, but in reality, it is not a pair, why? Because one of them in humans and mammals one of them is shrunk and made debilitated; that means, it is useless it is never utilized.

So, only one chromosome functions. And in human male the Y chromosome is there that contains a very few number of genes that is meant for maintaining the maleness. Like I told you one of the earlier classes that human body pattern is going to be feminine. To become a female body, you do not need to do anything, to become male body you need to have lots of genes especially the SRY Sex determinant Region of the Y chromosome that is called SRY which is constantly fighting against becoming body a feminine body.

If people who do not have SRY genes or who do not have proper functioning of their male hormones they will look exactly like female you cannot distinguish, they may not have the reproductive organs like ovary etcetera, but they have all the body features of a female you cannot distinguish whether that person is a male or a female unless you do chromosome preparation.

So, SRY gene is present in the Y chromosome and that is the major gene that major contribution of the Y chromosome in human males. So, technically in humans and other mammals only one X chromosome is representing the 23rd chromosome, there is no pairing actually exist.

So, males Y chromosome is well justified to prevent the body becoming feminine and whereas, in female the body anyways is female you do not need to struggle to make it a feminine body. So, the X chromosome will doing lots of housekeeping function as in the case of a male X chromosome. So, this is a strategy done in mammals. So, this is what is called dosage compensation.

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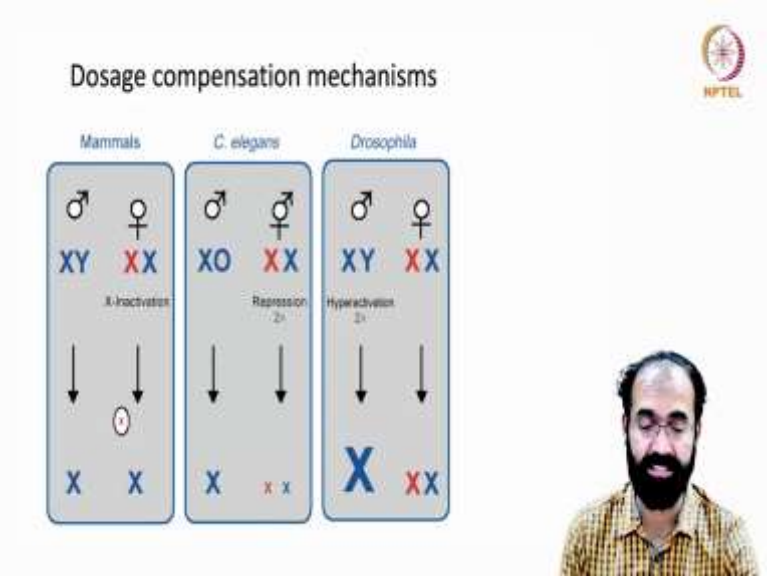


So, there are different ways of dosage compensation mammals, C. elegans and drosophila. Let us see how is it done. In the case of mammals males have X and Y whereas, females have got X and X.

So, mammals resort to inactivation. In males no inactivation because only one X is there in females there are two X, one of them is inactivated that is given in red in color. So, it is inactivated and net result in the individual cells you have only one X chromosome.

In C. elegans males have got X and no empty O means not a chromosome it is empty, no homolog or no pairing nothing in the place of y. Whereas, the there is no females in C. elegans they have they are called hermaphrodites, they have got both male and female body parts.

So, that is why this symbol is given not a typical female symbol. Female and male combined they have both X and X and they have two X two chromosome, but they are not inactivating it, what they are doing? They are reducing the expression of both the chromosome. So, it became smaller X; that means, they are repressed.

Since almost like you know you are cutting off a portion of it, not physical cutting, but you make sure that its strength is diluted. It is just like if you have more people to have one dish or so at home what or any place what they do is when you have more visitor

they pour some water into the [FL] or you pour some water into the milk. So, that you will get more it is a kind of dilution effect.

So, technically you do not have more milk, but it appears like you have more milk. So, 1 liter milk can be made into 2 liter milk without buying extra 1 more liter milk by adding one liter of water. But in quality is a 2 liter milk no, but in reality is a 2 liter milk, yes. Qualitatively it is low, but technically it is dilute, but you can fulfill and same kind of dilution will happen that is called gene repression. Dilution will happen onto this X chromosome that is why it is shown in smaller color, smaller size, red color and blue color is maintained.

So, in drosophila same system is there male have got X and Y female have got X and X similar to that of mammals, but they do a different strategy. What they do is they are both the females both the X chromosomes are working as it is there is no repression or nothing is happening then how do you match it? Because the male X chromosome is made hyperactive now.

So, male X chromosome is boosted up that is why given a bigger X shape here. So, now, male X chromosome is super strong and male X chromosome can do the job of both the regular 2X chromosome. It is just like if you have like normally in labor department and all or many like in in many Multinational Company or even many countries like if you want to make a huge ship or you want to make a rocket you normally refer to as how many man hours.

So, 1 man hour say 1 day you will work 8 hour. So, I want 80 man hour, if I want 80 man hour what I will do? I hand make one fellow work for 80 days or I can employ 80 10 people in 1 day. If I employ 10 people in 1 day, I get 80 man hour or I keep 1 fellow for 80 days then also I get 10 days sorry, not 80 days, one fellow works 8 hours.

So, keep that fellow for 10 days you get 80 man hour. So, you can choose whether I want to get this 80 man hours 80 man hours in 1 day or in 10 days. Accordingly, you choose the people. So, here 2X chromosome is working normal and to match it so that is a lot, to match it the male have got only 1 X chromosome. So, what it will do? It will cause hyper activation 2X here it was repression 2X, 2 times 2x basically mean 2 times, 2 times repression in C elegans, 2 times hyper activation in drosophila.

So, that it will match the females 2X chromosome. So, mammals it cause 1 X chromosome is blocked in C. elegans both the X chromosomes are decreased in their performance and in drosophila one of the males X chromosome is boosted. So, this is how they maintain the dosage compensation. We will learn more about the dosage compensation in the subsequent class.

Thank you.